Assessing Chronic Musculoskeletal Pain in Horses and the Effectiveness of the Administration of Paracetamol in addition to Non-Steroidal Anti-Inflammatories for Chronic Pain

Stefanie Louise Pratt

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List of Abbreviations

NSAID – non-steroidal anti-inflammatory drug

HRV – heart rate variability

ECG – electrocardiogram

SDS – simple description scale

VAS- visual analogue scale

NRS- numerical rating scale

CPS- composite pain scale

IMUs – inertial measurement units

MinDiff – minimum difference

MaxDiff- maximum difference

UpDiff – upwards difference

HHD – hip hike difference

COX - cyclooxygenase

HDmin - mean difference in head minimum

HDmax – mean difference in head maximum

PDmin- mean difference in pelvis minimum

SIup – symmetry index of upwards movement

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Attributions

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Adam Redpath BVMS PGCert(VetMed) DipECEIM MRCVS was an Assistant Professor of Equine Internal Medicine at the University of Nottingham. He is now working in private practice as an Equine Internal Medicine Specialist. Dr Redpath was a reviewer for the systematic review, assisted with data collection and interpretation during the in vivo study and contributed to manuscript preparation.

James Bailey BVetMed MRCVS is an Assistant Professor of Equine Sports Medicine at the University of Nottingham. Dr Bailey was a reviewer for the systematic review, assisted with data collection and interpretation, in particular contributing significantly to the IMU data analysis, during the in vivo study and contributed to manuscript preparation.

Mark Bowen BVetMed PFHEA CertVA CertEM(IntMed) PhD DipACVIM DipECEIM DipECVSMR FRCVS was a Professor of Equine Internal Medicine at the University of Nottingham. He is now an Equine Internal Medicine consultant and is the Director of Education at EBVS. Dr Bowen was integral to the idea and design of the thesis and in vivo study. He was a reviewer for the systematic review, assisted with data collection and interpretation during the in vivo study and contributed to manuscript preparation.

Gayle Hallowell MA VetMB CertVA DipACVIM PhD DipACVECC PFHEA FRCVS was a Professor in Large Animal Critical Care and Internal Medicine at the University of Nottingham. She is now the Head of Veterinary Professional Development for IVC Evidensia. Dr Hallowell assisted with statistical analysis and the power calculation.

Abstract

Equine lameness is a common problem which affects both equine athletes and geriatric horses, with geriatric horses being at increased risk of lameness (BlueCross, 2018, Ireland et al., 2011). Lameness leads to a shortened working career, increased financial losses and reduction in welfare (Ireland et al., 2012, Marshall et al., 2012). Lameness is also very important to owners, however there is significant under recognition of lameness by owners (Dyson et al., 2017, McGowan et al., 2010). As lameness is a manifestation of pain due to a musculoskeletal injury, when lameness is not recognised horses can continue to be worked leading to compromised welfare (Vinuela-Fernandez et al., 2011). Recognition of musculoskeletal pain relies on assessments including pain scales, subjective lameness grading scales and objective technologies including forceplates, kinematic technology and inertial measurement units. Subjective lameness grading scales have been shown to have marginal agreement between observers especially when considering mild or hindlimb lameness (Dyson, 2011, Hammarberg et al., 2016, Keegan et al., 2010). Objective gait analysis systems may be more reliable especially when considering mild lameness. A narrative review briefly compared both objective and subjective gait analysis systems (Crecan and Peștean, 2023), however there are currently no published systematic reviews which compare the effectiveness of subjective and objective gait analysis.

Once detected, lameness often requires analgesia, with phenylbutazone, a non-steroidal anti-inflammatory drug (NSAID), being most commonly used (De Grauw et al., 2014, Duz et al., 2019, Sabate et al., 2009). However, NSAID use can lead to side effects including gastrointestinal toxicity, with phenylbutazone being the most likely to induce adverse events (Bowen et al., 2020). Paracetamol is used in horses alongside NSAIDs for multi-modal analgesia (Bruniges et al., 2019, West et al., 2011), however to date there are only a few studies published which have investigated its analgesic effect in the horse as a monotherapy (Foreman et al., 2016, Mercer et al., 2023a, Mercer et al., 2023b, Mercer et al., 2022). As a single analgesic at 20mg/kg administered orally, paracetamol has been shown to significantly reduce lameness score and heart rate compared to a control in an inducible acute foot pain model, and was comparable to flunixin meglumine (Foreman et al., 2016). In another acute mechanically induced lameness model, paracetamol at

30mg/kg orally was shown to reduce the lameness score, however 20mg/kg did not (Mercer et al., 2022). This study also compared paracetamol to phenylbutazone, and showed that oral paracetamol at 30mg/kg and oral phenylbutazone at 2.2mg/kg did improve lameness scores compared to the control group, although this occurred at different times post treatment. Mercer et al. (2023a) investigated paracetamol as a monotherapy in horses with naturally occurring chronic lameness and showed that at 30mg/kg orally there was a transient improvement in lameness both subjectively and objectively. Lameness assessments were only performed after administration of 3 weeks of paracetamol at 30mg/kg twice daily orally. Further studies are required to determine the effect of paracetamol on chronic lameness in combination with an NSAID.

This thesis aims to improve our understanding of assessment of chronic musculoskeletal pain in horses and the effectiveness of the administration of paracetamol in addition to an NSAID for chronic pain. The literature review is therefore focused on discussing pain physiology in the horse and how pain is best assessed and recognised. There is a particular focus on musculoskeletal pain recognition including subjective and objective gait assessments. This is followed by a review of NSAID and paracetamol use in the horse. A systematic review was also conducted to review the literature to answer the question *'In lame horses does objective gait assessment versus subjective gait assessment improve the accuracy of lameness detection?'*. Objective and subjective gait analysis techniques were then used to assess the response to the addition of paracetamol treatment in chronically lame horses currently treated with NSAIDs in order to determine whether paracetamol reduced the lameness severity.

Chapter 1: Literature Review

Introduction

Equine lameness is a common problem worldwide and can lead to a reduced career, financial loss and most importantly compromised welfare (BlueCross, 2018, Marshall et al., 2012). Lameness can potentially affect all horses including both equine athletes and geriatric horses. It causes the majority of health problems seen in dressage horses (Murray et al., 2010) and lameness within the previous 3 months has been identified as a risk factor for catastrophic musculoskeletal injury in thoroughbred racehorses (Hitchens et al., 2018). Geriatric horses are also significantly more likely to become lame (Ireland et al., 2011). As the lifespan of horses is increasing, this presents a scenario where a large proportion of the population is at greater risk of developing lameness (Ireland et al., 2012). Although there are many causes of lameness, up to 50% of lame horses are thought to be affected by osteoarthritis (van Weeren and Back, 2016).

Although very important to owners, there is under recognition of lameness by owners and trainers (Dyson et al., 2017, McGowan et al., 2010). Dyson et al. (2016) demonstrated that in a population of sports horses assumed to be sound by the owner, 75% were lame of which 28% were obviously lame in a straight line. This highlights a key problem as lame horses are widely considered unfit to perform (van Weeren et al., 2017). Lameness is a manifestation of pain produced due to musculoskeletal injury, therefore continued use whilst lame is a real welfare issue (Vinuela-Fernandez et al., 2011). To avoid horses suffering from compromised welfare and a poor quality of life, improvements in pain recognition need to occur. To be able to adequately recognise, treat and monitor lameness in horses we must first understand how horses demonstrate pain and find robust ways to assess it.

Pain

The International Association for the Study of Pain (IASP) have defined pain as '*an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage'* (Raja et al., 2020*)*. They also highlight that an inability to communicate verbally does not reduce the pain an individual is experiencing or lessen the need for analgesia. However, this means pain assessment in

animals, who cannot communicate, is difficult. Molony and Kent (1997) use a definition of animal pain which relates the pain experience to the change in the animal's physiology and behavior to reduce damage, reoccurrence and promote recovery, which is a more usable definition for species that cannot communicate verbally. Animal pain is further complicated by the fact that many veterinary surgeons consider their ability to recognise pain and provide appropriate analgesic therapy to be insufficient (Dujardin and van Loon, 2011).

Pain is a complex experience generated initially by activated nociceptors and is designed as a physiological protective mechanism for the body (Gleerup and Lindegaard, 2016). Pain is composed of sensory, affective and cognitive components (Paul et al., 2005). The sensory component, involving the somatosensory cortices, allows the source, intensity, and duration of pain to be determined. The affective or behavioural component, which involves the prefrontal region and limbic system, includes neuroendocrine activation in addition to behavioural and physiological responses. This is affected by emotion and also affects emotion. The cognitive component interprets pain within the context of the environment. Animals feel physical and emotional responses to pain and therefore have both sensory and affective components to their nociceptive experience (Taylor et al., 2002). Although the cognitive component is difficult to determine in animals, it may not be specific to humans as previously thought (Paul et al., 2005).

Pain can have an acute or chronic nature. Acute pain is an adaptive response following a noxious insult leading to withdrawal from a potentially harmful stimulus, minimising potential tissue damage and promoting survival (Muir, 2005). When an acute pain episode persists beyond what is expected for tissue healing, it becomes chronic. Chronic pain is not associated with an ongoing noxious stimulus or healing process and so is considered maladaptive. It occurs due to abnormal sensory processing due to tissue or nerve damage, or abnormal nervous system function, termed neuropathic pain (Muir, 2005). Chronic pain has been recognised in horses, especially in musculoskeletal disease (Daglish and Mama, 2016). It has been associated with abnormal behaviours, including depression and aggression, reducing quality of life and potentially leading to distress and euthanasia (Fureix et al., 2010, Gleerup and Lindegaard, 2016).

Pain Physiology

The term 'nociception' encompasses all of transduction, transmission, modulation and perception of the nociceptive stimuli (Argoff, 2011). A stimulus is detected by peripheral receptors, called nociceptors. Nociceptors are free nerve endings of a first order afferent neuron which is a small diameter, minimally myelinated or unmyelinated Aδ or C fibre respectively (Almeida et al., 2004). The nociceptors can be activated by mechanical, thermal or chemical stimuli. The stimuli can also be characterised as inflammatory, neuropathic and nociceptive.

The nociceptive stimulus is converted to an electrical impulse via transduction. The stimuli causes specific ion channels to open, including transient receptor potential channels (TRP), acid sensing ion channels (ASIC) and voltage gated sodium channels (Nav). This alters the membrane potential of the cell leading to further voltage-gated ion channels opening, and depolarization of the afferent sensory nerve which generates an electrical signal (McEntire et al., 2016). This signal is transmitted to the central nervous system via primary sensory nerve fibres including unmyelinated C fibres and myelinated Aδ fibres. When considering a noxious stimulus affecting the skin, Aδ fibres propagate specific information quickly and are associated with the first acute pain and withdrawal reflex, C fibres propagate information more slowly allowing for summation and the sensation of dull pain, although this differentiation doesn't apply to other organs (Almeida et al., 2004). The cell bodies of these primary afferent nerve fibres are in the dorsal root ganglia or trigeminal ganglion and all terminate in the dorsal horn of the spinal cord. Here the primary afferent fibres synapse with second-order projection neurons which transmit the electrical signal via the spinothalamic and spinoreticular tracts to the thalamus for processing. In animals these tracts are thought to be bilateral, although these tracts are not well defined in horses (Wang et al., 2022). Additionally, interneurons are present within the spinal cord, which can have both a local inhibitory and excitatory function, or connect to reflex arcs (Almeida et al., 2004). From the thalamus, the signal is then transmitted to higher centres via the medial and lateral projection neurons, including to the somatosensory cortices and prefrontal cortex, allowing the perception of pain (Almeida et al., 2004). Once the pain signal reaches the cortices it triggers the descending pain modulatory system. This involves the periaqueductal gray and

rostroventromedial medulla which can produce an endogenous analgesic effect by inhibiting nociceptive inputs (Ossipov et al., 2014). This allows the pain response to be modulated, with the goal of enabling an animal to respond to the pain source.

Pain sensitisation is an increased sensitivity to stimuli. This can be peripheral or central in origin. Central sensitisation occurs when the nervous system is in a persistently high activity state. Increased recruitment of post-synaptic receptors secondary to an increase in excitatory neurotransmitters or a sensitisation of the interneurons of the adjacent primary afferents decreases the threshold for recruitment, leading to an increase in second order neuron activity. Nerve injury and dysfunction due to persistent sensitisation can lead to long term potentiation where intense or repeated painful stimuli can cause a persistent increase in the pain pathway sensitivity due to an increase in action potential firing. In addition, central sensitisation can affect multiple senses leading to light or sound sensitivity. Peripheral sensitisation involves the somatic nervous system or visceral nociceptors and occurs due to damage from inflammatory tissue byproducts including prostaglandins, bradykinin, cytokines (including IL-6 and IL-1β), endocannabinoids, eicosanoids and leukotrienes, which lower the nociception activation thresholds, allowing for recruitment of silent nociceptors or increasing the magnitude of responsiveness at the peripheral end of the sensory nerve fibre. This can cause repetitive activation at a lower stimulus intensity, more vigorous responses when stimulated, prolonged neuronal discharges and expand the area which can produce stimulation of that nerve (Graven-Nielsen and Arendt-Nielsen, 2002, Schaible and Richter, 2004).

Neuropathic pain is characterized by chronic pain which is initiated by dysfunction or lesions of the nervous system and can be maintained via different mechanisms including damage to inhibitory nociceptive pathways, excessive nociceptive pathway stimulation and pain signals produced in response to an innocuous stimulus (Graven-Nielsen and Arendt-Nielsen, 2002, Schaible and Richter, 2004). It can involve both the central and peripheral nervous system.

Referred pain is commonly reported in humans but is less well understood, and is not reported in animals (Jin et al., 2023). Both central sensitisation and peripheral reflexes may be implicated in the pathological mechanism. Other mechanisms include sympathetic autonomic interplay between visceral and somatic neurons, up-regulation

of pain projection neurons enhancing response from nociceptive neurons in another body region or formation of abnormal neuronal connections (Procacci and Maresca, 1999).

Although pain sensitisation, neuropathic pain and referred pain have distinct definitions as described, the distinction, particularly clinically, is not always well defined and they are often appreciated as a continuum.

Pain Recognition

As pain is a highly subjective and emotional experience, objective assessment is challenging. This is exacerbated in horses as in addition to being unable to verbally communicate, they are prey animals and therefore suppress overt signs of pain (Dalla Costa et al., 2014). Pain is also affected by the horse's personality, suggesting that when introverts feel pain it may be even more difficult to detect (Ijichi et al., 2014). However, pain has been shown to affect both behavioural and physiological variables in horses which may be used for assessment (Gleerup and Lindegaard, 2016).

To recognise pain, it is important to understand both normal and pain behaviours shown by horses. Signs of generalised pain include depression, reduced movement and appetite, low head carriage and a decrease in interaction with both the environment and humans (Dalla Costa et al., 2014, Graubner et al., 2011, Pritchett et al., 2003, Raekallio et al., 1997, Reid et al., 2017). In addition to a depressed and lethargic state, pain can also cause aggression and self-mutilation behaviours (Hausberger et al., 2016, McDonnell, 2008). Therefore, it is important to consider that a behavioral change may be a consequence of pain.

Multiple studies have identified specific behaviours which are thought to be linked to a pain type (Dalla Costa et al., 2016, Dyson et al., 2018, Grint et al., 2017, Graubner et al., 2011, van Loon et al., 2014). Studies investigating visceral pain highlight pawing, postural behaviour and interactive behaviour (Graubner et al., 2011, van Loon et al., 2014). In comparison, orthopaedic pain behaviours commonly cited include weight shifting, backwards ear position and partially closed eyes (Dalla Costa et al., 2016, Dyson et al., 2017, Grint et al., 2017). This suggests pain expression is dependent on the type and origin of pain, however this may be due to the fact research thus far has focused on

specific patient groups. A review by Gleerup and Lindegaard (2016) demonstrated that the same behavioural signs are included in pain scales, independent of pain type. Pain behaviours may also be able to indicate disease severity with regard to intestinal disease, as an association has been demonstrated between the behaviours expressed and whether horses exhibiting signs of abdominal pain required surgical or medical treatment (Sutton et al., 2013a, van Loon and Van Dierendonck, 2015).

Behaviour can be affected by unfamiliar environments and people, potentially masking pain signs (Gleerup and Lindegaard, 2016, Taylor et al., 2002). In addition, fear and pain behaviours overlap, and when both are present, signs corresponding to anxiety are shown in preference to pain behaviours (Hall et al., 2013, Reid et al., 2017). The individual and breed may also affect behaviours shown (Taylor et al., 2002). Therefore, it is important to ensure pain assessments using behavioural expression are robust and tested with multiple patient groups and in different environments to ensure validity.

Physiological indicators including heart rate and respiratory rate, have been investigated as a method to assess pain. However, many studies have demonstrated they have low sensitivity and specificity and are not useful in a clinical setting (Daglish and Mama, 2016, De Grauw and Van Loon, 2016, Gleerup and Lindegaard, 2016). Bussieres et al. (2008) evaluated non-invasive blood pressure when assessing orthopaedic pain and demonstrated it had good specificity and high sensitivity. However no further studies have confirmed this finding.

Heart rate variability (HRV) has also been investigated as a physiological indicator of pain. HRV is the fluctuation in time intervals between adjacent heartbeats, measured by using the R-R interval recorded using an electrocardiogram (ECG). In addition to being a marker of autonomic nervous activity it has been suggested that changes in HRV may indicate pain or stress in horses as seen in human infants (Rietmann et al., 2004). Reitmann et al. (2004) showed that although mean heart rate decreased with analgesia administration there was no significant difference seen in HRV. Reid et al. (2017) also demonstrated no change in HRV with pain alone, however reduced HRV was seen with anxiety. They also suggest that anxiety can alter a horse's response to pain, as restlessness increased in the anxiety-pain group compared to the pain group, potentially resulting in underestimation

of pain scoring for anxious horses. Therefore, HRV may be useful to measure the stress response to pain.

Pain Scales

A validated pain scale provides a reliable method which can be used by different observers, and the same observer on repeated occasions, to determine the severity of pain a horse is experiencing. Pain scales are essential to allow recognition of changing pain, enabling alteration of analgesia therapy (Dutton et al., 2009). Multiple pain scales have been investigated, with all including behavioural parameters and some using physiological parameters. Ideal features of a pain scale include being independent of the observer with a high agreement between observers, use of easily recognised well-defined behaviours, and to be able to be assessed within an appropriate length of time and to be practical (Ashley et al., 2005, van Loon and Van Dierendonck, 2018). Several limitations exist with pain scales, including subjective categories, arbitrary cut off values, use of a single modality during development to assess behaviour, such as video stills, and evidence that assessments work best without an observer present (Coles et al., 2018, Dalla Costa et al., 2016, Hausberger et al., 2016, van Loon et al., 2014).

Multiple tools have been investigated to determine an objective assessment of pain in horses including the simple descriptive scale (SDS), visual analogue scale (VAS), numerical rating scale (NRS), composite pain scale (CPS) and scales based on facial expressions. A SDS classifies pain by categories of severity including mild, moderate, and severe. VAS uses a 100mm line with no true scale, only two defined extremity points, and NRS utilises a discrete scale which can have any number of categories. These scales are all one-dimensional and inherently subjective when assessing an equine patient who cannot score themselves (van Loon, 2012). Instead scales utilising well-defined descriptors graded via simple descriptive scales combined to form an overall pain score are recommended, such as the CPS.

Various scales have been developed to differing effects. The Equine Acute Abdominal Pain Scale (EAAPS-1) was developed by Sutton et al. (2013a, 2013b) and later refined and revalidated (Sutton and Bar, 2016). This scale is a SDS, using an index of behaviours ascending in severity which correspond to a score. The presence of the most severe

behaviour manifested is the final pain score. This scale demonstrated good interobserver reliability and speed, when assessing horses with colic directly and using video footage, however agreement was superior with severe pain. The Equine Utrecht University Scale for Composite Pain Assessment (EQUUS-COMPASS) was also developed for pain assessment of horses with acute colic (van Loon and Van Dierendonck, 2015). In 2016 physiological parameters were removed and weighting factors were applied (VanDierendonck and van Loon, 2016). This scale performed with high interobserver reliability, significant differences in pain scores between control and painful groups and has been used by others effectively (Abass et al., 2018). Taffarel et al. (2015) developed a CPS for castration including assessing the effect of general anaesthesia. Initial inter- and intra-observer reliability was only shown to be satisfactory. Further refinements to the scale were made, however additional validation of this scale is now required. Bussieres et al. (2008) developed a composite scale for experimental acute orthopaedic pain which can be completed in less than 10 minutes with high interobserver reliability. It has been used effectively in clinical cases by others and suggestions have been made for scores corresponding to mild, moderate and severe pain to allow analgesic intervention to be determined (van Loon et al., 2010, van Loon and Van Dierendonck, 2019). There have also been multiple other composite pain scales reported which have not yet been validated (Dutton et al., 2009, Graubner et al., 2011, Pritchett et al., 2003, Sanz et al., 2009, Sellon et al., 2004).

Facial expression-based pain scales have also been developed for use in horses. Dalla Costa et al. (2014, 2016) developed a composite SDS which has been evaluated in horses undergoing castration and in horses with acute laminitis. The Horse Grimace Scale (HGS) correlated with a simultaneously assessed CPS and provided good interobserver reliability and is quick to perform (<2 minutes). It has the benefit that the horses are not required to move and emotional state was shown to have no significant effect on results (Dalla Costa et al., 2017). However, it has not yet been fully validated. A scale also based on facial action coding units was published by Gleerup et al. (2015). The Equine Pain Face was assessed using two experimental pain models live, using video footage and video stills and demonstrated consistency in pain observed when compared to a CPS. Though reproducibility was not assessed. Van Loon and van Dierendonck (2015) developed the

Equine Utrecht University Scale for Facial Assessment of Pain (EQUUS-FAP) to assess horses with acute abdominal pain. They later demonstrated its use in horses with other sources of pain, through assessing horses with pain originating from the head (van Loon and Van Dierendonck, 2017). The EQUUS-FAP showed excellent interobserver reliability and correlated well with a CPS. These facial scales also have the benefit that no physiological data is included therefore the scales can be used by all.

Assessment of facial expressions when horses are ridden has led to the development of the FEReq (facial expressions in ridden horses) scale (Mullard et al., 2017). Total FEReq score for lame horses was significantly higher than the scores observed in sound horses, however this was only the case when ridden. When stood lame horses had lower facial marker scores. This study was based on still images and minimal variables were controlled. Dyson et al. (2018) further developed this scale utilising video recordings and a controlled environment. They demonstrated a significant difference in 24 facial markers between lame and sound horses. Using these markers, they proposed an ethogram based on 24 behavioural markers assessed over 5 minutes of work, with a threshold above which is suggestive of musculoskeletal pain.

For all pain scales validation is important, as without appropriate validation scales are not considered accurate (Bussieres et al., 2008). However, it is hard to validate subjective pain scales as there is no gold standard scale to compare to (Dugdale, 2014). Therefore, as true validation is very difficult, if not impossible to achieve, it is even more important to consider how the pain scale has been evaluated and assess the robustness of the scale. The most robustly validated pain scale published to date is the EAAPS scale (Sutton and Bar, 2016), followed by the EQUUS-COMPASS scale (VanDierendonck and van Loon, 2016). All others are only partially evaluated with key steps in the attempted validation process being missed. Van Loon and Van Dierendonck (2018) outline how validation of pain scales can be achieved. Scales are often refined by eliminating behavioural features which are less sensitive and specific for the pain state being investigated. This also reduces the time required to assess the scales, making them more practical and user-friendly.

Musculoskeletal Pain Assessment Tools

When specifically considering musculoskeletal pain, lameness severity is thought to correlate to the degree of pain, as lameness is a behavioural expression of the underlying pathology (Ashley et al., 2005, Taylor et al., 2002). Lameness is a clinical sign of pathology as it presents when a loss of function or defect is present within the musculoskeletal system (van Weeren et al., 2017). There are multiple methods utilised for measuring lameness including subjective gait analysis and objective gait analysis.

Subjective Gait Analysis

Lameness grading systems are a numerical rating scale used to assess musculoskeletal pain. They assess head movement for forelimb lameness, and the symmetry of gluteal and pelvic movement for hindlimb lameness (Stashak, 1987). Although commonly used worldwide they have been shown to be subjective with only marginal agreement between observers, especially when lameness is mild (Dyson, 2011, Keegan et al., 2010). In fact vets only agree on which limb is affected 50% of the time (Keegan et al., 2010). It has also been demonstrated to be more difficult to assess hindlimbs compared to forelimbs. Hammarberg et al. (2016) demonstrated that while the inter-rater agreement for forelimb lameness was acceptable, the agreement seen for hindlimb lameness was poor. This has also been demonstrated when using near-realistic animations (Starke and Oosterlinck, 2019).

In addition, subjective grading scales are also subject to bias and operator experience. Lameness grade following regional limb anaesthesia was shown to be influenced by the clinicians knowledge of whether local anaesthesia had been administered (Arkell et al., 2006). It is suggested that clinician experience affects interobserver agreement (Hammarberg et al., 2016), although case load may be more important than years of experience (Starke and Oosterlinck, 2019). There are multiple subjective grading scales reported to be used which are not interchangeable (Hewetson et al., 2006), further complicating observational assessment of lameness. Many grading scales are also used incorrectly, for example the American Association of Equine Practitioners (AAEP) scale which consists of a 1-5 scale used following assessment of the horse under various circumstances (straight line, circling, different surfaces) is often used to just assess horses

in a straight line at trot (da Silva Azevedo et al., 2019, Donnell et al., 2015, Ishihara et al., 2005, Thomsen et al., 2010).

When considering intraobserver reliability, Fuller et al. (2006) demonstrated that scoring lameness over a 9 month period, is consistent and repeatable over time. However, as lameness grading systems provide poor interobserver reliability, a more objective, reliable method of detecting musculoskeletal pain is required.

Objective Gait Analysis

Force plate Technology

Force plates can be used to measure changes in limb loading in lame horses. The force applied to the ground by the lame limb is reduced in comparison to the sound limb, as they rely more heavily on the sound limb to reduce pain (Ashley et al., 2005, Bragança et al., 2018). The most commonly reported change is a reduction of peak vertical force in the predominantly lame limb (Bragança et al., 2018). Force plates provide an objective measure of lameness since they document a reluctance to load the limb due to pain. However, they require a dedicated location for a stationary force plate, and time and patience to obtain enough hoof strikes on the force plate for analysis. Smaller force plates which can be attached to horseshoes have been developed however few studies have investigated their application (Judy et al., 2001). Force plate technology has been shown to be repeatable and able to detect subtle lameness (Keegan et al., 2012), however is not yet practical in the field setting.

Kinematic Technology

Kinematic evaluations of gait using video technology have been utilised for experimental studies to measure lameness objectively (Buchner et al., 1996). These systems assess vertical displacement of the head, withers, tuber sacrale and tuber coxae. With increasing lameness the vertical displacement of the lame limb during stance phase is reduced with a contralateral increase on the other non-lame limb (Bragança et al., 2018). This is often presented as a symmetry index or change in amplitude. Depending on the facility, horses may also be required to be trained on a treadmill. The technology and expertise required for these studies make this technique impractical for clinical practice. More recently, an artificial intelligence marker-less motion tracking system has been used (Sleip AI), which

tracks the movement of the head, pelvis and hooves using a smartphone camera, making it more practical in a field setting (Lawin et al., 2023).

Inertial Measurement Units (IMUs)

IMUs enable kinematic data collection pertaining to head and pelvis movement symmetry whilst only requiring small body-mounted sensors connected wirelessly to a laptop. Similar to video kinematics they assess vertical displacement of the head and pelvis (Bragança et al., 2018). They remove bias allowing lameness to be measured objectively (Maliye et al., 2013). These systems produce real-time data which can be rapidly analysed in a clinical situation, enabling evidence-based decision making, through lameness detection and monitoring treatment response. They may be used alongside traditional lameness evaluations, including being used to assist with interpretation of flexion tests and diagnostic anaesthesia (Maliye et al., 2013, Marshall et al., 2012). There are several IMU systems available, and each offers a slightly different configuration of accelerometers, some also include a limb gyroscope (Pfau et al., 2016, Bosch et al., 2018).

Within one complete stride cycle in a straight line at trot, the head and pelvis of a sound horse move upwards and downwards twice forming a sinusoidal pattern (Buchner et al., 1996). This can be visualized in Figure 1.1. Asymmetry of the sinusodial pattern occurs when lameness is present, and by measuring the extent of the asymmetry, the lameness severity can be quantified. When a horse is lame, the amplitude of the vertical displacement of the head or pelvis decreases during/after the stance phase of the lame limb, for forelimb and hindlimb lameness, respectively. In comparison, the vertical amplitude reached during/after the stance phase of the sound contralateral limb increases (Buchner et al., 1996). Consideration of vertical amplitude difference is used to determine the limb which is lame, and the lameness severity.

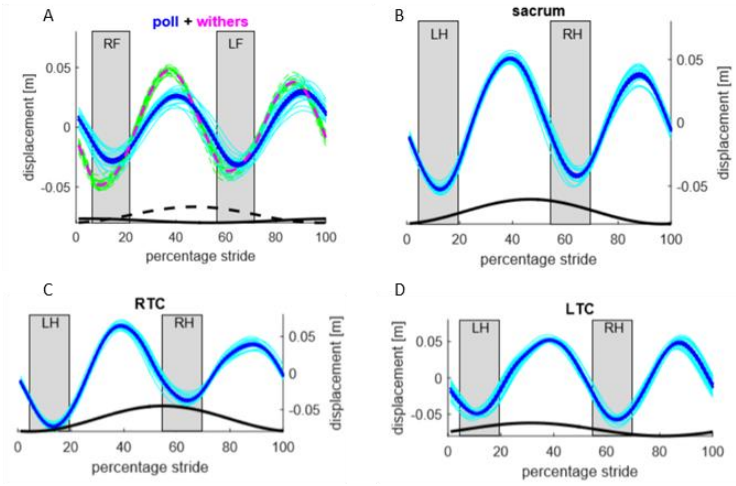

Figure 1.1 Graphs to show the vertical movement for each stride and the average for A) poll individual strides (cyan), average of poll strides (blue), individual withers strides (green) and average of wither strides (pink dashed), B) sacrum individual strides (cyan) and average of sacrum strides (blue), C) right tuber coxae individual strides (cyan) and average of right tuber coxae strides (blue), and D) left tuber coxae individual strides (cyan) and average of left tuber coxae strides (blue). The grey boxes highlight the stance phase and the white boxes highlight the swing phase of the stride. Abbreviations: right tuber coxae (RTC), left tuber coxae (LTC), left hind (LH), right hind (RH).

Kinematic observations of head and pelvis movement also provide insight into the kinetics occurring in the lame horse. Buchner et al. (1996) demonstrated reduced vertical movement amplitude is mirrored by a reduction in amplitude of vertical acceleration. Vertical acceleration of the head/pelvis can be related to the vertical force produced by the lame limb through consideration of Newtons second law of motion. IMUs measure head and pelvis acceleration in addition to displacement allowing both an assessment of the kinematics and an appreciation of the kinetics of the lame horse to be made.

To determine if a horse is lame, the difference in the minimum and maximum positions of the head/pelvis are considered (Bragança et al., 2018, Kramer et al., 2004), which is shown in Figure 1.2. The minimum difference (MinDiff) is determined from the minimum height reached by the head/pelvis during the stance phase of the lame limb, compared to the minimum height reached during the stance phase on the contralateral sound limb, which is lower. Similarly, the maximum difference (MaxDiff) is calculated from the maximum height reached by the head/pelvis following the stance phase for both limbs,

and maximum height is lower on the lame limb compared to the sound limb. When MaxDiff or MinDiff is zero, it indicates that phase of limb movement is symmetrical from left to right sides.

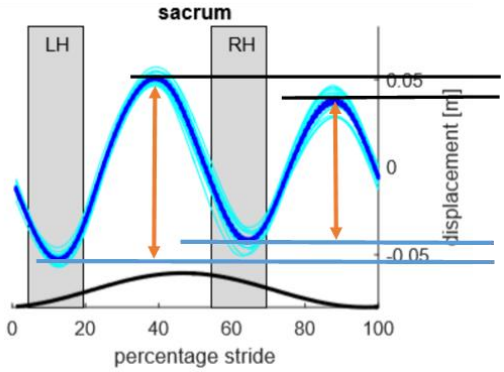

Figure 1.2 Graph demonstrating the maximum difference of the sacrum (difference between the black lines), minimum difference of the sacrum (difference between the blue lines) and the upwards difference (difference between the two orange arrows) in a lame horse. The grey boxes highlight the stance phase and the white boxes highlight the swing phase of the stride. Abbreviations: left hind (LH), right hind (RH).

MinDiff occurs during mid-stance phase when the body is producing the maximal vertical ground force, therefore, lameness affecting MinDiff are considered to be related to weightbearing or impact (Pfau, 2019). MaxDiff occurs following the horse pushing off from the ground in the aerial phase for that limb. Therefore lameness affecting the maximum position are related to a reduced propulsive 'push-off' force (Pfau, 2019). However, considerations must be made when horses have concurrent weightbearing and push-off asymmetry. When both are present, the MinDiff value is not zero. This means that as the head has a different starting position before each aerial phase, MaxDiff would underestimate the lameness severity. Instead the upwards difference (UpDiff) between the two vertical movement amplitudes is assessed, which is theoretically a better measure of push off lameness (Pfau, 2019). UpDiff is shown in Figure 1.2.

The final parameter determined is specific to tuber coxae movement and enables an evaluation of hip hike. Hip hike is the upwards movement of the tuber coxae before the stance phase of that limb, which is calculated for both left and right limbs. Hip Hike Difference (HHD) is the difference between these two amplitudes (Starke et al., 2012). In a symmetrical horse the hip hike of each limb is equal leading to a HHD of zero. In

comparison, a lame horse would have a larger movement amplitude of the tuber coxae of the lame limb, leading to a non-zero value.

Threshold values have been identified, being a vertical movement difference of greater than 6mm for the head when considering forelimb lameness and greater than 3mm for the sacrum when considering hindlimb lameness (McCracken et al., 2012). However, these thresholds are guidelines only, as they may be altered by the situation and normal variation seen in horses' movement. Rhodin et al. (2017) demonstrated 72.5% of 222 horses who were considered free from lameness by the owner exceeded the thresholds for one or more variables when trotting in a straight line. It is unknown if the asymmetry demonstrated by these horses was due to pain or normal variation. However, it does bring the validity of these thresholds into question. Normal variation between horses and between day repeat assessments for the same horse have been shown to change as much as 16mm for head movement and 11mm for sacral movement, although none of these horses were assessed by veterinary surgeons (Sepulveda Caviedes et al., 2018). Pfau (2019) suggests the thresholds can be used in populations with high lameness prevalence (i.e. horses seen by a veterinary surgeon for lameness or performance issues) where, due to the high positive predictive value, you are less likely to detect false positives. However, clinicians using IMUs diagnose lameness above 14.5mm for forelimb lameness and 7.5mm for hindlimb lameness as these values are more in line with the potential day-to-day variation seen (Pfau, 2019). Studies have also investigated the use of IMUs when lunging a horse and have demonstrated thresholds must be adjusted as circles affect symmetrical movement (Starke et al., 2012, Robartes et al., 2013).

Lameness can occur in a single limb, bilaterally or any forelimb and hindlimb combination. IMUs are very useful in multi-limb lameness cases especially where the horse is reassessed multiple times following diagnostic blocks (Pfau, 2019). IMUs are also able to enable detection of compensatory lameness, where horses can appear multi-limb lame when in fact there is a primary lameness with a compensatory mechanism. Following the *'law of sides'*, horses with a primary forelimb lameness can demonstrate a contralateral hindlimb lameness, and those with hindlimb lameness may demonstrate an ipsilateral forelimb lameness (Maliye et al., 2015, Maliye and Marshall, 2016).

As an objective lameness tool, IMUs have been investigated in detail to determine their usefulness as part of the lameness examination. However, they potentially have a multitude of other uses including to assess a horse's response to pharmacological or nutraceutical treatment, the progression of an orthopaedic condition over time, and to monitor lameness, and therefore potentially pain severity, to allow a determination of welfare.

Subjective versus Objective Gait Analysis

Objective gait analysis methods commonly rely on gait symmetry measurements. It is important to note that asymmetry does not mean lameness. However, it has been repeatedly demonstrated that movement asymmetries can respond to diagnostic anaesthesia, giving more confidence to the fact the asymmetries in these studies are caused by pain (Maliye et al., 2013, Leelamankong et al., 2020, Keegan et al., 1998, Rungsri et al., 2014, Pfau et al., 2014). Threshold values for objective gait analysis tools have been reported to identify the lame limb, however these should be considered guidelines in clinical situations as it has been demonstrated that normal horses fall outside of these thresholds (Rhodin et al., 2017). In a review by Bragança et al. (2018) they report the evidence from experimentally induced lameness models justifies the use of objective gait analysis alongside clinical assessment of lameness especially in mild lameness or to assist in comparison between interventions such as diagnostic anaesthesia.

Studies have suggested that IMUs are more reliable than subjective lameness grading especially when evaluating mild lameness (Donnell et al., 2015, Leelamankong et al., 2020). Leelamankong et al. (2020) has recently demonstrated agreement between live clinicians and IMUs improves with experience level although agreement with experienced clinicians was still only considered 'fair'. IMUs have been compared to technologies used to objectively evaluate lameness including forceplates and video-based kinematics and have been shown to be reliable and accurate (Donnell et al., 2015, Keegan et al., 2004). In order to further investigate whether objective gait assessment improves the accuracy of lameness detection compared to subjective gait assessment, a systematic review has been conducted as part of this thesis.

NSAIDs and paracetamol for analgesia of musculoskeletal pain

NSAIDS

When considering which analgesic agent to administer for musculoskeletal pain in the horse, the resounding consensus is an NSAID, of which phenylbutazone is most frequently chosen (De Grauw et al., 2014, Duz et al., 2019, Sabate et al., 2009). NSAIDs inhibit cyclooxygenase (COX) enzymes within the arachidonic acid cascade preventing the release of prostaglandins and thromboxanes (Knych, 2017). Production of prostaglandins, specifically PGE_2 and PGI_2, initiates an acute inflammatory process including rapid influx of neutrophils followed closely by monocytes. This leads to the four key signs of inflammation; redness, heat, swelling and pain (Ricciotti and FitzGerald, 2011). However, this is not their only function. COX enzymes are also homeostatic enzymes involved in blood flow regulation, gastrointestinal protective mechanisms and organ function (Knych, 2017).

Three isoforms of the COX enzyme have been identified so far in mammalian species. COX-1 is expressed in most tissues and plays a large role in physiologic functions including renal blood flow, gastrointestinal blood flow and mucosal integrity and platelet aggregation (Chen et al., 2013). Although historically, COX-2 was thought to be an inducible enzyme with pro-inflammatory functions this is no longer the case. COX-2-deficient mice have affected renal function, bone resorption and female reproductive function (Lim et al., 1997, Harris et al., 1994). COX-2 also has a key role in gastric mucosal defence and healing (Peskar et al., 2001). It is also important to the maintenance of blood flow through production of prostacyclin (Kirkby et al., 2014). When considering their role in inflammation, COX-1 has been shown to have an inflammatory role by driving acute inflammation, with COX-2 upregulation within hours of the inciting event (Smyth et al., 2009). Finally, a variant of COX-1, known as COX-3, was identified. COX-3 is structurally similar to COX-1 and 2 with the same catalytic features. This is most abundant in the brain and spinal cord and is sensitive to medications that are analgesic and antipyretic but not anti-inflammatory (Chandrasekharan et al., 2002).

Traditional NSAIDs inhibit both COX-1 and COX-2 function. It has been suggested that NSAIDs primarily affecting COX-2 may be advantageous as they would prevent pain and

inflammation whilst having minimal effect on normal homeostatic functions. However, it is not this straightforward. In humans there is an increased risk of cardiovascular side effects with COX-2 selective inhibitors due to the reduction in production of prostacyclin and the increase in production of thromboxane favoring thrombosis, vasoconstriction and platelet aggregation (Graham, 2006). This effect has not been demonstrated in horses although further larger clinical studies are required (Koene et al., 2010). Of the NSAIDs licensed for use in the horse, meloxicam and firocoxib have been shown to be more COX-2 selective than flunixin meglumine and phenylbutazone (Beretta et al., 2005, Fogle et al., 2021).

Adverse effects described with excessive NSAID use include duodenal and gastric erosions, right dorsal colon mucosal necrosis, central nervous system depression and renal medullary necrosis (Collins and Tyler, 1985, Gunson and Soma, 1983, Meschter et al., 1990, Read, 1983). Phenylbutazone has been shown to have the most toxic potential, followed by flunixin meglumine (Mozaffari et al., 2010). This is further supported by the BEVA analgesia guidelines, which outline that there is moderate evidence that phenylbutazone is the NSAID most likely to cause gastrointestinal side effects (Bowen et al., 2020), however it is also the most frequently administered and researched. In comparison, COX-2 specific inhibitors are presumed to have a superior safety profile. Meloxicam has been shown to affect mucosal integrity to a lesser extent than phenylbutazone. Although, this was only determined through assessment of sucrose permeability and plasma protein concentrations did not correlate (D'Arcy-Moskwa et al., 2012). Firocoxib may allow mucosal recovery in comparison to flunixin meglumine (Cook et al., 2009), suggesting a reduced risk of gastrointestinal adverse events in comparison to non-selective NSAIDs. Richardson et al. (2018) demonstrated firocoxib produced less severe gastric ulceration than phenylbutazone, although phenylbutazone was administered at 4.4mg/kg once daily compared to the licensed 2.2mg/kg twice daily. It is important to note that gastric ulceration was observed with firocoxib administration, regardless of whether it is compared to phenylbutazone. Additionally, Noble et al. (2012) demonstrated that when meloxicam was given at three to five times the licensed dose, renal and gastrointestinal damage was observed. Therefore, these studies highlight that COX-2 selectivity does not eliminate the possibility of adverse effects.

NSAIDs have been shown to be effective at reducing pain caused by musculoskeletal conditions (Erkert et al., 2005, Foreman et al., 2010, Olson et al., 2016, Orsini et al., 2012). In a recent study of NSAID use within the USA, Canada and the UK, phenylbutazone was the most commonly prescribed NSAID in all countries for orthopaedic pain followed by suxibuzone in the UK (Duz et al., 2019). Only a small proportion of NSAIDs prescribed included the COX-2 selective, meloxicam and firocoxib (Duz et al., 2019). Naylor et al. (2014) demonstrated meloxicam, at the recommended once daily administration provides suboptimal analgesia, and even when used twice daily for post-operative analgesia following colic surgery, flunixin meglumine provided superior analgesia. This potentially explains the lack of administration of this product in horses. In addition, the recently published BEVA analgesia guidelines suggest that phenylbutazone provides a better plane of analgesia than firocoxib and meloxicam for hoof pain, although they may provide a similar plane of analgesia for synovial pain (Bowen et al., 2020).

Phenylbutazone is licensed in the UK for both acute and chronic musculoskeletal pain, as well as soft tissue inflammation. It is available in an intravenous formulation or an oral formulation, as a paste or granules. It has been shown to be an effective analgesic for musculoskeletal conditions (Doucet et al., 2008, Raekallio et al., 1997), and has no long term effects on cartilage although it has been shown to transiently reduce collagen II synthesis marker (De Grauw et al., 2014). Due to risk of aplastic anaemia in humans, all horses who have received phenylbutazone must be excluded from the food chain, as no safe limit for human consumption has been established (Lees and Toutain, 2013).

Suxibuzone, which is also commonly prescribed for musculoskeletal conditions in the UK (Duz et al., 2019), is the pro-drug of phenylbutazone. It is available as oral granules and has been shown to be more palatable than phenylbutazone, making it preferable to owners (Sabate et al., 2009). In addition, it has been shown to have similar efficacy to phenylbutazone for improving lameness (Sabate et al., 2009) and causes significantly less gastric ulceration (Monreal et al., 2004). The current recommendation is that suxibuzone can be used to directly replace phenylbutazone to treat chronic orthopaedic pain (Bowen et al., 2020).

Paracetamol

Paracetamol is the most commonly used analgesic and antipyretic in human medicine (McCrae et al., 2018) and is essential for multimodal analgesia (Sharma and Mehta, 2014). It is used for chronic pain conditions including osteoarthritis in humans despite the effects being found as only modest when compared with a placebo (Graham et al., 2013, McCrae et al., 2018). It has a wide safety margin with few drug interactions although concerns have been raised over potential adverse effects (McCrae et al., 2018, Sharma and Mehta, 2014). One review suggests that in human medicine the most concerning potential side effects are hypertension and gastrointestinal bleeding, the latter which may have an additive effect when co-administered with NSAIDS (McCrae et al., 2018). A different review reports that at therapeutic doses it does not cause significant gastrointestinal toxicity, but instead the main adverse event seen is hepatotoxicity (Graham et al., 2013). In horses few studies have been performed which report paracetamol safety. Twice daily oral dosing of 25mg/kg for 30 days did not show any significant changes in renal or hepatic blood parameters (Foreman, 2018). Mercer et al. (2020) demonstrated statistically significant reductions in both total protein and platelet count with twice daily oral dosing at 20mg/kg for 2 weeks in addition to increases in albumin, alkaline phosphatase, calcium, creatine kinase and potassium. However, values remained within the laboratory reference range for all parameters. This study also reported increases in SDH and total bilirubin which were significant and ranged outside of the reference interval. There was no change in the level of gastric disease observed with paracetamol dosing in this study and liver biopsies revealed mild portal inflammation in all horses sampled, with irreversible changes seen in one case. However, biopsies were not taken prior to paracetamol dosing for comparison. In a different study there were no significant changes detected on liver biopsies following 3 weeks of oral paracetamol at 30mg/kg when compared to pre-treatment liver biopsies (Mercer et al., 2023a). This study also showed no significant differences in gastric disease score when compared to before paracetamol treatment.

Paracetamol can be administered orally, rectally or intravenously with analgesia occurring within 40 minutes after oral or rectal administration and within 5 minutes following intravenous administration in humans (Sharma and Mehta, 2014). It is a low molecular

weight lipid soluble molecule which is unionised at physiological pH meaning it has excellent penetration including into the CNS and aqueous humour of the eye (Graham et al., 2013, Kumpulainen et al., 2007, Peraza et al., 2022). In horses, paracetamol is rapidly absorbed and does not accumulate with multiple doses (Mercer et al., 2020). It has a higher bioavailability in horses than in other species (Neirinckx et al., 2010). Oral absorption in the study by Mercer et al. (2020) was variable, suggested to be due to gastric emptying as paracetamol is absorbed via passive diffusion in the proximal portion of the small intestine. Paracetamol is metabolised in the liver and in humans, this occurs mainly via glucuronidation and sulphation (Sharma and Mehta, 2014).

Despite best efforts the mechanism of action of paracetamol is still not fully understood (Oscier and Milner, 2009). It is thought that paracetamol provides its effects via several mechanisms. Paracetamol has been shown to inhibit both COX1 and COX2 enzymes when the peroxide concentration is low, inhibiting the rise of prostaglandin-E_2 (Graham et al., 2013, Oscier and Milner, 2009). This occurs in more centrally located areas, for example the brain, and not peripherally or at sites of inflammation, therefore only weak anti-inflammatory activity is observed (Oscier and Milner, 2009). Additionally its major affect appears to be on COX2 although it is not COX2 selective (Graham et al., 2013). One previous study has suggested that paracetamol may preferentially affect COX3, which is a splice variant of COX1, however further research is required to verify this finding (Chandrasekharan et al., 2002, Graham et al., 2013). Paracetamol also activates descending serotonergic inhibitory pain pathways and within the brain and spinal cord is metabolised to N-arachidonylphenolamine. This metabolite can activate cannabinoid receptors, activate vanilloid type 1 receptors and inhibit COX, nitric oxide and tumour necrosis factor-alpha (Oscier and Milner, 2009, Sharma and Mehta, 2014). It appears maintenance of the endocannabinoid system and the serotonergic system are essential to allow the analgesic effect of paracetamol, as antagonists to both systems have been demonstrated to reduce paracetamol efficacy (Graham et al., 2013).

In humans, the minimum suggested therapeutic concentration of paracetamol is 10µg/ml (Ward and Alexander-Williams, 1999). Using the data from two intravenous paracetamol studies which utilised a 10mg/kg dose, Ishii et al. (2020) suggested an effective plasma concentration (EPC) in horses was 12µg/ml. More recently Pesko et al.

(2022) determined an EPC of 8µg/ml using a 20mg/kg oral dosing regimen. Oral dosing with paracetamol at 30mg/kg and 20mg/kg achieved plasma concentrations greater than 8µg/ml for 5.8 ± 1.1 hours and 7.3 ± 1.1 hours respectively (Mercer et al., 2022). However, in this study when the change in lameness score was plotted against the plasma concentration it demonstrated a hysteresis loop revealing a lag between concentration and effect. This makes the determination of an EPC for paracetamol in the horse more challenging. Further higher-powered studies are required to calculate an analgesic therapeutic plasma concentration in the horse. Mercer et al. (2020) report an initial single dose elimination half-life of 2.78 hours, which lengthened with multiple doses to 3.99 hours, comparable with other published work (Pesko et al., 2022, Mercer et al., 2022). Accumulation is not reported following multiple doses at 12-hour intervals for up to 21 days (Mercer et al., 2020, Mercer et al., 2023a). Oral dosing with paracetamol at 30mg/kg increased the maximum plasma concentration compared to 20mg/kg but did not affect the time to maximum concentration or the elimination half-life (Mercer et al., 2022). Oral dosing in horses with endotoxaemia with 30mg/kg revealed a lower maximum plasma concentration although time to maximum plasma concentration was similar (Mercer et al., 2023b).

Paracetamol has been demonstrated to be an effective analgesic for acute pain associated with extremity compartment syndrome in one horse as part of a multimodal analgesia regime (Bruniges et al., 2019) and as a short term additional analgesic in a laminitic pony (West et al., 2011). When compared with flunixin meglumine and a control group in a model of inducible acute foot pain, Foreman et al. (2016) reported oral paracetamol at 20mg/kg significantly reduced the lameness score and heart rate for 5 hours and 11 hours respectively, compared to the control group. This was comparable to the effect of flunixin meglumine. When compared to phenylbutazone and a placebo, a dose of 30mg/kg orally demonstrated a significant improvement in subjective lameness grade at 2 and 4 hours post administration using an acute mechanically induced lameness model (Mercer et al., 2022). Paracetamol at 30mg/kg orally resulted in a more rapid improvement in lameness score compared to phenylbutazone although both groups had a similar level of reduction in lameness score. Improvement in lameness in this study was not seen with a 20mg/kg oral paracetamol dose. These studies suggest that paracetamol

may be an effective analgesic for acute musculoskeletal pain either as a standalone therapy or as part of a multimodal analgesia protocol, with 30mg/kg orally potentially being more efficacious. A transient improvement in subjective and objective lameness assessment in horses with naturally occurring chronic lameness has been shown with oral dosing of 30mg/kg paracetamol following 3 weeks of twice daily oral dosing (Mercer et al., 2023a).

In the United Kingdom the only licensed preparations of paracetamol for veterinary species are for dogs and pigs (Bardell, 2017). Therefore, all use of paracetamol in the horse is considered off-license and is prescribed under the cascade. There is a significant amount of anecdotal evidence for paracetamol use alongside NSAIDs for management of pain in the horse (Bowen et al., 2020) and its use in clinical practice is increasing. In humans, paracetamol is often given in combination with NSAIDs (Graham et al., 2013). Paracetamol is thought to work synergistically with NSAIDs aiding the overall analgesia efficacy (Graham et al., 2013, Pesko et al., 2022). No studies have yet investigated whether this effect is seen in horses, although their combined use is common (Bruniges et al., 2019, West et al., 2011).

Conclusion

Equine lameness is a major problem worldwide. To improve the welfare of these horses, the pain from which they are suffering must first be adequately recognised and assessed using objective tools. Over the past decade public and research interest in pain recognition and management in horses has increased, although it still lags behind human and companion animal research. Many studies have highlighted the need for more research (Gleerup and Lindegaard, 2016, Heleski et al., 2012).

Following pain recognition, an effective method of managing this pain is required. This is likely to involve pharmacological intervention alongside regular pain assessments both by the veterinary surgeon and owner.

This study aims to compare subjective and objective lameness gait analysis using a systematic review method in addition to assessing paracetamol efficacy as an additional analgesic in chronically lame horses already being treated with a NSAID.

Chapter 2: In lame horses does objective gait assessment versus subjective gait assessment improve the accuracy of lameness detection?

Abstract

Lameness is most commonly measured clinically by using subjective grading scales which are numerical rating scales used to assess musculoskeletal pain. However determination of the lame leg, and quantification of the lameness severity, can be challenging especially when the lameness is mild or affects multiple limbs. Objective gait assessments have been suggested to be a more reliable method of determining and monitoring lameness. The aim of this systematic review is to summarise and assess the evidence published to answer the question 'In lame horses does objective gait assessment versus subjective gait assessment improve the accuracy of lameness detection?'.

Following the PRISMA guidelines, systematic searches were conducted on Medline, CAB Abstracts, PubMed, ProQuest Dissertations & Theses A&I, Embase, Scopus, Web of Science and Google Scholar. The title and abstracts were screened, and records were excluded if they did not include the following: horses with lameness, subjective and objective gait analysis. Full text articles were assessed, and records were included if they met specific criteria which included being published in a peer-reviewed journal and being written in the English language. Four reviewers then assessed the studies to determine a consensus regarding the quality of the evidence and therefore each study's vetGRADE.

19 records met the inclusion criteria and were evaluated. Of these six studies were found to have a moderate vetGRADE, eight studies were considered low, two were very low and three were speculative. These studies demonstrated that objective gait analysis was able to consistently determine which leg was lame and sometimes outperformed the subjective lameness analysis especially in mild cases.

This review has demonstrated there is moderate evidence that objective gait analysis is more accurate than subjective gait analysis for lameness detection. This is particularly evident for the use of IMUs in comparison to other objective techniques, especially in clinical practice and for when using diagnostic anaesthesia.

Introduction

Lameness is a clinical sign which presents when a loss of function or defect is present within the musculoskeletal system (van Weeren et al., 2017). Lameness is most commonly measured clinically by using subjective grading scales which are numerical rating scales used to assess musculoskeletal pain. However, determination of the lame leg, and quantification of the lameness severity can be challenging especially when the lameness is mild or affects multiple limbs (Dyson, 2011, Keegan et al., 2010). Fuller et al. (2006) demonstrated that agreement between experienced clinicians using a 0-10 subjective grading system was only just above acceptable limits when grading severity of lameness, and Keegan et al. (2010) showed vets only agree on which limb is affected 50% of the time. When considering severity, interobserver agreement is reduced for mild lameness when compared to moderate or severe lameness (Hewetson et al., 2006, Keegan et al., 2010, Starke and Oosterlinck, 2019). Additionally, assessment of hindlimb lameness has been demonstrated to be more difficult (Hammarberg et al., 2016, Starke and Oosterlinck, 2019).

Subjective grading scales are also subject to bias and operator experience, particularly case load (Arkell et al., 2006, Hammarberg et al., 2016, Starke and Oosterlinck, 2019). Further complicating observational assessment of lameness is the presence of multiple subjective grading scales, which are not interchangeable (Hewetson et al., 2006). Many grading scales are also used incorrectly (da Silva Azevedo et al., 2019, Donnell et al., 2015, Ishihara et al., 2005, Thomsen et al., 2010).

Due to the many issues outlined with subjective lameness grading, a more objective, reliable method of detecting musculoskeletal pain is required. A recent review has determined there is a large body of robust evidence between individual gait parameters assessed with objective measurements and lameness at trot (Bragança et al., 2018). There are multiple methods of objectively assessing horse lameness including force plates, kinematics and inertial measurement units.

Force plates measure a reduction in limb loading in the lame limb (Ashley et al., 2005, Bragança et al., 2018), reported as a reduction of peak vertical force (Bragança et al., 2018). Force plate technology is repeatable and may be able to detect subtle lameness

(Keegan et al., 2012), however is not yet practical in a field setting as it requires a dedicated location for a stationary force plate, and time and patience to obtain enough hoof strikes on the force plate for analysis.

Kinematic evaluations of gait using video technology determine a symmetry index or change in amplitude using vertical displacement (Buchner et al., 1996). With increasing lameness the vertical displacement of the withers or tuber sacrale for forelimb and hindlimb lameness during stance phase respectively is reduced with a contralateral increase on the other non-lame limb (Bragança et al., 2018). However, the technology and expertise required for these studies make this technique impractical for clinical practice.

IMUs assess vertical displacement of the head and pelvis (Bragança et al., 2018). They are practical for use within clinical practice as they produce real-time data and can be used alongside traditional lameness evaluations, including assisting with interpretation of flexion tests and diagnostic anaesthesia (Maliye et al., 2013, Marshall et al., 2012).

It is important to note that objective gait analysis methods measure gait symmetry, and that asymmetry does not equal lameness. Although multiple studies have demonstrated that movement asymmetries can respond to diagnostic anaesthesia, suggesting these asymmetries are caused by pain (Keegan et al., 1998, Leelamankong et al., 2020, Maliye et al., 2013, Pfau et al., 2014, Rungsri et al., 2014), differentiation of a lameness from an asymmetry relies on a threshold value. As it has been demonstrated that normal horses fall outside of these thresholds (Rhodin et al., 2017), the reported threshold values should only be considered guidelines in clinical situations.

This systematic review aims to compare objective and subjective gait assessment in order to determine whether objective gait assessment improves the accuracy of lameness detection in lame horses in both induced and natural models of lameness. The hypothesis is that objective gait assessment will be more accurate and detecting lameness especially with milder lameness.

Methods

This systematic review was performed using the PRISMA guidelines (Shamseer et al., 2015) and the PRISMA checklist (Appendix 1) and PRISMA flow chart (Figure 2.1) were

completed. The aim of the systematic review was to answer the following question *'In lame horses does objective gait assessment versus subjective gait assessment improve the accuracy of lameness detection?'*, therefore a PICO search framework was used (Table 2.1). This framework was used to develop search terms and a search strategy (Appendix 2) which were used to search the following databases: Medline, CAB Abstracts, PubMed, ProQuest Dissertations & Theses A&I, Embase, Scopus, Web of Science and Google Scholar. All databases were searched on 13th October 2020. Table 2.2 demonstrates the search strategy used for Medline.

Table 2.1 PICO framework for systematic review to answer the following research question: '*In lame horses does objective gait assessment versus subjective gait assessment improve the accuracy of lameness detection?*

Population	Horses with Lameness
Intervention	Subjective Gait Analysis
Comparison	Objective Gait Assessment
Outcome	Lame or not lame

Table 2.2 Outline of Medline Search Strategy completed 13th October 2020

	Search	Results
1	Exp Equidae/	70942
2	Equi*	943104
3	(Pony or ponies)	3424
4	(Gelding* or Mare* or Stallion* or Horse*)	129671
5	Exp Lameness, Animal/	4075
6	Lame*	46273
7	(Asymmetr* adj2 (movement or gait))	998
8	Exp Gait Analysis/	613
9	(gait adj2 analys#s)	8296
10	(locomot* adj2 analys#s)	420
11	(Objective ADJ3 gait)	515

12	(Objective ADJ3 lame*)	74
13	(Inertial ADJ3 sensor*)	2320
14	ISS	10117
15	(Inertial ADJ3 measurement*)	1972
16	Force plat*	7349
17	Accelerometer*	15673
18	(quantitative adj3 gait)	574
19	(kine* adj3 gait)	2461
20	(kine* adj3 anayls#s)	1
21	(subjective or qualitative or visual or observational or empirical) adj3 (gait or lame*)	794
22	(Lameness ADJ3 grad*)	163
23	1 or 2 or 3 or 4	1040939
24	5 or 6 or 7	47220
25	8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22	45776
26	22 and 23 and 24	340

All records found through the search strategy were assessed for duplicates and these were removed. The title and abstracts were screened, and records were excluded if they did not include the following: horses with lameness, subjective and objective gait analysis. Full-text articles were then assessed for eligibility using the eligibility criteria. The records must include an equine population of any age which were lame, a subjective measure of lameness and an objective measure of gait analysis. The records must also have been in the English language. Records must have been published in a peer-reviewed journal up to and including the 13th of October 2020. The records that met these criteria were included in a qualitative synthesis.

All records which met the inclusion criteria were assessed by four reviewers to determine the quality of the evidence presented. Evidence was assessed using vetGRADE (Bowen et al., 2020) which is a veterinary equivalent of the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) system used to rate clinical

guidelines in human medicine. GRADE enables a rating of the certainty of the evidence based on criteria (Balshem et al., 2011, Schwingshackl et al., 2021). The evidence is initially classified by the study design, and then the evidence is assessed for risk of bias, indirectness, inconsistency, publication bias and insufficient precision. Using these criteria, the certainty of the evidence can be increased or decreased on the scale with the final outcome being the one that provides the lowest confidence (Guyatt et al., 2013). The GRADE system is highly regarded in human medicine as an excellent and transparent system for rating the certainty of evidence in relation to key clinical questions (Schwingshackl et al., 2021). In comparison to the GRADE guidelines, VetGRADE rates systematic reviews as exceptional and then includes high, moderate, low and speculative categories depending on the quality of the evidence (Table 2.3). This change is required for veterinary evidence as there is a lack of high grade evidence such as randomized controlled trials, therefore VetGRADE has expanded the grading system to include a wider range of study designs making it more suitable for veterinary medicine. VetGRADE has been used in one paper which created clinical guidelines for analgesia (Bowen et al., 2020). This demonstrates that VetGRADE can be used to assess a diverse and complex question in veterinary medicine (Bowen et al., 2020). The GRADE system has been used extensively to assess large bodies of evidence in human medicine including gait analysis (D'Souza et al., 2021), making it a suitable choice for this systematic review. All studies were assigned a vetGRADE depending on the type of study design and the rating was increased or decreased depending on the criteria outlined above; risk of bias, indirectness, inconsistency, publication bias and insufficient precision. The final VetGRADE was made by consensus of the four reviewers.

Table 2.3 Summary of the vetGRADE with examples

vetGRADE	Example of study design
Exceptional	Systematic review of randomised clinical trials
High	Randomised clinical trial
Moderate	Validated experimental model
Low	Observational study
Very Low	In vivo/ex vitro studies with potential clinical relevance

Speculative	Case studies with the support of a panel's expert experience

Results

The PRISMA flow diagram demonstrates the number of records identified including the number removed due to duplication and the number removed as the title and abstract did not include key criteria (Figure 2.1). There were 41 full-text articles which were assessed against the eligibility criteria. There were 19 records which met the eligibility criteria and were included in the qualitative synthesis.

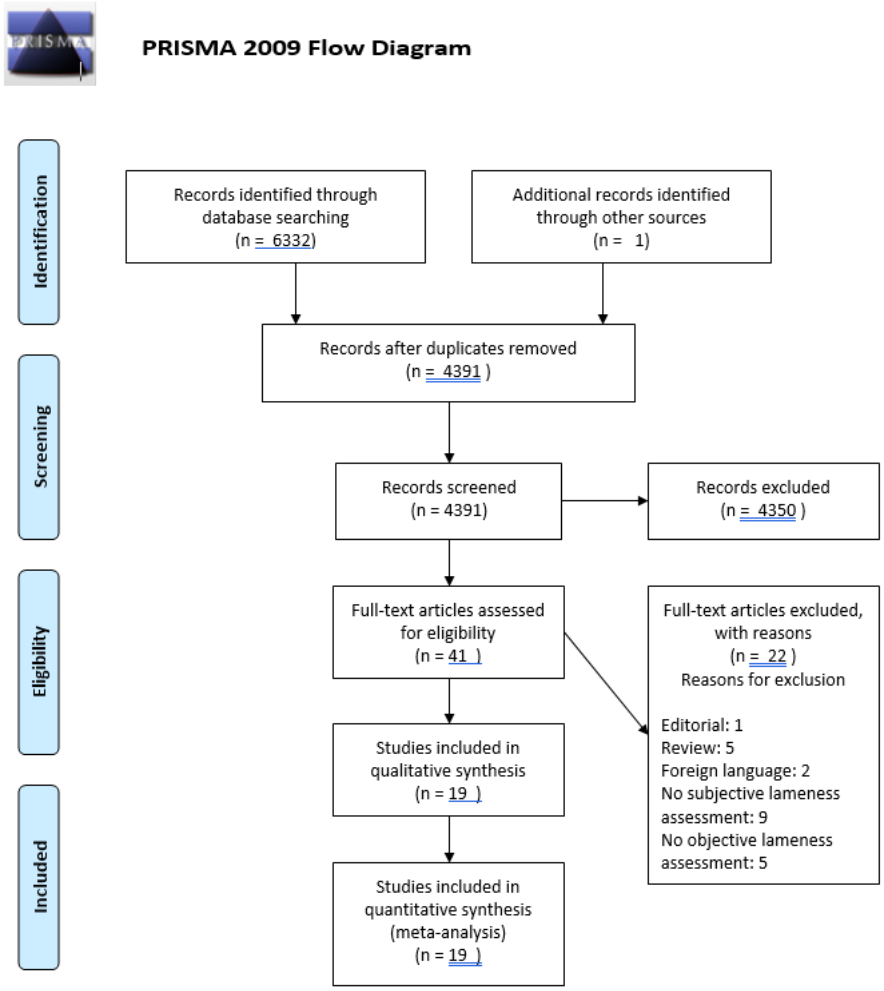

Figure 2.1 PRISMA flow diagram for systematic review to answer the following research question: *'In lame horses does objective gait assessment versus subjective gait assessment improve the accuracy of lameness detection?'*

The qualitative synthesis identified the experimental model, flaws and outcome for each of the 19 studies (Table 2.4). Four studies used accelerometery, one study used kinematics with Fourier analysis, one study used kinematics, three studies used force plates, one study used a locomotion analysis system called CODA-3 and 11 studies used inertial measurement sensors of which nine were dual sensors and two were multi sensors. Fourteen studies used horses with naturally occurring lameness whereas five studies created lameness using an experimental method. Overall, six studies were found to have a moderate vetGRADE, eight studies were considered low, two were very low and three were speculative. The studies are separated below first by vetGRADE then by model of lameness and finally by objective assessment.

Table 2.4. Extracted data, key findings, experimental flaws and vetGRADE outcome for the 19 included studies for the systematic review to answer the following research question: *'In lame horses does objective gait assessment versus subjective gait assessment improve the accuracy of lameness detection?* Abbreviations: American Association of Equine Practitioners (AAEP), inertial measurement unit (IMU), lipopolysaccharide (LPS)

Reference	Population	Lameness type	Subjective gait analysis	Objective gait analysis	Key findings	Experimental flaws	vetGRADE
Donnell et al. (2015)	16	Experimental (Carpal fragment) Forelimb	AAEP 4 experienced observers Video Blinded	IMU (lameness locator) and force platform	IMU and subjective assessment were more accurate than force platform. Strong evidence IMU can detect which leg is lame	Experimental lameness on a treadmill No Intervention	Moderate

Ishihara et al. (2005)	32	Experimental (LPS in fetlock) Forelimb	AAEP with 0.5 increments 1 experienced observer Live Blinded	Force platform	Vertical peak force appeared to best differentiate lame and sound horses with high specificity and sensitivity even with mild lameness.	Poor injection technique for LPS Some horses received phenylbutazone No Intervention	Moderate
Keegan et al. (1998)	24	Natural Forelimb	Bidirectional scale from 1-7 13 variable experienced observers Video Blinded	Kinematics	Suggests objective is more accurate and less variable than subjective Used an intervention (local anaesthetic blocks)	Only mild and moderate lameness Treadmill only Poor agreement between clinicians	Moderate
Leelamankong et al. (2020)	26	Natural Hindlimb	0-5 20 variable experience observers Live and video Blinded	IMU (Lameness locator)	Subjective agreement improved for live compared to video, with experience and with increasing severity Used an intervention (local anaesthetic blocks)	Horses only identified as lame by IMU were not included in study	Moderate
McCracken et al. (2012)	15	Experimental (Shoe providing variable sole pressure) Forelimb and Hindlimb	Not graded- only lame leg chosen 13 variable experience observers Live Blinded	IMU (lameness locator)	Supports objective can identify the lame leg	Subjective assessment required a consensus No intervention Statistics can be improved- means rather than median	Moderate

Thomsen et al. (2010)	5	Experimental (Saline injection into fetlock) Forelimb	AAEP scale 2 experienced observers Video Blinded	Accelerometery (Mega Electronics)	Significant relationship between mean visual scores of both observers and symmetry scores. Demonstrates objective is better at determining which leg is lame when lameness is mild. Intervention (saline injection)	Only used 8 strides which were chosen therefore likely significant manual bias. Interobserver agreement questionable for milder lameness	Moderate
Audigié et al. (2002)	25	Natural Forelimb and Hindlimb	0-4 scale (Dyson, 1991) 1 experienced observers Live Not blinded	Fourier analysis	Objective and subjective agree the horses are lame and which leg	No intervention Objective technique not available clinically Neurological cases included	Low
Barrey and Desbrosse (1996)	32	Natural Forelimb and Hindlimb	0-3 Unknown observers and method	Accelerometery (ICSensor)	Suggests symmetry and regularity increases with improving subjective lameness grade	Multi-limb lameness System not good at differentiating low grade lameness No intervention	Low
Maliye et al. (2013)	23	Natural Forelimb	AAEP 2 experienced observers Live Blinded	IMU (lameness locator)	Supports objective analysis agrees with subjective assessment following local anaesthesia Used an intervention (local anaesthetic blocks)	Retrospective study but a standard lameness protocol. Unknown how lame the horses were initially	Low

Marshall et al. (2012)	17	Natural Hindlimb	AAEP 1 experienced observer Live Blinded	IMU (lameness locator)	Objective correlates with subjective assessment	Flexion test No baseline data or severity for lameness Residual values very high	Low
Pfau et al. (2020)	25	Natural Forelimb and Hindlimb	0-5 6 experienced observers Video Blinded	IMU (EquiGait5)	More horses were detected as lame using objective analysis compared to subjective	Poor to fair agreement between observers Site of pain not determined Inconsistent observation Different thresholds to dual sensor papers Only mild lameness	Low
Pfau et al. (2014)	13	Natural Hindlimb	0-11 1 experienced observer Live Blinded	IMU (EquiGait3)	Correlation demonstrated between objective and subjective Intervention included (local anaesthesia)	Retrospective study with a small sample size. Multiple clinicians used with no agreement data presented. Only included mild lameness Different thresholds to dual sensor papers	Low
Rungsri et al. (2014)	24	Natural Forelimb	0-5 (Ross, 2003) 13 variable experience observers Video and live Blinded	IMU (Lameness locator)	Agreement between subjective and objective was fair to moderate. Agreement improved with experience. Agreement post local anaesthesia was also moderate.	Analysis of the data is poor. Means were used instead of medians and method of normalisation unusual.	Low

Weishaupt et al. (2001)	22	Natural Forelimb and Hindlimb	0-5 3 experienced observers Live Blinded	Accelerometry (Equimetrix) and force plates	Moderate correlation between the two objective methods individually and the clinical evaluation. No significant correlation found between the 2 objective methods or for degree of lameness for objective and subjective	Gait analysis performed on the treadmill Only mild lameness included	Low
Argüelles et al. (2019)	11	Natural Forelimb	AAEP scale 2 experienced observers Video Not blinded	Accelerometery (Equimetrix)	Improvement seen in both subjective and objective gait parameters	Bilateral lameness Mild lameness (up to grade 2/5) No intervention	Very low
Keegan et al. (2013)	106	Natural Forelimb and Hindlimb	AAEP 3 experienced observers Live Blinded	IMU (lameness locator)	Correlation between the IMU and subjective which was increased when only forelimbs were considered	Lameness severity unknown Incorrect data analysis (should be median not mean) Subjective assessor agreement low No Intervention	Very low
da Silva Azevedo et al. (2019)	29	Natural Hindlimb	AAEP 3 variable experience observers Video Not blinded	IMU (Lameness Locator)	Clinicians do not agree subjectivity 2 evaluators agreed with objective assessment	Flexion test No lameness severity given	Speculative
Back et al. (1993)	16	Experimental (LPS in radiocarpal joint)	0-5 scale with 0.5 increments	CODA-3	Correlation between clinical lameness	No intervention Objective technique	Speculative

		Forelimb	1 experienced observer Live Not blinded		grade and worsening objective parameters	poorly described and reliability unknown Poor data analysis	
Lopes et al. (2018)	22	Natural Forelimb and Hindlimb	A-C 3 experienced observers Live Blinded	IMU (Lameness locator)	Significant disagreement between the veterinary evaluation and standard IMU analysis	Non-standard subjective grading system Unknown which leg was affected No intervention Poor quality surface Questionable quality of observations Statistics not appropriate Not all horses with asymmetries detected were eliminated	Speculative

Of the six studies that had a moderate vetGRADE, one investigated accelerometery, one investigated force plates, two investigated IMU, one investigated force plates and IMU and one investigated kinematics. These have been separated into two distinct groups to enable better comparisons. Group one includes those where lameness was experimentally induced, and group two includes naturally occurring lameness however includes diagnostic anaesthesia as an intervention.

Group one includes four studies (Donnell et al., 2015, Ishihara et al., 2005, McCracken et al., 2012, Thomsen et al., 2010). Lameness was induced in this group therefore it is known which limb was lame. This enables comparison of how well both objective and subjective gait assessments can identify a lame limb. Thomsen et al. (2010) induced a unilateral forelimb lameness by injecting saline into the metacarpophalangeal joint and then assessed these horses using accelerometery and subjective lameness assessments. This study demonstrated a significant relationship between the mean subjective scores of both observers and the symmetry score as measured by the objective analysis. The

objective assessment, specifically the A score (a symmetry score calculated as the natural logarithmic quotient of the total positive accelerations during stance of the right and left diagonals), performed better at determining the lame leg than the subjective assessment as it was able to determine the correct diagonal on every trial, even when considering mild lameness. Agreement between both observers was good for more severe lameness but moderate for low grade lameness, and in one horse both observers incorrectly identified which limb was lame. Donnell et al. (2015) induced a unilateral forelimb lameness experimentally using arthroscopy to create a carpal fragment, these horses were then assessed with force plate technology and a dual-sensor IMU. The data showed that both subjective assessment and IMU were consistently better at identifying the lame leg than the force plate, and all assessments were poor at assessing severity. When just considering which leg was lame at any time point, agreement was highest between the peak vertical force from the force plate and the angle of the vector sum determined from the IMU data. Percentage agreement between the subjective assessment and IMUs was 53% and 50% for unblinded and blinded subjective assessment respectively. Ishihara et al. (2005) induced forelimb lameness which was assessed by force plates. Lameness was induced via a lipopolysaccharide injection into the metacarpophalangeal joint. This study demonstrated that peak vertical force best differentiated lame and sound horses even in mild lameness. All other values assessed only changed with moderate and severe lameness. The final study in group one assessed IMUs and it demonstrated that the objective assessment can identify the lame leg every time, agrees with the subjective and suggested objective may identify the lame leg sooner than the subjective evaluation (McCracken et al., 2012). This paper used a novel experimental model to induce lameness using a shoe which provided variable sole pressure creating a range of lameness grades.

Group two includes two studies (Keegan et al., 1998, Leelamankong et al., 2020). Leelamankong et al. (2020) used IMUs to assess natural hindlimb lameness. Additionally, this study utilised diagnostic anaesthesia until the lameness was abolished. This study demonstrated moderate agreement between live subjective assessment and the objective assessment and slight to fair agreement for video assessment depending on clinician experience. When considering mild lameness this agreement reduced to fair and slight to poor respectively. Horses only identified as lame by the IMU were excluded from

the study. In the supplementary material from this study percentage disagreement between the objective and subjective assessment revealed that for live clinical evaluation there was no disagreement between the subjective and objective on whether the horse was left hindlimb or right hindlimb lame. However, for the video evaluation disagreement varied from 32.5-49% depending on the experience level. When considering whether a horse was sound or lame, the objective and subjective assessments disagreed between 31-47% of the time, with the objective more likely to determine a horse as sound. The other paper in group two investigated kinematics. Keegan et al. (1998) demonstrated that when using kinematics on a treadmill the objective is more accurate and less variable that the subjective assessment when assessing natural forelimb lameness. This study used diagnostic anaesthesia as an intervention and demonstrated that interobserver agreement for subjective assessments was poor for determining whether lameness had stayed the same, improved or worsened following diagnostic anaesthesia.

Eight studies received a low vetGRADE including one study investigating accelerometery, one study investigating accelerometery and force plates, five studies investigating IMU and one study investigating Fourier analysis. These studies have also been split into two groups, group three includes naturally occurring lameness (Audigié et al., 2002, Barrey and Desbrosse, 1996, Marshall et al., 2012, Pfau et al., 2020, Weishaupt et al., 2001), whereas group four includes naturally occurring lameness in addition to the use of diagnostic anaesthesia (Maliye et al., 2013, Pfau et al., 2014, Rungsri et al., 2014).

In group three, Barrey and Desbrosse (1996) used accelerometery to assess both naturally occurring forelimb and hindlimb lameness. This study indicated that symmetry and regularity scores moderately correlated with subjective lameness scores. When assessing the side of lameness, the objective assessment was able to detect the correct limb in 13/17 cases. This system appeared to struggle to differentiate low grade lameness via subjective or objective assessment, as demonstrated by Table 2.4, but was able to identify severe lameness at trot. The second study assessing accelerometery by Weishaupt et al. (2001) investigated naturally occurring forelimb and hindlimb lameness and compared this to subjective assessment in addition to using force plates. This study demonstrated a significant correlation between the subjective assessment and the objective assessments individually when considering whether the horse was lame or not,

and whether it was a forelimb or hindlimb lameness. This was a moderate correlation with a correlation coefficient of 0.51 and 0.47 respectively. Additionally, a significant correlation was demonstrated between the force plate score and the subjective lameness assessment for the choice of lame limb. This was a strong correlation with a correlation coefficient of 0.65. There was no correlation between the final force plate or accelerometery grouping, however there was a significant moderate correlation between the raw data for the peak vertical force and dorsoventral acceleration when considering hindlimb lameness. There was no correlation for any gait analysis combination when considering degree of lameness. Marshall et al. (2012) investigated a dual-sensor IMU in hindlimb flexion tests. The objective analysis using the IMU correlated with the subjective analysis when considering if the result of the flexion test was positive or negative, however there was a large amount of overlap in the data and this correlation was not significant. This study does not report the subjective assessment for the baseline trials, therefore it is unknown which limb the horses were lame on prior to the flexion tests. Pfau et al. (2020) used a multi-sensor IMU to assess naturally occurring lameness in forelimbs and hindlimbs and demonstrated that more horses were detected as lame with the objective assessment. Like the other studies there was fair to poor agreement between the observers and only horses with mild lameness were included. The final study in group three assessed naturally occurring forelimb and hindlimb lameness. This demonstrated that Fourier analysis of kinematic data agrees with the subjective assessment when determining which horses are lame and which leg is affected when assessing forelimb and hindlimb lameness (Audigié et al., 2002).

In group four, Maliye et al. (2013) used a dual sensor IMU system in naturally occurring forelimb lameness. This demonstrated a significant decrease in maximum and minimum head height and vertical head movement asymmetry following diagnostic anaesthesia which correlated with a subjective assessment that the horse had responded positively to the diagnostic anaesthesia. Rungsri et al. (2014) also assessed dual-sensor IMU analysis in naturally occurring forelimb lameness. They did not look at lameness severity but just determination of the lame limb. They demonstrated that live subjective assessment has greater agreement than video assessment however there were only two clinicians assessing the horses live compared to 13 using video analysis. Agreement

between the live subjective and IMU was moderate and agreement between the video subjective assessment and IMU was fair to moderate with agreement improving with clinician experience. Agreement between the subjective and objective analysis post-diagnostic local anaesthesia was also moderate. Using a multi-sensor IMU, Pfau et al. (2014) assessed naturally occurring hindlimb lameness using local anaesthesia and demonstrated a correlation between objective and subjective assessment when assessing whether there was a positive or negative response to the diagnostic anaesthesia.

Two studies had a very low vetGRADE, both investigated naturally occurring lameness and did not include diagnostic anaesthesia. Argüelles et al. (2019) investigated naturally occurring bilateral forelimb lameness using accelerometery and demonstrated an improvement in objective scores in horses that showed an improvement in subjective scores, however there is a lack of agreement data presented within this study. Keegan et al. (2013) demonstrated that dual-sensor IMUs were weakly associated with the subjective assessment and that agreement improved with forelimbs compared to hindlimbs in naturally occurring lameness. However, it is unknown how lame the horses were, and data assessment was poor as the categorical AAEP subjective scores have been summed.

The final three studies had a speculative vetGRADE. da Silva Azevedo et al. (2019) demonstrated that when assessing natural hindlimb lameness using flexion tests, two of the three clinicians agreed with the IMU objective analysis. However there were many flaws with this study including a lack of lameness severity, use of flexion tests and small reference values to differentiate lameness and asymmetry. Lopes et al. (2018) compared an A-C subjective scale with the dual-sensor IMU during an endurance race and demonstrated significant disagreement between the two methods. However, this study did not require the lame leg to be stated and had poor quality subjective observations. Back et al. (1993) used an objective gait assessment system attached to the left front limb which produces joint angle diagrams used to measure lameness. This technique was poorly described, and its reliability is unknown. This study investigated experimentally induced lameness with no intervention and demonstrated a moderate correlation between clinical lameness grade and worsening objective parameters.

Discussion

There is currently no gold standard method for lameness assessment in horses although objective methods are suspected to be more reliable than subjective due to reduced bias and variability. This review is the first to compare objective and subjective gait assessment tools. All studies included in this review assessed both subjective and objective gait analysis however there was often a lack of data presented which was required to determine the answer to the question as establishing agreement between the modalities was not the main study objective. This was taken into account in addition to the assigned vetGRADE when considering the question.

All of these studies have limitations which impacted on their assigned vetGRADE as discussed in the materials and methods. The study by Thomsen et al. (2010) may have an element of bias as for the objective assessment eight strides were manually chosen from the 25-metre trot up. Multiple studies did not include interventions (Argüelles et al., 2019, Audigié et al., 2002, Barrey and Desbrosse, 1996, Donnell et al., 2015, Ishihara et al., 2005, Keegan et al., 2013, McCracken et al., 2012, Pfau et al., 2020, Weishaupt et al., 2001). An intervention would improve the quality of the study as it confirms which is the lame limb, enabling greater confidence in the evidence presented for both subjective and objective assessments. Some studies completed the subjective assessments on a treadmill (Donnell et al., 2015, Keegan et al., 1998), which may mean the data is not relevant to clinical practice. In particular the study by Keegan et al. (1998) used only a lateral view on the treadmill from a video recording for the subjective assessment, potentially contributing to the poor agreement between clinicians when completing the subjective assessment. In comparison other studies completed a full orthopaedic assessment, as would be performed in clinical practice, including lunging and flexion tests before determining subjective scores (Weishaupt et al., 2001).

Experimental and clinical techniques between studies were variable. In the study by Ishihara et al. (2005) although there was a good range of lameness severities, lameness was not observed in eight horses following injection. Also, certain horses received non-steroidal anti-inflammatories during this trial. However, this was not discussed in detail in the discussion, therefore it is unknown if these eight horses received this medication which is why lameness was not observed. The range of lameness present within the

studies was variable with many just including mild lameness (Argüelles et al., 2019, Maliye et al., 2013, Pfau et al., 2014, Weishaupt et al., 2001), or no statement of lameness severity (da Silva Azevedo et al., 2019, Keegan et al., 2013, Marshall et al., 2012). The study by Barrey and Desbrosse (1996) included many horses with multi-limb lameness, making it harder to compare the subjective with the objective. Multiple studies required a consensus for the subjective assessment, did not report interobserver reliability or demonstrated poor quality of agreement between observers (da Silva Azevedo et al., 2019, McCracken et al., 2012, Thomsen et al., 2010, Weishaupt et al., 2001). Additionally, some studies used different clinicians for the subjective assessment for different horses, therefore potentially affecting our ability to compare the data. This is because as seen in other studies poor agreement is suspected with subjective grading scales.

When using IMUs, studies used different thresholds for the same parameters to determine if a horse was lame or asymmetric, making it harder to compare the results. In both the multi-sensor IMU studies, thresholds of 14.5mm for mean difference in head minimum (HDmin) and 7.5mm for mean difference in pelvis minimum (PDmin) were used (Pfau et al., 2014, Pfau et al., 2020). Multiple studies using dual-sensor IMUs used thresholds of 6mm for HDmin and 3mm for PDmin which are considerably lower (Donnell et al., 2015, Leelamankong et al., 2020, McCracken et al., 2012, Rungsri et al., 2014). Another study used a threshold of 4mm for mean difference in head maximum (HDmax) and 0.19mm for HDmin (Maliye et al., 2013). This variation affects the ability to compare these studies and highlights importance of considering the population or patient when utilising these devices in clinical practice. As outlined by Pfau (2019), when assessing a horse which has been presented for poor performance, using a strict threshold gives a high sensitivity and therefore a high positive predictive value. Therefore, this test will be more likely to correctly identify lame horses. However, when used in the general population, horses may be wrongly identified as lame.

There were also concerns with the data handling and statistics in multiple papers. In the study by McCracken et al. (2012) when considering the number of half turns of the screw which were required prior to lameness detection, the ranges described have a large overlap, objective range 1-11 and subjective range 2-13, which does not provide much confidence in their claim that it can detect the lameness sooner. Although the statistics

appear adequate, presentation of more data within this study would have improved the strength of the evidence. In the study by Rungsri et al. (2014) the data analysis was affected by the fact they subtracted the score for the left forelimb from the right forelimb for each observer. This is an odd way to normalise the data and creates a mean suggesting the data is continuous which is incorrect. Marshall et al. (2012) reported very small changes within the data with large residuals. Within this study there is only a small difference in the median value between groups with large overlap in the ranges presented. Audigié et al. (2002) included neurological cases in their statistical analysis, which affects the validity of their data. These should have been excluded from their data analysis. Additionally, it is not possible to compare the strength of the correlation presented in the study by Keegan et al. (2013). This is because the subjective scores, which are categorical data, were subtracted from each other. This is not appropriate as the spacing between the categories is uneven. For some studies determining agreement between objective and subjective gait analysis was not their main objective, therefore minimal data is presented (Argüelles et al., 2019).

Two of the studies which were assigned a speculative vetGRADE had significant flaws. The study by Lopes et al. (2018) used a non-standardised subjective assessment, horses were trotted on an uneven dirt surface, statistics were not appropriate, and the data was overinterpreted. Additionally, 21 horses demonstrated asymmetry at some point during the race and were not eliminated, calling into question the quality of the observation. In the study by Back et al. (1993) the description of the methods was poor, and the reliability of the technique is unknown as no other group appears to have published on this technique. This study was not randomised or blinded and utilised a non-standard subjective grading system.

When only considering the studies which were found to have a moderate vetGRADE the objective gait assessments were consistently able to determine which leg was lame (Donnell et al., 2015, Leelamankong et al., 2020, McCracken et al., 2012), and in some cases outperformed the subjective assessment especially when considering mild lameness (Ishihara et al., 2005, Keegan et al., 1998, Thomsen et al., 2010). Of these studies four investigated experimentally induced lameness models. Experimentally induced lameness, although less clinically realistic, provides a sound method for

producing a controllable singular lame limb. In particular the studies by McCracken et al. (2012) and Thomsen et al. (2010) provided a lameness model of variable severity over time. It has also been suggested that experimental lameness may improve interobserver agreement due to visual perception of a similar lameness and horse type (Thomsen et al., 2010), making a subjective gait analysis consensus more likely. Subjective interobserver agreement has been shown to improve with clinician experience and with live assessment rather than video recordings (Leelamankong et al., 2020). When the data shows that the objective gait analysis agrees with the subjective analysis, and there is good interobserver agreement, this further increases the confidence in the objective assessment. This occurred when lameness severity was moderate or severe and with experienced clinicians in the studies reviewed (Ishihara et al., 2005, Leelamankong et al., 2020). With mild lameness interobserver agreement is often poor (Hewetson et al., 2006, Keegan et al., 2010) which casts doubt upon the reliability and accuracy of subjective analysis. Therefore, it is when assessing mild lameness where the objective gait assessment is truly needed and would considerably add to the clinical assessment.

Multiple studies implied that objective methods were more accurate than subjective assessment when assessing mild lameness. Ishihara et al. (2005) demonstrated that peak vertical force, detected by a force plate, correlated with mild and moderate forelimb lameness and has a potential role in detecting subclinical lameness, as there were changes from baseline in horses that were not classed as lame by subjective assessment. In comparison, the study by Donnell et al. (2015) showed better agreement between IMU and subjective assessment than between force plates and subjective assessment. This is likely due to the fact IMUs determine lameness based on movement asymmetry, like subjective assessment, rather than ground reaction forces. Force plates also use fewer strides than IMUs, reducing variation in the stride but also potentially limiting the ability to detect lameness. Another difference between these studies is that the study by Donnell et al. (2015) used subjective assessment by video analysis on a treadmill potentially reducing interobserver agreement, whereas the other study completed live subjective assessments over solid ground. McCracken et al. (2012) suggested that the IMU was able to identify the lame leg sooner than the subjective assessment potentially indicating that it would be able to detect subclinical lameness. Though this is affected by

the fact that all three observers in this study had to come to a consensus prior to the limb being selected, and there is significant overlap in the data. Although observers were blinded to which limb was lame, they were aware of the study protocol and therefore understood they were looking for a worsening limb and that the lameness would continue to worsen until all three observers agreed. This may have caused bias, prompting observers to choose a limb sooner than they would in a normal lameness assessment, or change their choice if the horse kept presenting, as a consensus had not yet been reached. Thomsen et al. (2010) demonstrated that when Fourier analysis was used to calculate symmetry score S, calculated using Fourier coefficients, there was a significant relationship between the accelerometric data and the subjective score however, the S score was more accurate when assessing lameness severity as it detected a decline in lameness between time points where the subjective assessment did not. The symmetry score A, calculated using the upwards acceleration during the stance phase, correctly identified the lame leg in all cases including those with mild lameness where the subjective assessment disagreed. This study provides good evidence to suggest that detecting lameness using accelerometery is more effective than using subjective lameness assessments for mild and moderate lameness. All these studies investigated experimentally induced lameness models. These studies suggest that objective gait analysis is more accurate than subjective gait analysis however further good quality studies with naturally induced lameness are required to confirm this.

When assessing accuracy of detection of lameness improvement post-local anaesthesia, Keegan et al. (1998) demonstrated that kinematics identified a change in minimum poll height difference which was positively correlated with a reduction in lameness score following local anaesthesia in horses. Interobserver agreement post-local anaesthesia was poor, suggesting objective assessment may be less variable than subjective. Although Leelamankong et al. (2020) demonstrated that following local anaesthesia, the agreement between the IMU and live subjective assessment increased to strong, this data was not presented within the paper therefore interobserver agreement post-local anaesthesia is unknown. However, using diagnostic anaesthesia techniques to abolish lameness gives added credence to these studies as demonstration of improvement in the

predominantly lame limb is evidence the objective and subjective assessments can identify lameness. This improves the quality of the data and the confidence in the results.

The results from the studies which have a low vetGRADE agree with these findings. Multiple studies demonstrated the objective assessment was able to correctly determine the lame limb (Audigié et al., 2002, Barrey and Desbrosse, 1996, Pfau et al., 2014, Pfau et al., 2020, Weishaupt et al., 2001), and objective assessment was shown to be more accurate in one study as more horses were detected as lame with this technique (Pfau et al., 2020). This study also demonstrated that IMUs were able to determine the most affected limb correctly in horses with multi-limb lameness using the head to withers relationship, making this data more clinically relevant than the experimental studies where there is only one site of lameness (Pfau et al., 2020). Two studies demonstrated a reduction in lameness score following local anaesthesia (Maliye et al., 2013, Rungsri et al., 2014) and Marshall et al. (2012) demonstrated that objective assessment correlated with subjective assessment when assessing hindlimb flexion tests, although the usefulness of IMUs for flexion tests must be questioned. For objective assessment with IMUs 25 strides are recommended, following the flexion test only 10-15 strides are obtained and due to the nature of the response to a flexion test these strides will not be consistent. Additionally, there may be variation in when the start button is pressed on the objective software as the first stride should probably be ignored. All these studies investigate natural causes of lameness and therefore provide evidence that objective assessment agrees with subjective assessment when assessing naturally derived and multi-limb lameness.

Even when considering the studies with very low and speculative vetGRADEs, correlations were seen between the objective gait assessment and subjective assessment in all but one study (Argüelles et al., 2019, Back et al., 1993, da Silva Azevedo et al., 2019, Keegan et al., 2013). The study which demonstrated significant disagreement between the subjective and objective assessments was highly flawed and is of a questionable quality therefore should not be used (Lopes et al., 2018).

From this evidence, IMUs appear to be the most effective objective gait analysis tool and can be used both to determine which leg is lame and to determine improvement following diagnostic anaesthesia. Donnell et al. (2015) demonstrated that IMUs were

consistently better at identifying the leg lame in comparison to force plates. However, no other studies compared IMUs to other objective techniques therefore more evidence is required to give confidence to this conclusion. Force plate technology is considered the gold standard for objective gait analysis (Weishaupt et al., 2004, Weishaupt et al., 2006) although it is largely an experimental tool and therefore may no longer be gold standard for assessing clinical cases.

Another aspect to consider is whether there are certain types of horse where objective gait analysis may be inferior to subjective gait analysis, for example in young horses who repeatedly throw their heads during evaluation. No study mentions excluding horses due to the inability to obtain adequate data using objective gait analysis methods. Therefore, this is unknown and would need to be assessed in future studies with different study populations.

This review was limited by the lack of randomised control trials which are few and far between in veterinary medicine. Of the studies suitable for inclusion in this review no studies were classed as providing exceptional or high quality of evidence. Exceptional studies include systematic reviews of randomised control trials and high-grade studies are randomised control trials. Additionally, many of the studies did not directly answer the question and so although may provide suggestive evidence, more high-quality studies directly assessing objective and subjective gait analysis in naturally derived lameness would be useful. However, this review has demonstrated that objective gait analysis can determine the lame limb and may be more sensitive at detecting which limb is lame compared to subjective gait assessment especially when considering mild lameness. Objective gait analysis is effective at identifying changes in lameness following local anaesthesia and may be more useful than subjective assessment in cases of multi-limb lameness, although a large-scale clinical trial is required to confirm this. There is no evidence that objective gait analysis can accurately determine lameness severity although this may be due to the fact it is compared to subjective gait assessment which, due to poor interobserver agreement, is inherently flawed as a gold standard to compare to. The evidence from these studies does suggest that objective gait analysis can assist in determining the predominantly lame limb in horses, and therefore should be used alongside subjective assessment in clinical practice. Clinicians should be aware that at

this time objective gait analysis cannot establish if gait asymmetry is due to an underlying pathological orthopedic process and therefore these techniques should be used to assist the clinician in decision making. Of the technologies discussed the most accessible to clinical practice is the IMUs which are readily available. Additionally, these appear to be the most reliable form of objective gait analysis.

Conclusion

This review is the first to compare objective and subjective gait assessment tools and presents a positive outcome for objective gait assessment. It has demonstrated there is moderate evidence that objective gait analysis is more accurate than subjective gait analysis, particularly for the use of IMUs in comparison to other objective techniques especially in clinical practice and for when using diagnostic anaesthesia.

Chapter 3: Paracetamol and Chronic Musculoskeletal Pain in Horses

Abstract

Paracetamol is used in horses alongside NSAIDs for multi-modal analgesia, however to date there are few studies published which have investigated its analgesic effect in the horse (Bowen et al., 2020).

Horses were recruited to this study from a population of geriatric horses at The Horse Trust. Horses were selected based on the following inclusion criteria; documented naturally occurring chronic lameness and being treated with an NSAID at the licensed dose and dosing interval. This population was assessed with a 5-point subjective lameness grading system performed live by two observers and an objective gait assessment tool (EquiGait5). Assessments were performed weekly during the eight-week study period with two weeks of baseline assessments. Following this paracetamol was administered for a period of 4 weeks at 20mg/kg per os twice daily. Paracetamol was then discontinued, and horses monitored for two further weeks.

Descriptive statistics for subjective assessments are presented based on the more experienced observer's subjective grades. Interobserver agreement between observers was analysed using Cohen's Kappa index. Objective data was separated into forelimb and hindlimb lame groups. Horses were excluded if the asymmetry was <6mm for the poll or <3mm for the sacrum for the forelimb and hindlimb groups respectively for each parameter in weeks 1 and 2, and if the lameness was not consistent between these two weeks. Additionally, horses were excluded if they switched from a left to right sided lameness during the data collection period. Minimum difference and upwards difference for the poll and sacrum ($Poll_{min\ diff}$, $poll_{up\ Diff}$, $sacrum_{min\ diff}$, $sacrum_{up\ diff}$) and hip hike difference (HHD) were assessed with all horses meeting the criteria above being included in each group. Median and interquartile range were reported for each parameter. A Friedman test was performed for each parameter to determine if there was a significant difference between the groups at any time point. Significance was set at <0.05.

Twenty-five horses were included. Median subjective lameness grade before treatment was 1 (range 1-3) and did not change during the treatment period. Interobserver

agreement was moderate to very good for forelimb lameness and was moderate to good for hindlimb lameness. There was no statistically significant difference between time points when assessing lameness with the objective gait analysis.

In a population of mild chronically lame retired horses oral paracetamol at 20mg/kg did not significantly reduce the lameness severity when assessed with both subjective and objective gait analysis. However as recently published evidence has demonstrated a reduction in chronic lameness severity with 30mg/kg oral paracetamol, further studies are required to assess the effect of oral paracetamol at 30mg/kg in chronically lame horses. These studies should include a larger sample size, a greater initial lameness severity and multiple doses.

Introduction

Paracetamol is a commonly used analgesic in human medicine and is used for chronic painful conditions such as osteoarthritis although its effects so far in this population have only been found to be modest compared to placebo (McCrae et al., 2018, Graham et al., 2013). As with NSAIDs there are concerns regarding adverse events in humans including gastrointestinal toxicity and hepatotoxicity (Graham et al., 2013, McCrae et al., 2018) however a large systematic review of the human literature revealed that there was no evidence for hepatic failure when a therapeutic dose was used (Dart and Bailey, 2007). Equally there are publications which do not report gastrointestinal toxicity at therapeutic doses (Graham et al., 2013). Due to this conflicting evidence, it is unknown whether gastrointestinal toxicity and hepatotoxicity are true concerns when considering the adverse effects of paracetamol. Currently the mechanism of action of paracetamol is not fully understood. It acts differently to NSAIDS, with its analgesic effects thought to be due to its effect on opioid and cannabinoid receptors alongside the 5-hydroxtryptamine system (Oscier and Milner, 2009, Graham et al., 2013). Therefore due to its differing action, wide safety margin and low reported adverse event rate it is commonly combined with NSAIDs to provide analgesia in humans (Graham et al., 2013).

In horses there is a large amount of anecdotal evidence for paracetamol use alongside NSAIDs for the management of acute and chronic pain. However, to date, there are few studies published which evaluate its clinical effects or its potential side effects (Bowen et

al., 2020). Paracetamol has been shown to be an effective analgesic for acute pain as part of a multimodal analgesia regime (Bruniges et al., 2019, West et al., 2011). As a single oral analgesic at 20mg/kg, it has also been shown to significantly reduce lameness score and heart rate compared to a control in an inducible acute foot pain model, and was comparable to flunixin meglumine (Foreman et al., 2016). In another study with an acute mechanically induced lameness model, oral paracetamol at 30mg/kg was shown to reduce the lameness score, however 20mg/kg orally did not (Mercer et al., 2022). Mercer et al. (2023a) investigated paracetamol as a monotherapy in horses with naturally occurring chronic lameness and showed that at 30mg/kg orally there was a transient improvement in lameness both subjectively and objectively. Lameness assessments were only performed after administration of 3 weeks of oral paracetamol at 30mg/kg twice daily orally. When considering adverse events, Foreman (2018) reported that 25mg/kg orally twice daily for 30 days did not cause any significant changes in renal or hepatic blood parameters. Mercer et al. (2020) demonstrated statistically significant reductions in both total protein and platelet count with twice daily oral dosing at 20mg/kg for 2 weeks. They reported increases in sorbitol dehydrogenase (SDH) and total bilirubin which were significant and ranged outside of the reference interval. Liver biopsies revealed mild portal inflammation in all horses sampled, with irreversible changes seen in one case. However, biopsies were not taken prior to paracetamol dosing for comparison. In a different study there were no significant changes detected on liver biopsies following 3 weeks of oral paracetamol at 30mg/kg when compared to pre-treatment liver biopsies (Mercer et al., 2023a). This study also showed no significant differences in gastric disease score when compared to before paracetamol treatment.

Lameness can be assessed both subjectively and objectively. The most commonly used objective gait analysis system clinically is the inertial measurement units (IMUs) which measure asymmetry. These systems remove bias and produce real-time data although consideration must be given that asymmetry does not equal lameness. However, movement asymmetries have been shown to respond to diagnostic anaesthesia which reinforces that asymmetry can be caused by pain (Leelamankong et al., 2020, Maliye et al., 2013, Pfau et al., 2014). Threshold values are reported for >6mm vertical movement for the poll and >3mm for the sacrum to help identify the lame limb (McCracken et al.,

2012), although normal horses can fall outside of these thresholds (Rhodin et al., 2017). IMUs are suggested to be more reliable than subjective lameness grading especially when evaluating mild lameness and can determine the most affected limb correctly in multi-limb lameness (Donnell et al., 2015, Leelamankong et al., 2020, Pfau et al., 2020). IMUs can also enable detection of compensatory lameness. When considering the poll and withers the direction they move indicates whether the lameness is a true forelimb lameness or a compensatory forelimb lameness due to an ipsilateral hindlimb lameness. When the poll and withers agree on the side of asymmetry this is a true forelimb lameness. If the withers indicate asymmetry to the opposite side to the poll, then this is a referred forelimb lameness due to an ipsilateral hindlimb lameness, the poll indicates the side of the lameness in this case.

This study aims to use subjective and objective gait analysis to assess the efficacy of paracetamol as an additional analgesic in chronically lame horses already being treated with an NSAID. Our hypothesis was that paracetamol will reduce the lameness severity observed.

Materials and Methods

Horses were recruited to this study from a population of geriatric horses at The Horse Trust. This population of horses were housed at a charity and the purpose of this study was to assess the efficacy of paracetamol in chronically lame horses already receiving NSAID therapy. Full informed owner consent, ethical approval (The University of Nottingham's Ethical Review Committee, ethical review number: 2608 181016) and animal testing certificates (ATC-s) were obtained for this study.

An a priori power analysis was conducted using free online software (Clincalc.com) to estimate the sample size required. Results indicated that the required sample size to achieve 80% power for detecting an effect size of 20% with a significance criterion of $\alpha = 0.05$ based on results from previous studies was 16 horses. The studies used for the power calculation were human paracetamol studies (Bradley et al., 1991, Singhal et al., 2021) and canine osteoarthritis studies (Bui and Bierer, 2003, Innes et al., 2003), as no appropriate studies had been performed in horses.

Population

Horses were selected for the study based on the following inclusion criteria; documented naturally occurring chronic lameness and being treated with an NSAID at the licensed dose and dosing interval. NSAID dose was calculated based on an accurate weight. Chronic lameness was defined as a minimum AAEP lameness grade of 1/5 for greater than 3 months duration. All included horses previously had the lameness confirmed using diagnostic analgesia and the disease process causing the lameness was established using radiographs in the majority of cases. Horses must have been on the NSAID for >30 days prior to the beginning of the study. Horses were excluded if they were not on the licensed dose and dosing interval of the NSAID, had surgery in the last 120 days, had received intrasynovial medication within the last 6 months or received systemic steroids in the last 7 days.

Horses were assessed at the beginning of the study by physical exam to confirm they were healthy other than the presence of chronic lameness. Horses were maintained at pasture during the study period. They were regularly seen by a farrier at their normal shoeing interval. All horses were retired and therefore did not undergo any exercise other than turnout during the study period.

Lameness evaluation

This population was assessed with objective and subjective lameness evaluations.

The subjective analysis was completed live using a numerical 0–5 grading scale (Appendix 3) with a degree of lameness assigned to one or multiple limbs by two independent observers with one being very experienced and the other less experienced at subjective lameness assessments. This was completed on a tarmac surface in a straight-line in hand with each horse being trotted away and towards the observer four times over an approximate total distance of 50m.

The objective assessment was performed with an inertial measurement unit (EquiGait5; EquiGait Ltd, UK). Data were collected at trot over a sequence of 25 or more strides in a straight line on a tarmac surface. This data was processed using the gait analysis software to produce the following parameters: minimum difference and upwards difference for the poll and sacrum ($Poll_{min\ diff}$, $poll_{up\ Diff}$, $sacrum_{min\ diff}$, $sacrum_{up\ diff}$) and hip hike difference

(HHD). Threshold values of 6mm for the poll and 3mm for the sacrum were used based on previous studies (Pfau, 2019). Stride time and speed were recorded to ensure the horse was trotted at a consistent speed despite changes in handler.

Objective and subjective lameness assessments were performed weekly during the eight-week study period on the same day each week. Initially two weeks of baseline assessments were completed during which time the horses only received the NSAID. Following this paracetamol was administered for a period of 4 weeks. Following the treatment period, paracetamol was discontinued, and assessments continued for two further weeks. Horses were monitored daily by the onsite vet for any adverse reactions and to ensure their welfare.

Paracetamol dosing

Paracetamol (500mg tablets, Crescent Pharma Limited) were administered orally at 20mg/kg every 12 hours for a total of 56 doses. Tablets were mixed with 30mL of water and administered orally using a catheter tip syringe within 30 minutes of mixing. Horses were weighed using an electronic scale prior to the beginning of the study and were weighed monthly during the study.

Statistical analysis

When considering the subjective lameness data, descriptive statistics are presented as medians with interquartile range (IQR) and range based on the more experienced observer's subjective grades. Interobserver agreement between the more experienced and less experienced observer was analysed using weighted Cohen's Kappa index which is reported alongside 95% confidence intervals. To enable assessment of both the interobserver agreement in lameness grade and in agreement of the side of lameness, right limb lameness grades were considered positive and left limb lameness grades were multiplied by -1 to make them negative. A value of <0.2 was considered poor agreement, 0.21-0.4 fair agreement, 0.41-0.6 moderate agreement, 0.61-0.8 good agreement and >0.8 very good agreement. Calculations were undertaken using an online statistical calculator (http://vassarstats.net/kappa.html).

When considering the objective data, all values were converted to absolute values to pool left and right limb lameness. Horses were placed into a predominantly forelimb lame

group and predominantly hindlimb lame group. If horses were both forelimb and hindlimb lame they could be in both groups however horses were excluded from the forelimb group if comparison of the poll to withers data suggested the lameness was compensatory. Horses were excluded if the asymmetry was <6mm for the poll or <3mm for the sacrum for the forelimb and hindlimb groups respectively for each parameter in weeks 1 and 2, and if the lameness was not consistent between these two weeks. Additionally, horses were excluded if they switched from a left to right sided lameness during the data collection period. IMU data was assessed for normality by visual assessment of a histogram, this data was not normally distributed therefore non-parametric statistical tests were used.

$Poll_{min\ diff}$, $poll_{up\ Diff}$, $sacrum_{min\ diff}$, $sacrum_{up\ diff}$ and hip hike difference (HHD) were assessed with all horses meeting the criteria above being included in each group. Median and interquartile range (IQR) were reported for each parameter. Week 1 and week 2 (pre-treatment) and weeks 7 and 8 (post-treatment) were compared with a Wilcox signed rank test, weeks 3, 4, 5 and 6 (treatment) were compared using a Friedman test to determine if there was a significant difference of an individual horse's lameness between these weeks for each parameter. Following a non-significant result, weeks were grouped in pre-treatment (week 1 and 2), early treatment (week 3 and 4), late treatment (week 5 and 6) and post-treatment (week 7 and 8). A Friedman test was used to compare the 4 groups for each parameter to determine if there was a significant difference between the groups at any time point. Significance was set at <0.05. All statistical analysis was performed using GraphPad Prism version 9.5.1 for Windows, GraphPad Software, San Diego, California USA.

Results

There were 25 horses which met the inclusion criteria and started the trial. This included 19 geldings and 6 mares, mean age was 23 years (standard deviation +/- 5 years), and age range was 15-37 years. Breeds included were twelve Irish Sports Horses, three Cobs, three Thoroughbreds, two Shires, one Fjord, one Belgium Draught, one Dales Pony, one Clydesdale and one Warmblood. All horses were receiving NSAIDs daily with eighteen receiving phenylbutazone twice daily per os (2.2mg/kg), five receiving suxibuzone twice daily per os (3.1mg/kg) and two receiving meloxicam once daily per os (0.6mg/kg). NSAID

therapy remained unchanged throughout the trial period. Twenty-three horses had diagnosed osteoarthritis and two had suspected osteoarthritis based on diagnostic analgesia and presentation (Table 3.1). All horses had been lame for >3 months. One horse was excluded as it developed cellulitis in week 8.

Table 3.1 Table showing signalment data, diagnosis, diagnostic tests performed, and the non-steroidal anti-inflammatory (NSAID) medication administered for each of the 25 horses in the study. Abbreviations: Irish Sport Horse (ISH), Thoroughbred (TB), osteoarthritis (OA), distal interphalangeal joint (DIPJ), Tarsocrural joint (TCJ), proximal interphalangeal joint (PIPJ), radiocarpal joint (RCJ), tarsometatarsal joint (TMTJ), distal intertarsal joint (DITJ), twice daily (BID), once daily (SID), per os (PO)

Horse	Sex	Age (years)	Breed	Diagnosis	Diagnostic tests performed	NSAID medication
1	Gelding	20	ISH	Left fore DIPJ OA	Diagnostic anaesthesia Radiography	2.2mg/kg phenylbutazone PO BID
2	Gelding	27	Fjord	Right TCJ OA	Diagnostic anaesthesia Radiography Previously responded to intrasynovial steroid medication	2.2mg/kg phenylbutazone PO BID
3	Gelding	25	ISH	Right fore DIPJ and PIPJ OA	Diagnostic anaesthesia Radiography	2.2mg/kg phenylbutazone PO BID
4	Gelding	22	Shire	Right RCJ OA	Diagnostic anaesthesia Radiography Previously responded to intrasynovial steroid medication	2.2mg/kg phenylbutazone PO BID
5	Gelding	17	Cob	Left TMTJ OA	Diagnostic anaesthesia	2.2mg/kg phenylbutazone PO BID
6	Gelding	27	TB	Left RCJ OA	Diagnostic anaesthesia Radiography	2.2mg/kg phenylbutazone PO BID
7	Mare	19	Belgian Draught	Right hind PIPJ OA	Diagnostic anaesthesia Radiography	2.2mg/kg phenylbutazone PO BID
8	Gelding	26	ISH	Right fore DIPJ OA	Diagnostic anaesthesia Radiography	2.2mg/kg phenylbutazone PO BID
9	Gelding	22	Cob	Left hind PIPJ OA	Diagnostic anaesthesia Radiography	2.2mg/kg phenylbutazone PO BID

10	Mare	16	TB	Left fore DIPJ OA	Diagnostic anaesthesia Radiography	2.2mg/kg phenylbutazone PO BID
11	Gelding	15	Cob	Left TMTJ and DITJ OA	Diagnostic anaesthesia Radiography	2.2mg/kg phenylbutazone PO BID
12	Gelding	28	Dales Pony	Right TMTJ and DITJ OA	Diagnostic anaesthesia Radiography	2.2mg/kg phenylbutazone PO BID
13	Gelding	19	ISH	Bilateral TMTJ OA	Diagnostic anaesthesia Radiography	2.2mg/kg phenylbutazone PO BID
14	Mare	28	ISH	Bilateral fore and hind DIPJ OA	Diagnostic anaesthesia Radiography	3.1mg/kg suxibuzone PO BID
15	Gelding	27	ISH	Left TMTJ OA	Diagnostic anaesthesia Previously responded to intrasynovial steroid medication	3.1mg/kg suxibuzone PO BID
16	Mare	26	Shire	Right fore DIPJ and PIPJ OA	Diagnostic anaesthesia Radiography	3.1mg/kg suxibuzone PO BID
17	Mare	19	ISH	Bilateral fore DIPJ and PIPJ. Bilateral TMTJ and DITJ OA	Diagnostic anaesthesia Radiography	3.1mg/kg suxibuzone PO BID
18	Gelding	19	ISH	Bilateral fore DIPJ OA	Diagnostic anaesthesia Radiography Previously responded to intrasynovial steroid medication	2.2mg/kg phenylbutazone PO BID
19	Mare	22	ISH	Right TMTJ and DITJ OA	Diagnostic anaesthesia Radiography	2.2mg/kg phenylbutazone PO BID
20	Gelding	27	ISH	Right fore DIPJ OA	Diagnostic anaesthesia Radiography	2.2mg/kg phenylbutazone PO BID
21	Gelding	23	Clydesdale	Left TMTJ OA	Diagnostic anaesthesia Radiography	2.2mg/kg phenylbutazone PO BID
22	Gelding	20	TB	Left TMTJ OA	Diagnostic anaesthesia Radiography	2.2mg/kg phenylbutazone PO BID
23	Gelding	30	ISH	Right TMTJ OA	Diagnostic anaesthesia Radiography	0.6mg/kg meloxicam once daily PO SID
24	Gelding	21	Warmblood	Bilateral fore DIPJ OA	Diagnostic anaesthesia Radiography	3.1mg/kg suxibuzone PO BID

					Previously responded to intrasynovial steroid medication	
25	Gelding	37	ISH	Right TMTJ and DITJ OA	Diagnostic anaesthesia Radiography	0.6mg/kg meloxicam once daily PO SID

Subjective Gait Analysis

Median subjective lameness grade for all horses before treatment was 1 (range 1-3). Two horses presented with hindlimb only lameness, all other horses had both forelimb and hindlimb lameness. When considering the most lame leg, 10 horses presented with a forelimb lameness and 15 horses presented with a hindlimb lameness. There was an even split of left and right limb lameness, with 12 horses having a left limb primary lameness and 13 horses having a right limb primary lameness.

When assessing the single main observer's lameness grade for the most lame leg following treatment with paracetamol there was no statistically significant difference in the lameness score throughout the study period as the median and IQR remained the same (Table 3.2). This was despite more horses being assigned a lameness grade of 0 during the study period when paracetamol was administered (Figure 3.1).

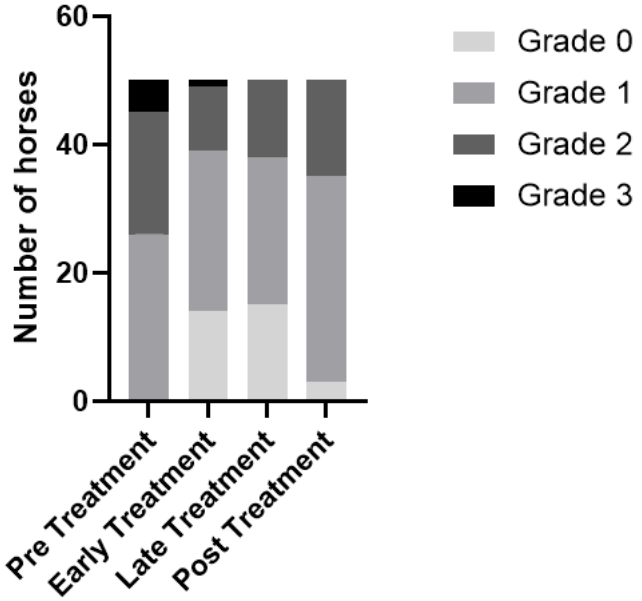

Figure 3.1 Subjective lameness grades for 25 horses from main observer for the most lame leg grouped into pre-treatment (week 1 and 2), early treatment (week 3 and 4), late treatment (week 5 and 6) and post-treatment (week 7 and 8) groups.

Table 3.2 Median, IQR and range for subjective lameness grades for 25 horses from main observer for the most lame leg grouped into pre-treatment (week 1 and 2), early treatment (week 3 and 4), late treatment (week 5 and 6) and post-treatment (week 7 and 8) groups

Time point	Median	IQR	Range
Pre-treatment	1	1-2	1-3
Early treatment	1	0-1	0-3
Late treatment	1	0-1	0-2
Post treatment	1	1-2	0-2

Interobserver agreement was moderate to very good when considering forelimb lameness and was moderate to good for hindlimb lameness (Table 3.3, Table 3.4).

Table 3.3 Cohen's Kappa analysis with 95% confidence intervals for each week between observer one and observer two for forelimb lameness

	Weighted Cohen's Kappa	95% Confidence Intervals (%)
Week 1	0.5	0.23-0.78
Week 2	0.7	0.52-0.92
Week 3	0.7	0.52-0.89
Week 4	0.9	0.76-1.00
Week 5	0.7	0.45-0.92
Week 6	0.8	0.59-1.00
Week 7	0.7	0.48-0.87
Week 8	0.7	0.43-0.89

Table 3.4 Cohen's Kappa analysis with 95% confidence intervals for each week between observer one and observer two for hindlimb lameness

	Weighted Cohen's Kappa	95% Confidence Intervals (%)
Week 1	0.5	0.31-0.76
Week 2	0.6	0.39-0.75
Week 3	0.6	0.33-0.85
Week 4	0.6	0.30-0.84
Week 5	0.7	0.57-0.90
Week 6	0.7	0.45-0.96
Week 7	0.7	0.52-0.87
Week 8	0.8	0.58-0.92

Objective Gait Analysis

Median degree of asymmetry, IQR and range for all horses prior to treatment is reported in Table 3.5.

Table 3.5 Median degree of asymmetry, IQR and range for each parameter for all horses prior to treatment

Parameter	Median (mm)	IQR (mm)	Range (mm)
$Poll_{Min\ Diff}$	11.0	3.0-22.5	0-40
$Poll_{Up\ Diff}$	15.0	6.0-37.0	0-66
$Sacrum_{Min\ Diff}$	5.0	2.0-10.0	0-23
$Sacrum_{Up\ Diff}$	10.5	4.0-15.0	0-50
HHD	11.0	5.5-17.5	0-56

Asymmetry data was assessed, and horses were placed into a forelimb lame and/or hindlimb lame group based on published asymmetry thresholds. 11 horses met the inclusion criteria for both $Poll_{min\ diff}$ and $Poll_{up\ Diff}$ which were included in the forelimb group. In the hindlimb group there were 17 horses which met the inclusion criteria for

HHD, 16 met the inclusion criteria for $Sacrum_{up\ diff}$ and 13 met the inclusion criteria for $Sacrum_{min\ diff.}$

Median and IQR for $Poll_{min\ diff}$ and $Poll_{up\ Diff}$ in the forelimb lame group are reported in Table 3.6. Median, IQR and range are reported in graphs in Figure 3.2. When compared with a Friedman test there were no significant differences between pre-treatment, early treatment, late treatment and post treatment time points for either parameter (Table 3.7).

Table 3.6. Median and interquartile range for forelimb asymmetry parameters (11 horses) for pre-treatment, early treatment, late treatment and post treatment time points

	Pre-treatment		Early treatment		Late treatment		Post-treatment	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR
$Poll_{min\ diff}$ (mm)	23.0	20.0-28.5	17.5	8.5-26.5	18.0	11.0-29.5	13.5	8.0-32.5
$Poll_{up\ Diff}$ (mm)	31.5	17.5-43.5	25.0	13.0-40.5	24.5	15.5-33.0	26.0	11.5-41.0

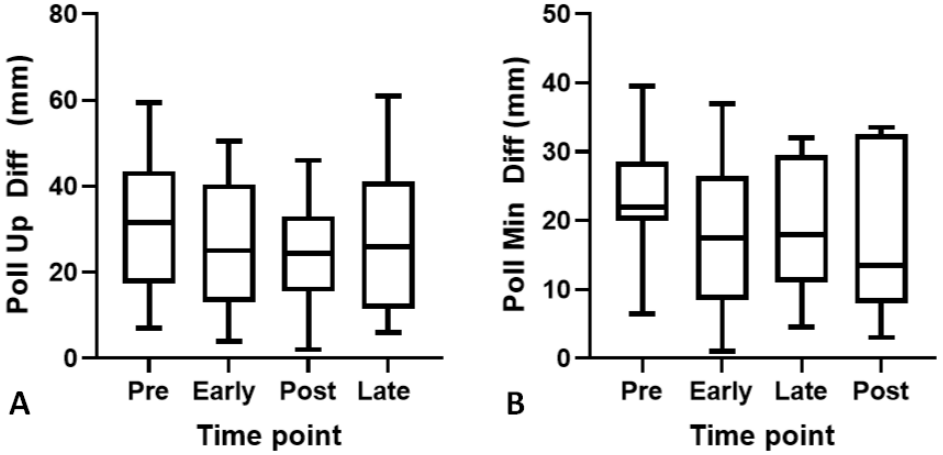

Figure 3.2 Box and whisker plot for 11 horses which were forelimb lame to show median, interquartile range and range for pre-treatment, early treatment, late treatment and post treatment time points for A) Poll Up Diff and B) Poll Min Diff parameters

Table 3.7 P values for each objective gait analysis parameter for the forelimb lame group (11 horses) comparing pre-treatment, early treatment, late treatment and post treatment time points determined by a Friedman test.

Parameter	P value
$Poll_{min\ diff}$	0.161
$Poll_{up\ Diff}$	0.147

Median and IQR for $Sacrum_{Min\ Diff}$, $Sacrum_{Up\ Diff}$ and HHD are reported in Table 3.8. Median, IQR and range are reported in graphs in Figure 3.3. When compared with a Friedman test there were no significant differences between pre-treatment, early treatment, late treatment and post treatment time points for any parameter (Table 3.9).

Table 3.8 Median and IQR for hindlimb asymmetry parameters; HHD (17 horses), $Sacrum_{up\ diff}$ (16 horses) and $Sacrum_{min\ diff}$ (13 horses) for pre-treatment, early treatment, late treatment and post treatment time points

	Pre-treatment		Early treatment		Late treatment		Post-treatment	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR
$Sacrum_{min\ diff}$ (mm)	9.0	5.5-13.5	7.0	5.0-12.0	5.0	4.0-11.0	8.5	3.5-10.5
$Sacrum_{up\ diff}$ (mm)	11.5	8.5-17.5	13.5	7.4-19.3	10.3	3.0-16.0	13.0	6.0-20.0
HHD (mm)	15.5	12.0-24.0	18.0	8.0-25.0	14.0	7.5-21.5	19.5	9.6-27.1

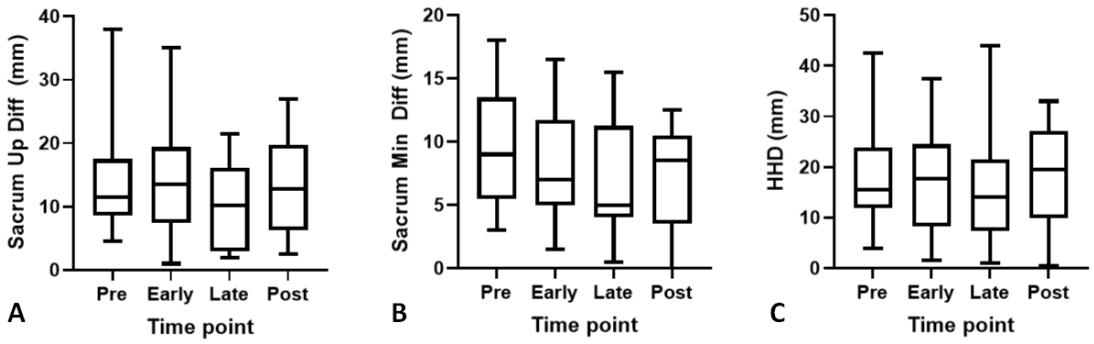

Figure 3.3. Box and whisker plot of hindlimb lame group to show median, interquartile range and range for pre-treatment, early treatment, late treatment and post treatment time points for A) Sacrum Up Diff (16 horses), B) Sacrum Min Diff (13 horses) and C) Hip Hike Difference (HHD, 17 horses) parameters

Table 3.9 P values for the objective gait analysis parameters; HHD (17 horses), $Sacrum_{up\ diff}$ (16 horses) and $Sacrum_{min\ diff}$ (13 horses) for the hindlimb lame group comparing pre-treatment, early treatment, late treatment and post treatment time points determined by a Friedman test.

Parameter	P value
$Sacrum_{min\ diff}$	0.092
$Sacrum_{up\ diff}$	0.052
HHD	0.068

Retrospective power analysis

A retrospective power analysis was conducted using the same free online software (Clincalc.com) to determine the required sample size using the median and IQR for the pre-treatment and late treatment groups reported in this thesis.

In order to detect a treatment effect with paracetamol in the forelimb group the required sample size to achieve 80% power with a significance criterion of $\alpha = 0.05$ is 130 horses when considering $Poll_{min\ diff}$ and 190 horses when considering $Poll_{up\ Diff}$. For the hindlimb group, the required sample size is 115 horses, 140 horses and 315 horses for $Sacrum_{min\ diff}$, $Sacrum_{up\ diff}$ and HHD respectively.

Discussion

In this population oral administration of paracetamol at 20mg/kg twice daily in addition to an NSAID at the licensed dose did not significantly affect chronic lameness when assessed with subjective or objective lameness analysis. However, when considering the subjective lameness analysis data, more horses were allocated scores of zero during the paracetamol treatment than during the pre and post-treatment phases, although median lameness score did not change over time. When considering the objective gait analysis

data, both median $Poll_{min\ diff}$ and median $Poll_{up\ Diff}$ decreased from pre-treatment values with paracetamol treatment, although this difference was not statistically significant. The hindlimb objective data was much more variable, with only median $Sacrum_{Min\ Diff}$ showing a decrease during the paracetamol treatment compared to during the pre and post-treatment phases. Again, this difference was not statistically significant. The lack of significance seen in this study may be due to the small sample size, as the retrospective power analysis revealed that this study was underpowered. It may be that if this population was larger, these observations would have reached significance. Additionally, the lameness severity observed in this study was mild, likely due to these horses already receiving a NSAID. Due to the animal test certificate (ATC) stipulations we were only authorised to use paracetamol in addition to a NSAID due to the concern paracetamol alone would not provide adequate analgesia for horses with chronic lameness. It may be that with a more severe lameness it may have been possible to detect a significant difference with oral paracetamol.

When considering acute lameness, paracetamol monotherapy at 20mg/kg orally has been shown in one study to significantly reduce lameness score in an experimental model (Foreman et al., 2016). However, another study did not demonstrate a significant difference in acute lameness at 20mg/kg orally but did demonstrate a reduction in lameness severity with 30mg/kg orally (Mercer et al., 2022). The reduction in lameness severity with 30mg/kg paracetamol was statistically significant although was small, with a maximum of 1 lameness grade on a 10-point scale. The reduction in lameness severity with 30mg/kg paracetamol orally did not differ significantly from horses treated with 2.2mg/kg phenylbutazone orally. Both studies only included a small sample size and used an experimental foot pain model. In both studies lameness assessments were performed repeatedly on the same day of therapy whereas in the current study lameness assessments were performed once weekly over a longer period with the aim to determine the effects of long-term administration of oral paracetamol on chronic lameness. The examinations in this study were performed at a similar time of day each week, however, as only one examination was performed per week this may mean that changes in lameness severity at other times of day may have been missed, as it has been

demonstrated by Mercer et al. (2022) that lameness grade following paracetamol dose varies with time post-administration.

Horses with naturally occurring chronic lameness are potentially more challenging to assess response to analgesia than acute experimental induced models as there is likely to be natural variation. Kaido et al. (2016) has shown objective assessment of naturally occurring lameness using forceplates does not differ significantly with 3 repetitions or if sessions have an interval of at least 3 hours, suggesting naturally occurring lameness may be as useful as experimentally induced lameness under these conditions. However this study was performed over one day, whereas the current study was performed over 8 weeks therefore it is unknown how much the lameness severity was affected by natural variation over this time. Mercer et al. (2023a) showed an improvement in chronic lameness with 30mg/kg paracetamol orally however lameness grading was only performed prior to treatment and after 21 days of treatment. Like the current study, this creates a wide window during which many other variables including shoeing, ridden exercise and activity during turnout were not controlled, all of which may have affected lameness severity. To minimise these potential confounding factors, more horses should be included in these studies.

Recent evidence suggests 30mg/kg paracetamol orally twice daily provides an increased reduction in lameness severity compared to 20mg/kg (Mercer et al., 2023a, Mercer et al., 2022). In the current study 20mg/kg was chosen, as at the time of study proposal, this was the current suggested dose. It may be that there would have been a statistically significant difference in lameness severity in this population with 30mg/kg which is a potential future study.

Horses in this study were receiving NSAIDs in addition to paracetamol due to ATC requirements. Horses were either receiving phenylbutazone, suxibuzone or meloxicam. Suxibuzone is a prodrug of phenylbutazone and has been shown to have no significant difference in alleviating lameness when compared to phenylbutazone (Sabate et al., 2009). Meloxicam has been shown to be less efficacious than phenylbutazone in a hoof pain model however was more effective at reducing lameness in a synovitis model (Banse and Cribb, 2017). Horses in this study had chronic lameness most likely due to osteoarthritis in one or multiple joints therefore pain may have been caused by

osteophyte formation, subchondral bone change or synovitis. Currently no studies have compared phenylbutazone, suxibuzone and meloxicam in osteoarthritic horses so comparable efficacy is unknown. Additionally, the interaction of paracetamol with each of these NSAIDs is unknown. To try to reduce any difference in effect from NSAID treatment, all horses received the same NSAID at the same dose for the duration of this study however using the same NSAID for all horses would have further reduced any difference in effect.

Subjective lameness assessments although used commonly in clinical practice have only been shown to have marginal agreement between different observers especially when considering both mild and hindlimb lameness (Dyson, 2011, Keegan et al., 2010). In the current study there were two live observers with different experience levels. Interobserver agreement was superior to previously published studies (Bragança et al., 2020, Leelamankong et al., 2020). Observers were asked independently for their score and therefore did not influence the other.

Lameness severity in this study was mild with a median subjective lameness grade of 1/5. Horses with a grade 1 lameness were included as horses were already being treated with a NSAID, reducing their lameness grade. However, this meant it was very difficult to observe a reduction in lameness grade, particularly with the subjective assessment as in order to improve a horse would have to be completely sound on that limb. This is a limitation of this study and in future studies it would be prudent to only include horses with a subjective lameness grade of 2 or greater. It may also be useful to use a more detailed subjective grading system, for example a 10-point scale, as in the study performed by Mercer et al. (2023a).

IMU's are the most commonly used objective gait analysis tool in clinical practice. This study used the EquiGait5 system which is easy to use and well tolerated by the horse. This system has been previously validated (Pfau et al., 2005). It must be noted that this system is not comparable with the other commonly used system (The Lameness Locator, Equinosis, Columbia, MO, USA) as shown by Pfau et al. (2016). This study showed the Lameness Locator consistently underestimates the movement asymmetry compared to the EquiGait5 system and provides widths of limits of agreement values between the two systems. These values should not be seen as a change in asymmetry when using both

systems on the same horse. Therefore, care must be taken not to directly compare results from these systems. It is also important to remember IMUs measure gait asymmetry not lameness. Rhodin et al. (2017) demonstrated that 72.5% of owners who thought their horses were lameness free had gait asymmetries. The question therefore remains, how do we know if these asymmetries are caused by pain? Thresholds have been developed to help guide lameness quantification with IMUs and although it has been shown that normal variation can exceed these thresholds (Sepulveda Caviedes et al., 2018), they are currently the accepted standard (Pfau, 2019). In this study horses were only included if they exceeded the reported thresholds in both week 1 and 2 (Pfau, 2019). Although this reduced the sample size it meant horses included in the analysis had asymmetry values that were stable.

When considering the forelimb lameness group, the objective parameters demonstrated a reduction in both median $Poll_{min\ diff}$ and $Poll_{up\ Diff}$ between pre-treatment and the early treatment and late treatment groups. Additionally, this difference remained when comparing the pre-treatment and post-treatment group, suggesting the forelimb lameness did not return to the original severity following discontinuing paracetamol. Although this reduction did not reach significance, it suggests that paracetamol may reduce chronic lameness in horses and that further studies with a larger sample size and higher doses are required. In the hindlimb lameness group, only median $Sacrum_{Min\ Diff}$ showed a decrease during the paracetamol treatment compared to during the pre and post-treatment phases. The parameters $Sacrum_{up\ diff}$ and HHD showed no pattern. This differs from a previous study, which revealed the largest changes when using the IMUs to monitor response to diagnostic anaesthesia were observed for HHD and symmetry index of upwards movement (SI_{up}) (Pfau et al., 2014). Although they cannot be compared directly as they are calculated differently, $Sacrum_{up\ Diff}$ in this study represents the same movement as SI_{up} in the study by Pfau et al. (2014).

This study has multiple limitations. Although a prospective power calculation was performed, the retrospective power calculation determined that the sample size was too small and the study was underpowered. Therefore, although promising trends were observed in the data no comparisons reached statistical significance. The lameness severity in this study was mild making it difficult to detect mild changes in lameness

severity with subjective and objective gait analysis. All horses were already receiving a NSAID which was essential to meet ATC requirements however meant the lameness severity was reduced and does not enable us to see paracetamols effect as a monotherapy. However paracetamol is often used as part of a multimodal therapy (Bruniges et al., 2019, West et al., 2011), therefore this data is relevant to clinical practice. Although all horses included were suspected to have osteoarthritis this was not confirmed with radiography in two cases. The majority of horses included had multi-limb lameness, we removed the horses with observable compensatory lameness, however multi-limb lameness is more challenging to assess than single limb lameness (Maliye et al., 2013). This study may have been improved if horses were only included if they had a single limb lameness. The population included retired horses only, therefore this data may not be applicable to horses in work.

Conclusion

In a population of mild chronically lame retired horses oral paracetamol (20mg/kg BID) did not significantly reduce the lameness severity when assessed with both subjective and objective gait analysis. However promising trends were observed and with recently published evidence which has demonstrated a reduction in chronic lameness severity with 30mg/kg, further studies are required to assess the effect of oral paracetamol in chronically lame horses. These studies should include a larger sample size, a greater initial lameness severity and multiple dosages.

Chapter 4: Final Comments

This thesis reviewed the literature to answer the question *'In lame horses does objective gait assessment versus subjective gait assessment improve the accuracy of lameness detection?'.* This revealed there is moderate evidence that objective gait analysis is more accurate than subjective gait analysis. In particular, the review found IMUs were more accurate than subjective gait analysis, especially in clinical practice and when using diagnostic anaesthesia.

Additionally, this thesis investigated the efficacy of paracetamol when used alongside an NSAID for chronic musculoskeletal pain in horses. Both subjective and objective gait analysis tools were used. For the objective gait analysis IMUs were chosen as the systematic review determined that there was moderate evidence this technology is more accurate than subjective gait analysis. In a population of mild chronically lame retired horses oral paracetamol (20mg/kg BID) did not significantly reduce the lameness severity when assessed with both subjective and objective gait analysis. However as recently published evidence which has demonstrated a reduction in chronic lameness severity with 30mg/kg, further studies are required to assess the effect of oral paracetamol in chronically lame horses. These studies should include a larger sample size, a greater initial lameness severity and multiple dosages.

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Appendix 1

PRISMA Checklist for the systematic review to answer the following research question: '*In lame horses does objective gait assessment versus subjective gait assessment improve the accuracy of lameness detection?*'

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	36
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	36
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	37-38
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	38-39
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	38-39
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	40
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	39-40
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	38-39
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	39-40
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	40-41

Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	40-41
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	40-41
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	N/A
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	N/A
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	N/A
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	N/A
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	42
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	43-48
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	48-52
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	43-48
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	N/A
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	N/A
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	N/A
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	53-60
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g.,	59-60

		incomplete retrieval of identified research, reporting bias).	
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	60
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Acknowledgements

Appendix 2

Full search strategy for systematic review to answer the following research question: '*In lame horses does objective gait assessment versus subjective gait assessment improve the accuracy of lameness detection?*'

MEDLINE

	Search	Results
1	Exp Equidae/	70942
2	Equi*	943104
3	(Pony or ponies)	3424
4	(Gelding* or Mare* or Stallion* or Horse*)	129671
5	Exp Lameness, Animal/	4075
6	Lame*	46273
7	(Asymmetr* adj2 (movement or gait))	998
8	Exp Gait Analysis/	613
9	(gait adj2 analys#s)	8296
10	(locomot* adj2 analys#s)	420
11	(Objective ADJ3 gait)	515
12	(Objective ADJ3 lame*)	74
13	(Inertial ADJ3 sensor*)	2320
14	ISS	10117
15	(Inertial ADJ3 measurement*)	1972
16	Force plat*	7349
17	Accelerometer*	15673
18	(quantitative adj3 gait)	574
19	(kine* adj3 gait)	2461
20	(kine* adj3 anayls#s)	1
21	(subjective or qualitative or visual or observational or empirical) adj3 (gait or lame*)	794
22	(Lameness ADJ3 grad*)	163
23	1 or 2 or 3 or 4	1040939
24	5 or 6 or 7	47220
25	8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22	45776
26	22 and 23 and 24	340

CAB Abstracts

	Search	Results
1	Exp Equidae/	80257
2	Equi*	264951
3	(Pony or ponies)	3097
4	(Gelding* or Mare* or Stallion* or Horse*)	114320
5	Exp Lameness/	5786
6	Lame*	12896
7	(Asymmetr* adj2 (movement or gait))	22
8	Exp Gait/	131
9	(gait adj2 analys?s)	26
10	(locomot* adj2 analys?s)	15
11	(Objective ADJ3 gait)	19
12	(Objective ADJ3 lame*)	34
13	(Inertial ADJ3 sensor*)	263
14	ISS	36
15	(Inertial ADJ3 measurement*)	129
16	Force plat*	504
17	Accelerometer*	8
18	(quantitative adj3 gait)	37
19	(kine* adj3 gait)	0
20	(kine* adj3 anayls?s)	40
21	((subjective or qualitative or visual or observational or empirical) adj3 (gait or lame*)	63
22	(Lameness ADJ3 grad*)	299674
23	1 or 2 or 3 or 4	13096
24	5 or 6 or 7	1163
25	8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22	119
26	22 and 23 and 24	

Pubmed

	Search	Results
1	Equidae	71040
2	Equine	89505
3	Pony	1722
4	Ponies	2299
5	Gelding	1258
6	Mare	9599
7	Stallion	76216
8	Horse	88703
9	Lameness	7163
10	Asymmetry	56523
11	Movement asymmetry	6669
12	Gait asymmetry	1428
13	Gait analysis	22773
14	Gait analyses	24416
15	Locomotion analysis	81423
16	Locomotion analyses	19870
17	Objective gait assessment	6767
18	Objective lameness assessment	659
19	Objective lameness evaluation	926
20	Inertial sensor system	2040
21	Inertial measurement system	2245
22	ISS	14114
23	Force plate	9568
24	Force platform	8609
25	Accelerometer	14448
26	Quantitative gait assessment	1226
27	Kinematic gait assessment	4205
28	Kinetic gait assessment	1017
29	Quantitative gait analysis	1538
30	Kinematic gait analysis	8111
31	Kinetic gait analysis	2148
32	subjective gait assessment	5006
33	subjective lameness assessment	164
34	subjective lameness evaluation	183
35	subjective lameness grading	55
36	Qualitative gait assessment	267
37	Qualitative gait analysis	314
38	Visual lameness assessment	106
39	Observational gait assessment	794
40	1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8	107527
41	9 OR 10 OR 11	63565

42	12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23 OR 24 OR 25 OR 26 OR 27 OR 28 OR 29 OR 30 OR 31 OR 32 OR 33 OR 34 OR 35 OR 36 OR 37 OR 38 OR 39	145987
43	40 AND 41 AND 42	741

ProQuest Dissertations & Theses A&I

	Search	Results
1	Equi*	25000
2	(Pony or ponies)	27531
3	(Gelding* or Mare* or Stallion* or Horse*)	511495
4	Lame*	344517
5	(Asymmetr* N/2 (movement or gait))	2876
6	(gait N/2 analys?s)	5663
7	(locomot* N/2 analys?s)	1723
8	(Objective N/3 gait)	224
9	(Objective N/3 lame*)	113
10	(Inertial N/3 sensor*)	3778
11	ISS	77398
12	(Inertial N/3 measurement*)	4490
13	Force plat*	4458
14	Accelerometer*	29281
15	(quantitative N/3 gait)	1536
16	(kine* N/3 gait)	24
17	(kine* N/3 anayls?s)	865
18	(subjective or qualitative or visual or observational or empirical) N/3 (gait or lame*)	108
19	(Lameness N/3 grad*)	575
20	1 or 2 or 3	527690
21	4 or 5	347039
22	6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19	116633
23	20 and 21 and 22	9675
		606

Embase

	Search	Results
1	Equidae	69792
2	Equine	65191
3	Pony	1075408
4	Ponies	3469
5	Gelding	125999
6	Mare	4756
7	Stallion	48796
8	Horse	1242
9	Lameness	54913
10	Asymmetry	11735
11	Movement asymmetry	508
12	Gait asymmetry	923
13	Gait analysis	97
14	Gait analyses	2544
15	Locomotion analysis	17268
16	Locomotion analyses	1923
17	Objective gait assessment	8818
18	Objective lameness assessment	20497
19	Objective lameness evaluation	808
20	Inertial sensor system	3225
21	Inertial measurement system	0
22	ISS	1104
23	Force plate	186
24	Force platform	1175455
25	Accelerometer	49984
26	Quantitative gait assessment	102722
27	Kinematic gait assessment	595
28	Kinetic gait assessment	
29	Quantitative gait analysis	
30	Kinematic gait analysis	
31	Kinetic gait analysis	
32	subjective gait assessment	
33	subjective lameness assessment	
34	subjective lameness evaluation	
35	subjective lameness grading	
36	Qualitative gait assessment	
37	Qualitative gait analysis	
38	Visual lameness assessment	
39	Observational gait assessment	
40	1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8	
41	9 OR 10 OR 11	

42	12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23 OR 24 OR 25 OR 26 OR 27 OR 28 OR 29 OR 30 OR 31 OR 32 OR 33 OR 34 OR 35 OR 36 OR 37 OR 38 OR 39	
43	40 AND 41 AND 42	

Scopus

	Search	Results
1	Equi*	3778003
2	(Pony or ponies)	4860
3	(Gelding* or Mare* or Stallion* or Horse*)	207975
4	Lame*	117561
5	(Asymmetr* W/2 (movement or gait))	2093
6	(gait W/2 analys?s)	19087
7	(locomot* W/2 analys?s)	1287
8	(Objective W/3 gait)	1108
9	(Objective W/3 lame*)	168
10	(Inertial W/3 sensor*)	12435
11	ISS	27490
12	(Inertial W/3 measurement*)	12404
13	Force plat*	102781
14	Accelerometer*	56513
15	(quantitative W/3 gait)	784
16	(kine* W/3 gait)	3898
17	(kine* W/3 anayls?s)	0
18	((subjective or qualitative or visual or observational or empirical) W/3 (gait or lame*)	1297
19	(Lameness W/3 grad*)	258
20	1 or 2 or 3	3927676
21	4 or 5	119586
22	6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19	223613
23	20 and 21 and 22	609

Web of Science

	Search	Results
1	TS=Equi*	47528
2	TS= (Pony or ponies)	5670
3	TS=(Gelding* or Mare* or Stallion* or Horse*)	158496
4	TS=Lame*	94658
5	TS=(Asymmetr* NEAR3 (movement or gait))	4231
6	TS=(gait NEAR3 analys?s)	15879
7	TS=(locomot* NEAR3 analys?s)	4351
8	TS=(Objective NEAR3 gait)	3110
9	TS=(Objective NEAR3 lame*)	550
10	TS=(Inertial NEAR3 sensor*)	11331
11	TS=ISS	16340
12	TS=(Inertial NEAR3 measurement*)	10606
13	TS=Force plat*	84204
14	TS=Accelerometer*	40115
15	TS=(quantitative NEAR3 gait)	1145
16	TS=(kine* NEAR3 gait)	6043
17	TS=(kine* NEAR3 anayls?s)	1
18	TS=((subjective or qualitative or visual or observational or empirical) NEAR3 (gait or lame*)	1720979
19	TS=(Lameness NEAR3 grad*)	321
20	1 or 2 or 3	183537
21	4 or 5	98791
22	6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19	1886902
23	20 and 21 and 22	3324

Google scholar

Search	Results
equi* subjective and objective gait analysis	No new studies
horse subjective and objective gait analysis	No new studies
horse subjective and objective gait assessment	1 new study
equi* subjective and objective gait assessment	No new studies

Appendix 3

Lameness grading scale

The lameness grading scale used in this study for subjective gait analysis was the same as that used in Pfau et al. (2020). This was chosen as it was able to be assessed in a straight line compared to the AAEP lameness grading scale which consists of a 1-5 scale used following assessment of the horse under various circumstances including straight line, circling and different surfaces.

Grade	Descriptor
0	Not lame
1	Lameness is difficult to observe and not consistently apparent in a straight line at trot. No observable lameness at walk.
2	Lameness is not apparent at walk but is consistently apparent at trot in a straight line.
3	Lameness is inconsistent or very mild at walk and consistently apparent at trot in a straight line.
4	Lameness is obvious at walk.
5	Non-weight bearing on affected limb.