Paracetamol in Horses: Current use and future perspectives

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Impact of Covid-19

During the global pandemic all face-face research activities (including post-graduate research) at the University of Nottingham were ceased during the various phases of lockdown placed on the country. This resulted in significant delays to the clinical research aspects of this study, particularly the pharmacokinetic work and, therefore, delays to completion and submission of this thesis.

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Summary

Pain is a leading welfare concern in horses and the management of pain is a critical component of equine practice. The neurobiology of pain is complex, with numerous potential targets for analgesic drugs. The intricate interactions between central and peripheral pain pathways highlight the need for a multi-modal approach to pain management. Traditional analgesic agents target local pain pathways and their use can be associated with life-threatening gastrointestinal complications, especially when used at high doses in horses with severe pain. The use of paracetamol in horses has increased significantly over the past decade, although its mechanism of action is still poorly understood. There is increasing scientific evidence supporting is use as an effective analgesic when administered orally in horses. A survey conducted in 2020 provided an overview of how paracetamol was being used in equine practice. Although paracetamol was being used most commonly for the management of pain associated with laminitis, it was still being used relatively infrequently when compared to traditional non-steroidal anti-inflammatory drugs (NSAIDs). Paracetamol was being used most commonly at a dose of 20mg/kg, which was in line with published literature at the time. However, more recent evidence suggests that more effective analgesia is provided when paracetamol is given at 30mg/kg. It is important to note that this survey was conducted prior to the release of the BEVA Primary Care Guidelines: Analgesia (Bowen et al, 2020), which may encourage more widespread uptake amongst equine practitioners. Although the pharmacokinetics of oral and intravenous paracetamol have been described, to date there has been no publications describing its use under general anaesthesia or its bioavailability when given by alternate routes of administration. These routes of administration may increase its utility in the hospital setting, or in horses that cannot receive oral medication. Pharmacokinetic studies conducted as part of this thesis suggest that intravenous paracetamol has favourable pharmacokinetics for short-term analgesia in horses under general anaesthesia for routine surgery. However, the bioavailability of paracetamol when administered per rectum is very low and cannot be recommended for analgesic use at the current most commonly used dose and formulation of this drug in horses. However, further dose optimisation studies and use of a different vehicle base for the suppository may improve the pharmacokinetics of paracetamol when administered by this route. Paracetamol shows great promise as an adjunctive analgesic in horses. However, further studies are required to improve our understanding of the full potential of paracetamol in equine pain management.

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Attributions

The research and writing associated with this manuscript would not have been possible without aid from colleagues. Contributions are defined below.

Kate White is a Professor of Veterinary Anaesthesia at the University of Nottingham and is one of my primary supervisors in this degree. Kate contributed towards study design and methodology, attainment of ATC-S certificates and manuscript preparation.

Sarah Freeman is a Professor of Veterinary Surgery at the University of Nottingham and is a primary supervisor in this degree. Sarah contributed to study design and methodology and manuscript preparation.

Stuart Paine is an Associate Professor of Veterinary Pharmacology at the University of Nottingham and is a co-supervisor in this degree. Stuart contributed towards methodology and data and statistical analysis, particularly in the pharmacokinetics studies. Stuart also contributed towards manuscript preparation.

Dr Mark Bowen is a world-renowned specialist in Equine Internal Medicine, colleague and professional mentor. Mark contributed towards final manuscript preparation and has continued to encourage and support me to submit this thesis. He also contributed towards statistical analysis of the data used in this thesis.

Dr Stephanie Pratt is a now an Assistant Professor in Equine Anaesthesia and Critical Care at the University of Nottingham. Stephanie helped with sample collection from horses enrolled in the study at Oakham Veterinary Hospital during her residency.

Dr Hannah Wilson is now a first opinion ambulatory vet at Hoermann Equine, professional colleague and friend. During her internship at Oakham Veterinary Hospital Hannah helped enormously with sample and data collection from the horses enrolled in the study at Oakham Veterinary Hospital.

Dr Hannah Stanley is currently completing an internship in Australia having graduated this year. During her third year at the University of Nottingham School of Veterinary Medicine and Science, Hannah helped with designing and collating the study data from the survey investigating current paracetamol use amongst equine practitioners.

Publications and Original Research Included in this thesis

BEVA Primary Care Guidelines: Analgesia
Bowen, I.M., Redpath, A., Dugdale, A., Burford, J.H., Lloyd, D., Watson, T. and Hallowell, G.D., 2020. BEVA primary care clinical guidelines: Analgesia. *Equine veterinary journal*, *52*(1), pp.13-27.

How equine veterinary surgeons are currently using paracetamol in horses. Hannah Stanley and Adam Redpath, University of Nottingham, School of Veterinary Medicine and Science (unpublished data)

Contents

Chapter 1: Introduction

“Pain is an unpleasant sensory and emotional experience associated with, or resembling that associated with actual or potential tissue damage” (Raja et al 2020). It is more than nociception, the activity of pain neurons, reflecting the interpretation of these nociceptive stimuli. The processing of these pain signals can be influenced by learnt characteristics, social and psychological factors (Garland, 2012). Pain can be adaptive or can have maladaptive traits and, in human patients, may impact on mental well-being (Garland, 2012). In animals, and veterinary medicine, pain is assessed as an aversive response to damage, or the threat of damage, to normal functions (Viñuela-Fernández et al, 2007). It may impact both physiological processes and animal behaviours that are designed to limit further damage (Molony et al, 1997). Intervention is often required when the intensity of the experience is beyond what is appropriate for the damage in order to maintain welfare standards. When those stimuli to pain are a result of human intervention, veterinary surgeons have a professional obligation to make all attempts to alleviate pain and, therefore, suffering (RCVS Code of Professional Conduct, 24-hour emergency first aid and pain relief)

Pain manifests in horses in a number of scenarios, either in response to disease, injury, or in a veterinary context, in relation to surgery. Colic is the manifestation of pain associated with abdominal disorders and is often referred to as visceral pain. This can results from intestinal distension or mesenteric tension. Visceral pain can also be associated with muscular contraction (spasm), ischaemia and inflammation (Robertson et al, 2010). Pain can be both a cause and effect of colic; with pain having direct effects on intestinal motility (Koenig et al, 2006) that can worsen abdominal distension. In some cases, surgical intervention is required in order to resolve physical and/or functional disorders of the intestinal tract (Southwood, 2023). Visceral pain can also be referred, where nociceptive input from visceral structures is projected as pain into more distal somatic structures that share segmental innervation (Bogduk et al, 2009). This can have the effect of conscious perception of pain reflecting the musculoskeletal system, thus creating clinical signs that may localize to other locations in horses. While this is well documented in human studies, there are no veterinary studies that document this theory in animals, and therefore these conclusions are assumptions, relating from clinical findings and outcomes in horses, such as those with gastric disease (Kjaerulff and Lindegaard, 2022). Somatic pain is pain associated with the musculoskeletal system and is a leading welfare concern in the horse, with a survey revealing that a third of horses recorded with health problems are lame (National Equine Health Survey, 2016). This is most commonly associated with joint disease, which may be acute (e.g. synovial sepsis) or chronic (e.g. osteoarthritis), or is associated with disorders of the hoof capsule, such as laminitis, that can be both acute or chronic. Muscle disease is also a significant cause of somatic pain e.g., rhabdomyolysis (RER, PSSM), atypical myopathy and over exertion Neuropathic pain is poorly understood in the horse, but is most closely defined in association with trigeminally mediated headshaking (Roberts, 2019). The pahophsyiology of pain in equine laminitis is complex and some believe may have a neuropathic pain component (Driessen et al, 2010)

Analgesia, the pharmacological control of pain, is relatively rudimentary in equine practice, with the management of chronic pain being highly dependent on traditional cyclo-oxygenase (COX) inhibiting non-steroidal anti-inflammatory drugs (NSAIDs) (Mama et al, 2019, Flood et al, 2022),

while acute pain management may also include opiate analgesia, ketamine, alpha-2 agonists, constant rate infusions of these drugs and/or lidocaine and locoregional administration of compounds such as local anaesthetics (Sanchez et al, 2014). Recent studies have identified the potential value of paracetamol in the management of chronic pain, although the extent to which it is being applied in equine practice is not clear. Furthermore, its value in the management of acute pain has not been investigated.

This study will explore the potential value of paracetamol in horses and attempt to document the current use of paracetamol in equine practice and identify possible additional methods of administration for the management of acute pain in the hospitalised setting.

Chapter 2: Literature Review

Declarations: Sections of this review contain extracts from previously published work in which Adam Redpath was the main author of those sections.
Bowen, I.M., Redpath, A., Dugdale, A., Burford, J.H., Lloyd, D., Watson, T. and Hallowell, G.D., 2020. BEVA primary care clinical guidelines: Analgesia. Equine veterinary journal, 52(1), pp.13-27.

The Neurobiology of Pain

Nociception is a term used to describe the neural processes for the transduction (detection), transmission, modulation, projection and central processing of actual or potential tissue-damaging stimuli (White et al, 2010). This complex process begins with the stimulation of free nerve endings called nociceptors. These specialised sensory fibres occur as free nerve endings within the tissue and are activated specifically by 'painful' stimuli (Vanderah, 2007). Most nociceptors are ion channels that readily respond to different noxious modalities that have the potential to cause damage to cells, but generally do not respond to non-noxious stimuli (Vanderah, 2007). Thermal, mechanical and chemical stimuli activate these ion channels, resulting in an influx of sodium or calcium ions, leading to the depolarisation of the plasma membrane (Costigan et al, 2000). This results in a generator potential. When this generator potential is of a sufficient magnitude, voltage gated sodium channels are activated, further depolarising the membrane and causing a burst of action potentials (Woolf, 2004). These action potentials are conducted from the periphery to the dorsal horn of the spinal cord or the brainstem along the axons of primary afferent nociceptive fibres.

Aδ and C fibres are principal nociceptive primary afferents. These fibres have differential activity responsible for fast and slow pain. Aδ fibres are large, myelinated and conduct impulses rapidly and are responsible for the initial sensation of pain (first pain), which is sharp, localised and transient (Giordano, 2005, Steeds, 2009). C fibres are small and unmyelinated with slower conduction velocities (Steeds, 2009). They are responsible for 'second pain', which is the poorly localised, burning, gnawing sensation that persists after termination of the noxious stimuli (Giordano, 2005)

Aδ and C fibres enter the dorsal horn grey matter of the spinal cord, where they synapse via interneurons with other neurons in the pain pathway, providing an opportunity for amplification or inhibition of the pain impulse (Vanderah,2007). The amount of neurotransmitter released by the primary afferents is proportional to the strength of the original stimulus. These neurotransmitters include glutamate, substance P, calcitonin gene-related peptide (CGRP), galanin, vasoactive intestinal peptide (VIP), nitric oxide (NO), adenosine triphosphate (ATP), prostaglandin and neurotropins (Yam et al, 2018). Glutamate and substance P are directly excitatory, while most other substances only modify post-synaptic membrane excitability. On entering the dorsal horn, primary pain afferent neurons synapse with several other neuron types. Here, they relay messages by releasing neurotransmitters such as glutamate, substance P and CGRP, which result in activation of second-order neurons (Todd et al 2010). These second-order neurons cross the spinal

cord to the contralateral side and travel up the spinothalamic tract to the thalamus. The spinothalamocortical pathway (spinothalamic tract) conveys pain signals from the dorsal horn to the contralateral thalamus (Craig, 2003). In veterinary species, a distinction has been made between the true spinothalamic tract as a conveyer of first pain and thermal stimuli, and the spinoreticular tract as a conveyer of second (true) pain (King, 1987). Anatomically, the two tracts lie adjacent to one another and are often considered as a single unit and simply referred to as the spinothalamic tract. From the thalamus, divergent pathways project to the somatosensory cortex, which is involved in the sensory discriminative elements of pain. The somatosensory cortex is also involved in the recognition and learning memory of painful events (Woolf, 2004). Other functional units such as the insular cortex and the cingulate gyrus are involved in the autonomic and behavioural responses to pain respectively (Schnitzler and Ploner, 2000)

The sensory nervous system is adaptable and initially adjusts to minimize or prevent tissue damage, and later through mechanisms that aid in the healing and repair of the injured body part. Chemical substances such as prostaglandins, leukotrienes, bradykinin, nerve growth factors and histamine are produced when there is tissue damage and inflammation (Abdulkhaleq et al, 2018. These inflammatory mediators activate and sensitize nociceptors, which results in a lower firing threshold. This process is known as peripheral sensitization, which can lead to hyperalgesia (exaggerated pain response) and allodynia (pain response to non-painful stimuli). Severe or prolonged pain can result in central sensitization. This is often initiated by peripheral sensitization and is characterized by hyperalgesia, allodynia, and secondary hypersensitivity (pain outside the injured area) (Woolf, 2004).

When a noxious stimulus is strong enough to cause significant tissue damage, it can lead to persistent sensory disturbances. These may include ongoing pain, an exaggerated and prolonged response to painful stimuli (hyperalgesia), and pain even after exposure to normally non-painful stimuli (allodynia). These clinical symptoms are caused by changes in the nervous system, both in the peripheral and central regions (Lamont, 2008).

Descending pathways from the brain (amygdala, hypothalamus) and relayed via the brainstem and medulla to the spinal cord release inhibitory neurotransmitters like 5-hydroxytryptamine, norepinephrine, and endogenous opioids. This provides inhibitory control of nociceptive input. Activation of central alpha-2 receptors leads to the release of inhibitory neurotransmitters, gamma amino-butyric acid (GABA) and lysine (Heinricher et al, 2009).

Peripheral sensitization, central sensitization, and disinhibition (increased nociceptive stimulation due to amplification or failure to suppress ascending neuronal signals) are part of a continuum of the pain process. Pain causes stress, which for most horses is short-lived because of the short duration of exposure to the stressor (e.g. surgery and anaesthesia). However, osteoarthritis and laminitis can produce pain that lasts for months or years, and can lead to suffering (Taylor, 1989; Haussler et al, 2007; Jones et al, 2007). Suffering occurs when horses are forced to endure untreated or chronic painful conditions, which is maladaptive. It initiates neural and endocrine responses that negatively affect homeostatic functions critical to the animal's well-being. Maladaptive pain is uncoupled from the noxious stimulus or healing, is spontaneous or recurrent, and results from the abnormal operation of the nervous system. It is pain as a disease (Carstens et al, 2000).

Pain therapy

Non-steroidal anti-inflammatory drugs (NSAIDs)

Pain therapy is essential in equine practice, and non-steroidal anti-inflammatory drugs (NSAIDs) such as flunixin and phenylbutazone are commonly used for pain management in horses (Duz et al, 2019, Citarella et al, 2023). NSAIDs work by inhibiting the production of prostaglandin (as shown in figure 1) which is a vital mediator in the inflammatory pathway of pain. The inhibition of prostaglandin production contributes to the analgesic effects of these drugs. Prostaglandins act as pro-inflammatory mediators that can lead to inflammation. They also increase the excitability of the peripheral somatosensory system which can exacerbate the pain (Jang et al, 2020).

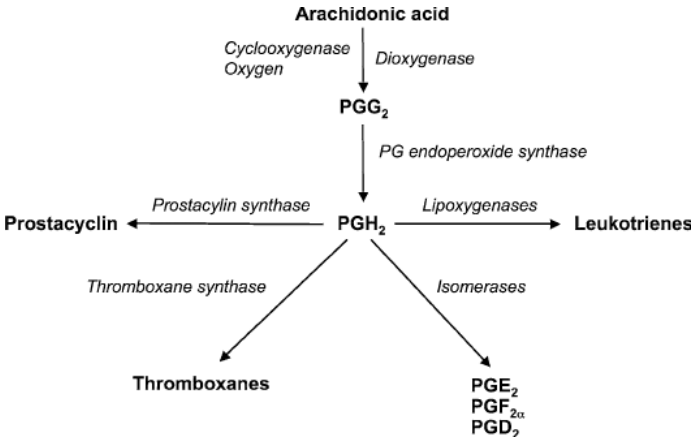

Figure 1: Simplified diagram showing the Arachidonic metabolism pathways (Weinberg et al, 2007)

Mechanism of action

NSAIDs work through their inhibition of cyclooxygenase (COX) enzymes (Vane et al, 1995). Two COX enzymes, COX-1 and COX-2, are responsible for the first steps in prostaglandin synthesis. In general, COX-1 is considered to have more of a 'housekeeping' role. It is constitutively expressed in most tissues and provides prostanoids required for homeostasis, such as vascular tone, in many organ systems. COX-2 is constitutively expressed in some tissues, such as the kidney, vas deferens and the brain. However, in most tissues, COX-2 is upregulated in inflammatory conditions. In the periphery, COX-2 expression is induced in cells involved in inflammation (macrophages, monocytes, synoviocytes) and is primarily responsible for the synthesis of prostaglandins in acute and chronic inflammatory states (Ziegler et al, 2017). However, human studies suggest that both isoforms, not only COX-1, are present in many normal tissues, and that both isoforms, not only COX-2, are upregulated in various pathological conditions. Although specific data for this in the horse is lacking, the concept of 'constitutive' and 'inducible' COX isoforms may need to be revised (Zidar et al, 2009).

There are two types of NSAIDs, selective and non-selective. Non-selective NSAIDs inhibit both COX-1 and COX-2 enzymes significantly, whereas selective NSAIDs will preferentially inhibit COX-2, which is expressed at sites of inflammation (Fadel et al, 2023). This theoretically allows more targeted treatment whilst reducing the risk of adverse effects by allowing maintenance of COX-1 expression. However, as discussed above, the distinction between constitutive and inducible COX isoforms is far more complicated than this, and there is undoubtedly a greater degree of overlap than previously thought.

Commonly used NSAIDs in equine practice

This review will focus mainly on the evidence relating to the use of NSAIDs in controlling musculoskeletal disease.

Phenylbutazone

Phenylbutazone is a non-selective COX inhibitor and is the most widely used NSAID in equine clinical practice (Duz et al. 2019). It is available as an IV injection, oral paste and granule. It is authorized for the treatment of acute and chronic musculoskeletal disease including laminitis in a number of countries, but can also be used for the control of soft tissue inflammation and as an antipyretic outside of those marketing authorizations. In the European Union and its trading partners such as the UK, it cannot be used in animals that may subsequently enter the human food chain since no safe limit for human consumption (maximum residue limit) has been determined (Lees et al, 2013). Phenylbutazone has been shown to be effective in reducing pain in placebo-controlled studies of arthroscopy (Raekallio et al, 1997) and in the heart bar shoe model of lameness (Foreman et al, 2008). Suxibuzone is the prodrug of phenylbutazone and is available as an oral medication. It has identical clinical effects to phenylbutazone but with possibly an improved safety profile and, therefore, can be used as a direct replacement for phenylbutazone in chronic orthopaedic pain (Sebate et al, 2009).

Flunixin meglumine

Flunixin is a non-selective COX inhibitor, which is widely used for the treatment of pain in horses. It is available as an IV injection or as an oral paste. In horses, it is authorized to alleviate inflammation and pain related to musculoskeletal disorders and colic. However, there is no significant evidence that suggests one medication is better than the other for pain relief, whether phenylbutazone or flunixin. (Bowen et al, 2020). There are several studies investigating the efficacy of flunixin in orthopoedic pain in horses (Foreman et al, 2012; Erkert et al, 2005; Foreman et al, 2010) that showed that flunixin meglumine provided comparable analgesia to phenylbutazone in musculoskeletal pain relating to joint disease or mechanical pain. However, two of these studies used experimental models of pain (Foreman et al 2012 & 2010), while only one investigated clinical lameness in naturally occurring palmer heel pain (Erkert et al, 2005). Few studies compare flunixin to phenylbutazone in the management of pain associated with abdominal disease. A clinical study comparing flunixin to metamizole favoured flunixin for the relief of signs associated with colic (Cristina et al, 2012), but lacked sufficient detail about presenting disease to draw conclusions regarding disease management. A second study comparing flunixin to sedative and opiate agents in the control of abdominal pain, suggested that flunixin provided less analgesia

compared to two doses of detomidine (Jochle et al, 1989). However, this study used poor outcome measures of pain such that it is difficult to make recommendations in clinical cases based on this study. Studies comparing flunixin to other NSAIDs are discussed below.

Meloxicam

Meloxicam is a type of COX-2 inhibitor that preferentially targets the inducible form of cyclo-oxygenase, which makes it more effective in inhibiting its activity compared to the constitutive form. (Berretta et al, 2005). Although this may suggest enhanced safety, there are no large-scale, long-term studies investigating the relative safety of any NSAID in the horse. Meloxicam is available in oral forms for the treatment of acute and chronic musculoskeletal disease in the horse and an injectable form that is also available for the treatment of colic (Bowen et al, 2020). However, studies suggest that the analgesic potency of meloxicam is not equivalent to flunixin in horses with colic or to phenylbutazone in horses with mechanical musculoskeletal hoof pain (Naylor et al, 2014; Toutain et al, 2004, UCVM class of 2016 et al,2017). There is weak evidence to suggest meloxicam is at least equivalent to phenylbutazone for control of inflammatory joint pain and therefore may be useful in the management of chronic osteoarthritis and acute inflammatory arthritis (Toutain et al, 2004; Banse and Cribb, 2017; Olsen et al, 2016; Walliser et al, 2015)

Firocoxib

Firocoxib is a type of NSAID that selectively targets COX-2. It's recommended for treating osteoarthritis in horses and has been authorized for use in the EU through oral and injectable routes. There is not much information available regarding the usage of firocoxib in horses. According to studies comparing firocoxib to phenylbutazone for treating osteoarthritis and lameness, it was found that firocoxib was less effective than phenylbutazone in managing mechanical lameness (Foreman 2014), but there was no significant difference between the two when it came to naturally occurring joint disease (Doucet et al, 2008). However, it was found that firocoxib and flunixin had similar pain-relieving effects in horses with colic, according to Zieglar's research in 2019.

Other NSAIDs, such as carprofen, ketoprofen, etodolac and ketorolac have been studied in horses, but these are not commonly used in equine practice in the UK. Studies investigating the use of these drugs in equine orthopaedic disease are comprehensively reviewed elsewhere. (Jacobs et al, 2022)

Adverse effects of NSAIDs

When using NSAIDs, the most frequently occurring adverse events include enteropathy, nephropathy, and bone marrow suppression (Flood et al, 2022; Bowen et al, 2020). Out of all NSAIDs, phenylbutazone appears to be the one most often linked to adverse events (Jacobs et al, 2022). It's important to note, however, that this information should be considered in the context that phenylbutazone is also the most studied of the NSAIDs available to horses (Davis et al, 2017, Monreal et al, 2004). These adverse events include renal necrosis, gastric ulceration and right dorsal colitis (Davis 2017). Meloxicam and firocoxib as preferential inhibitors of COX-2 in the

horse are often considered to have a superior safety profile (Davis et al., 2017. However, clinical signs of right dorsal colitis have been reported with firocoxib (Davis 2017) and gastric ulcerative effects of meloxicam were found to be no different from phenylbutazone at standard doses (Noble et al., 2012). There is limited evidence indicating that meloxicam may have an improved safety profile in foals. This is due to its faster clearance and shorter half-life compared to adult horses. In normal healthy foals, more frequent dosing intervals have been used without any apparent adverse events (Raidal et al, 2013). Given the potential for adverse effects with use of all the non-steroidal anti-inflammatory drugs, there is clearly a need for additional, safe analgesics in horses.

Opioids

In equine clinical practice, opioids are frequently used alongside alpha-2 adrenoreceptor agonists to improve sedation in standing horses. There are multiple clinical studies investigating the role of various opioids in combination with the different alpha-2 agonists, including detomidine (Clarke et al, 1988, Taylor et al, 1988, Love et al, 2011). These studies show that when combined, these drugs produce both sedation and analgesia. However, many of these studies don't specifically mention the pain-relieving effects of opiates alone or when used with other analgesic drugs.

Numerous studies have explored the impact of opiate pain relief on reducing the minimum inhibitory concentrations (MAC) of inhalational analgesic agents in horses (Doherty et al, 1997, Gozalo-Marcila et al, 2014, Knych et al, 2009, Matthews et al, 1990). Whilst such studies may demonstrate an analgesic effect of these agents, it is worth noting that these findings may not be directly applicable to conscious horses, as pain perception cannot be observed during anesthesia. Opioids are widely accepted as effective analgesics in horses. These can be categorised as either pure u-agonists (such as morphine, methadone and fentanyl) partial u-agonists (like buprenorphine) and k-agonist/u-antagonists (such as butorphanol). All of these types have been used in horses. They have a range of possible therapeutic benefits that may coincide with the risk of side effects and toxicity. They can be used in conjunction with sedative or tranquillizing drugs, where they have a beneficial role of modifying behaviour. However, when given intravenously alone as a sole agent they may cause excitement (Muir et al., 1979b, Combie et al., 1981, Robertson and Muir, 1983, Hellyer et al., 2003). The ability of these agents to cause ataxia and hyperexcitability has been well documented (Kamerling 1993). Although their role as analgesics is less clear, studies suggest that when used at low doses, these agents may have a calming effect on horses experiencing pain and may improve their recovery from anaesthesia (Taylor, 1986, Clark et al., 2008). However, little is known about the horse's potential to develop tolerance or delayed hyperalgesia to opioid agonists. It is believed that the pain-relieving properties of opioids are influenced by the mu (u) and kappa (k) opioid receptors (Kamerling 1993). Drugs like butorphanol (agonist-antagonist) and buprenorphine (partial agonist) produce similar effects to fentanyl, morphine, and methadone, but less profound analgesia.

Butorphanol

Butorphanol is a partial opioid agonist that primarily acts at kappa receptors. While its primary use is to enhance the sedative effects of alpha-2 adrenoceptor agonists, it is also authorised as a short-term analgesic agent for horses. There are several clinical studies evaluating the analgesic properties of butorphanol in the horse, all relating to visceral pain (Love et al, 2009; Sanz et al,

2009, Jochle et al 1989; Bettschart-Wolfensberger et al, 2011; Rigotti et al, 2014; Taylor et al, 2016). However, the only study comparing butorphanol to a no-treatment control failed to demonstrate an analgesic effect of butorphanol in the management of visceral pain associated with castration (Love et al, 2009). Butorphanol was inferior to buprenorphine in the management of surgical pain in another study (Taylor et al, 2016) and equivalent to phenylbutazone when given every 4 h in horses undergoing castration in another study (Sanz et al, 2009). In the management of naturally occurring colic, butorphanol was considered to provide inadequate analgesia (Jochle et al, 1989). There are several experimental studies that assess the analgesic effects of butorphanol as a visceral analgesic using balloon inflation in the caecum or duodenum (Boatwright et al, 1996; Kalpravidh et al, 1984; Muir et al 1985; Sanchez et al 2008). While these findings suggest a possible pain-relieving effect, it is important to note that this may be a reflection on changes in gastrointestinal motility.

There have been no clinical studies on the use of butorphanol for somatic pain in horses. According to a study by Carregaro et al in 2014, when tested against morphine and methadone in models of synovitis, butorphanol was found to be less effective and had minimal impact on lameness grade. In fact, out of six horses, two required rescue analgesia. A recent study examined the impact of sedation using xylazine and butorphanol on lameness grade during lameness assessments. However, the study was not intended to document any direct analgesic effect of butorphanol, and no such effect was found (Beck et al, 2019).

Two studies (Queiroz et al, 2013; Spadavecchia et al, 2007) examined the anti-nociceptive effects of butorphanol in horses. The results of these studies are conflicting. One study displayed pain-relieving benefits while the other did not show any such advantages. Many experts believe that butorphanol has limited effectiveness as an analgesic for both visceral and somatic pain, as there is no sufficient evidence to support its efficacy at clinical doses. Therefore, if opiate analgesics are required, alternative and more potent options should be considered (Bowen et al, 2020). However, as discussed below, disappointing results have also been observed in studies using very potent opioids in horses.

Buprenorphine and pure mu-agonists

Buprenorphine is another partial opiate agonist with a greater effect at mu-opiate receptors than butorphanol. Its marketing authorisation includes post-operative analgesia and potentiation of sedative agents in the horse. Currently, there are no clinical studies on the use of buprenorphine for managing clinical pain, except during the perioperative period (Emanual et al, 2022). However, a single case report documented the sublingual administration of buprenorphine to a horse experiencing severe cervical pain for a 5-day period (Walker 2007). Two studies have examined the bioavailability of buprenorphine when administered sublingually in horses (Messenger et al, 2011, Grubb et al, 2019). One study showed good absorption when measuring plasma concentrations from the jugular vein. However, the study also found significantly higher concentrations of buprenorphine in blood samples from the jugular vein compared to those from the lateral thoracic vein. Pharmacokinetics were not determined from these samples (Messenger et al., 2011). In a study involving foals, it was found that only 25% of the drug was absorbed into the body when measured through samples taken from the cephalic vein. Additionally, when a sublingual dose of 0.02 mg/kg was tested, it failed to reach the necessary plasma concentrations

for pain relief in experimental models (Grubb et al., 2019). The dose used in the case report described above was one-third of this dose (0.006 mg/kg). Clinical evidence of efficacy of sublingual administration is not known currently and is not recommended.

Two experimental studies have been conducted to assess the response of horses to adverse stimuli, and both have documented an analgesic effect of buprenorphine (Carregarro et al, 2007; Love et al 2015). Two studies have compared the analgesic effects of buprenorphine and butorphanol in horses undergoing elective surgery. Another study evaluated the use of buprenorphine in horses undergoing routine castration (Rigotti et al 2014; Taylor et al, 2016; Love et al., 2013). These studies indicate that buprenorphine is an effective analgesic agent. However, there are no studies comparing buprenorphine to pure mu-agonists in a clinical or experimental setting. According to these studies, systemic buprenorphine may be a useful treatment option for pain management in horses. However, its effectiveness compared to other medications is yet to be determined.

There are no clinical studies evaluating the analgesic effects of any of the pure mu-agonists. Several experimental studies have examined the impact of pethidine, methadone, and morphine on induced visceral and somatic pain models in horses (Foreman et al, 2013; Muir et al, 1985; Figueiredo et al., 2012; Lopes et al., 2016; Ruiz et al., 2015). Pethidine (meperidine) was previously authorised in the UK for the management of spasmodic colic. There are two studies investigating the analgesic effects of pethidine, both in experimental models of pain (Foreman et al, 2013; Muir et al, 1985). Following intramuscular injection, pethidine provided short-lived (3–4 h) and inconsistent analgesia in a heart bar shoe model of lameness (Foreman et al., 2013). According to a study by Muir et al in 1985, intravenous administration of pethidine for visceral pain resulted in a very brief period of relief, lasting only 30 minutes.

Methadone is authorized in the UK for the control of pain in dogs and cats. It has been shown to provide superior analgesia to buprenorphine in dogs undergoing orthopoedic surgery (Hunt et al., 2013). It has effects at NMDA as well as mu-receptors and therefore may have potential advantages over other opiate analgesic agents. Morphine is a pure mu-agonist that has no veterinary marketing authorisation. According to thermal and electrical threshold models of pain, normal clinical doses of morphine and methadone did not have any measurable analgesic effects. However, higher doses of methadone (0.5 mg/kg) have been found to affect thermal thresholds Figueiredo et al., 2012; Lopes et al., 2016) but these doses were associated with severe excitement reactions when given prior to the onset of painful stimuli in conscious horses (de Oliveira et al.,2014). In a carpal synovitis model of pain, both morphine and methadone were effective in reducing lameness severity, although there was no difference between the two drugs (Ruiz et al., 2015). It is possible that the limited effects observed in previous studies regarding the effectiveness of pure mu-agonists in treating pain in horses could be due to the experimental techniques used. Therefore, it is important for clinical studies to be conducted to assist clinicians in selecting the appropriate pure mu-agonist for horses that do not respond to NSAID treatment (Bowen et al, 2020)

Adverse Effects of Opioids

Opioid analgesic agents have been associated with adverse events, predominantly a reduction in gastrointestinal motility and excitability, leading to spontaneous locomotor activity. There are several experimental studies investigating both of these events in horses, predominantly identifying adverse events when these agents are administered to normal horses, usually before the onset of pain induction (Clutton 2010, Combie et al 1979). Butorphanol, buprenorphine, morphine, methadone and pethidine have all been shown to reduce gastrointestinal motility in horses in a variety of experimental settings using different techniques to document motility (Carregaro et al., 2014; Figueiredo et al., 2012; Davies et al., 1983; Sojka et al, 1988; Merritt e al, 1989; Merritt et al., 1998; Sutton et al., 2002; Boscan et al., 2006; Levionnois et al., 2018). There are five studies evaluating the clinical effect of opiates on the development of post-operative ileus and post-operative colic in the horse (PAC) (Bailey et al., 2016; Senior et al, 2004; Anderson et al., 2006; Jago et al., 2015; Mircica et al, 2003; Senior et al., 2007). One study suggested that butorphanol reduced the occurrence of PAC (Bailey et al., 2016), and three did not identify significant associations between the use of morphine and PAC (Bailey et al., 2016, Anderson et al., 2006, Jago et al., 2015). The use of morphine was associated with a fourfold increased risk for the development of PAC in one study in horses undergoing orthopaedic surgery (Senior et al, 2004), although this study does not differentiate whether the presence of pain requiring opiate analgesia or the effect of that medication was responsible for the development of colic.

Excitement reactions and spontaneous locomotor activity reactions have been described in horses in a variety of experimental and clinical studies of the use of different opiates (Rigotti et al.2014; Queiroz Neto et al., 2013; Carregaro et al, 2007; Combie et al., 1979; Nolan et al 1994; de Oliveira et al, 2014; Sellon et al, 2001; Szoke et al 1998). Several studies, including one clinical study and multiple experimental studies in the horse, have shown that there is a higher occurrence of spontaneous locomotor activity when given prior to surgery or before experiencing painful stimuli (Carregaro et al., 2014; Ruiz et al., 2015). Although comparative locomotor effects have been assessed in experimental studies (Combie et al., 1979), they have not been compared in a clinical setting. It has been suggested that pre-emptive administration of potent opiates is more likely to result in spontaneous motor activity and that this is more likely to occur when opiates are given in horses not demonstrating signs of severe pain. These effects can be reduced by the administration of opiates to horses that have been sedated with alpha-2 adrenoceptor agonists. Administering opiate analgesia locally, such as through intra-articular or epidural methods, can potentially lower the risks of negative effects that come with opiate use.

NMDA antagonists

Ketamine is a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist and is traditionally used as a dissociative anaesthetic, but its use as an analgesic is gaining recognition. Human studies have indicated that administering low doses of ketamine could potentially provide beneficial pain relief. Additionally, studies conducted on rats have suggested that combining ketamine with opioids and alpha-2 agonists could enhance their pain-relieving properties. (Himmelseher et al, 2005; Grant et al, 1981). The analgesic effects of ketamine are thought to be through a combination of its actions as a NMDA receptor antagonist, an opioid receptor agonist

and an activator of the descending inhibitory pain pathways (Muir, 2010). However, ketamine appeared to produce minimal analgesic effects when administered alone as the sole analgesic drug post-operatively in humans (DeKock et al, 2007). This may be related to the role of the NMDA receptor in intense or sustained pain.

Studies using animal and human pain models have shown that NMDA receptor antagonists may have potential as pain relievers (Hewitt, 2000). However, these studies have mainly shown their ability to reduce hyperexcitability in pain-sensing neurons and provide secondary pain relief (which depends on NMDA receptors) (Latremoliere et al, 2009). They have not yet been shown to influence the body's initial response to painful stimuli (Muir, 2010). Despite this, the potential benefits of using ketamine or other NMDA antagonists to treat painful conditions in horses should not be dismissed. This is because it is widely recognized that CNS sensitization, which can result from extensive surgical procedures or degenerative diseases, is a crucial factor in the development of chronic pain (Latremoliere et al, 2009). More research is needed to objectively assess the pain-relieving properties of ketamine for horses with naturally occurring diseases.

Magnesium ions act as both NMDA receptor antagonist and calcium channel blockers. These properties are believed to contribute to the analgesic effects of magnesium by altering the perception and duration of pain (Tramer et al, 1996). It has been shown to reduce postoperative pain, decrease opioid requirements and delay tolerance development following various surgical procedures in people (Albrecht et al, 2014, Guo et al., 2015). It has also been demonstrated to enhance the effects of general and local anaesthetics in preclinical and clinical trials (Thomson et al, 1998., Soave et al, 2000., Nechifor et al 2011., Morrison et al, 2013). It has not been evaluated as an analgesic agent in horses. Synergistic interactions between magnesium and ketamine have been demonstrated (Liu et al, 2001). Combining ketamine and magnesium could be more effective in treating acute pain than ketamine alone. This is because they block NMDA receptor activation through different mechanisms of action. Additionally, using both drugs could allow for more significant clinical benefits without exceeding safe dosage levels.

Drugs that cause NMDA antagonism may be more effective in preventing hyperalgesia and central sensitization caused by severe or chronic tissue damage. However, they may not be as effective in managing pain resulting from mild to moderate acute surgical or accidental events. However, numerous studies in rats have demonstrated that when given in sub-analgesic doses, ketamine can enhance the analgesic effects of opioids and alpha-2 agonists, and vice versa (Roh et al, 2010; Shulte et al, 2004; Zang et al, 2009; Lauline et al, 2002). It is possible that Ketamine could be useful in preventing or reducing the occurrence of tolerance or negative effects that may arise from opioid usage (Lauline et al, 2002; Inturrisi 1994).

Paracetamol

Paracetamol is an analgesic and an anti-pyretic, but despite its widespread use in human medicine, the mechanism of action of paracetamol is still relatively unclear. There is evidence to suggest that it influences various sites within the central nervous system. This includes inhibiting the synthesis of prostaglandins and interacting with both serotonergic and cannabinoid pathways (Oscier & Milner, 2009). However, many of these studies have been carried out in laboratory animals, and it is still unclear whether species differences exist in receptor site activity of paracetamol. Even though paracetamol can inhibit both isoforms of COX in the central nervous system, its anti-

inflammatory activity is weaker than that of NSAIDs. This is because paracetamol has significantly reduced peripheral cyclo-oxygenase inhibition when compared to traditional NSAIDs (Oscier & Milner 2009). The benefits of paracetamol stem from its limited impact on the gastrointestinal system and a minor dose-dependent change in platelet function Graham et al, 2005., Munsterhjelm et al 2005).

Human studies suggest that paracetamol reinforces descending serotonergic inhibitory pain pathways and studies have found that co-administration of a 5-HT_3 antagonist completely blocks the analgesic effect of paracetamol (Pickering et al 2006 & 2008). Furthermore, depletion of brain serotonin has been shown to reduce the analgesic effect of paracetamol in laboratory animal studies (Graham & Scott, 2005). Research also suggests that paracetamol may also involve indirect activation of cannabinoid CB1 receptors in rats (Bertolini et al, 2006), where a metabolite of paracetamol, N-arachidonylphenolamine (AM404) inhibits cellular uptake of anandamide, an endocannabinoid, and is an agonist at the vanilloid receptor TRPV1, which is believed to play a central role in nociception. The analgesic effect of paracetamol has been shown to be completely prevented by the addition of a CB1 receptor antagonist in rats (Ottani et al, 2006). A recent study showed a notable distribution of cannabinoid and cannabinoid-related receptors (e.g., 5-HT receptors) in the dorsal root ganglion of the horse. The mechanism of action has not been investigated in horses.

Paracetamol is well absorbed from the proximal small intestine in monogastric species and is not subject to significant first-pass metabolism in the liver (Ward et al, 1999). It has been previously used in horses as a marker of gastric emptying, due to its poor gastric and rapid small intestinal absorption (Lohmann et al 2002). Pharmacokinetic studies in horses demonstrated that paracetamol was rapidly absorbed, with a high bioavailability of 91%, lower clearance and longer elimination half-life (2.4 h) than other species; potentially due to high levels of plasma protein binding (Neirinckx et al, 2010). Metabolism is mostly by hepatic glucuronidation but remaining reactive metabolites are conjugated with glutathione. Depletion of the liver and red blood cell stores of glutathione can result in methaemoglobinaemia, haemolysis and hepatocellular damage or failure (Court 2001)

Recent case reports have demonstrated the efficacy of paracetamol as an adjunctive treatment for laminitis in one pony and as an effective analgesic agent in combination with NSAIDs in a model of inducible foot pain (West et al., 2011; Foreman et al., 2016). These clinical reports use paracetamol at a dose of 20mg/kg, which is twice the dose that has been reported in previous pharmacological studies, and in these studies the administration of paracetamol was only for a brief period of time, or for a single dose (West et al. 2011, Foreman et al. 2016, Neurinckx et al 2010, Engelking et al, 1987). Paracetamol has been shown to be safe following multiple dosing over a 14-day period (Mercer et al, 2019). It has an elimination half-life of four hours following multiple dosing (Mercer et al, 2020) and, therefore, more frequent dosing may be both safe and more appropriate. However, its efficacy in naturally occurring clinical diseases has not been fully investigated. Further studies are required to determine optimal dosing regimens.

Adverse effects of paracetamol

Few adverse effects have been reported following paracetamol use in horses. One study reports the occurrence of a transient ventricular arrhythmia (ventricular premature complexes) in one horse following an intravenous infusion of a combination of tramadol and paracetamol (Tavanaeimanesh et al, 2018). This resolved spontaneously without treatment following the infusion. The study did not report any cardiac adverse effects following infusion of either drug alone, suggesting that if this was a true adverse drug effect, then it may be a result of a synergistic effect or interaction between the two drugs. In addition, cardiac effects have been reported following higher doses of tramadol in horses but has not been reported following intravenous paracetamol infusion.

The study by Mercer et al (2019) identified evidence of mild hepatic inflammation on liver biopsies in horses that had received paracetamol. However, it is unclear whether these changes were due to paracetamol administration. It is difficult to interpret the mild histological changes observed on liver biopsy samples in the study, as these were only obtained at a single time point after treatment with paracetamol. Mild liver inflammation is a common finding on liver biopsy in horses and represents a non-specific change (Durham 2019). It is also not clear whether this was already present prior to drug administration. In addition, in all but one of these horses, the changes were reversible. Further studies are required to determine the risks of detrimental effects of paracetamol administration on liver function.

Conclusions

There is significant anecdotal evidence of the value of the use of paracetamol alongside traditional NSAIDs and other analgesic agents for the management of acute and chronic pain following on from its value in other species (man and dogs) and growing clinical experience with its use in equine medicine. Despite this, there are few studies evaluating its effects in experimental scenarios and its use in clinical cases or regarding medium to long-term side effects. Species differences do exist for other analgesic drugs, such as opioids, and, therefore, there is a need for more species-specific research in horses to determine the benefits of paracetamol for pain management in equine clinical practice.

Traditional analgesic agents target local pain pathways, and their use can be associated with life-threatening gastrointestinal complications, especially when higher doses are required in horses with severe pain. Supplemental analgesics, including paracetamol, have the potential to enhance the degree of analgesia while reducing the occurrence of adverse events by targeting under-exploited therapeutic targets. Although preliminary work describing the pharmacokinetics of oral and intravenous paracetamol has been described, to date there have been no publications describing its use under general anaesthesia or its bioavailability when given by alternate routes of administration.

The overall aims of this research were to document the current clinical use of paracetamol in equine practice and to investigate the pharmacokinetics of novel routes of administration to determine their feasibility for use in the hospitalised horse.

Data on clinical use will be collected through the use of a survey to determine how practitioners rank paracetamol compared to other commonly used analgesics for the management of pain associated with common orthopoedic conditions in horses, with further questions focused on the practical use of this drug. Pharmacokinetic studies will focus on intravenous administration of paracetamol under general anaesthesia and rectal administration in post-operative colic patients. The objectives of these studies will be to determine whether these more novel routes of administration have favourable pharmacokinetics for the provision of analgesia in horses in the hospital setting and to determine whether these routes of administration are both safe and practical.

Chapter 3: Current clinical use of paracetamol in horses

Contributions: Adam Redpath was the main researcher with primary responsibility for project conceptualisation, study design and methodology. Hannah Stanley (University of Nottingham, Year 3 undergraduate veterinary student) contributed to the development of the questionnaire and collection of this data as part of an undergraduate project. Dr Mark Bowen contributed towards statistical analysis of this data.

Introduction

Analgesic use is an important part of equine practice and paracetamol has potential value as an adjunctive analgesic in equine pain management, where pain is not adequately controlled with our traditional analgesic agents. When used in combination with non-steroidal anti-inflammatory drugs, it may allow for reduced dose requirements and, therefore, decreases the risk of adverse effects of these drugs (Hopster and Eps, 2019). Paracetamol is not currently authorised for use in the horse and, although supplemental analgesia with paracetamol appears to be safe, there is limited evidence on optimum dosing regimens and clinical indications.

The aim of this study was to survey equine practitioners' opinions on the use of paracetamol as an analgesic agent.

The objectives of this study were:

To recruit a sample of equine veterinary practitioners to participate in an online survey of the use paracetamol in the horse

To determine how equine veterinary practitioners rank paracetamol compared to other analgesic drugs for the management of pain associated with common orthopaedic conditions

To determine what dose of paracetamol equine veterinary practitioners are prescribing and the maximum quantity prescribed.

Materials and methods

Study Design

A cross-sectional study was carried out using an online survey created using Microsoft Forms. The survey was created to include 23 questions across four main sections: participant demographic information, the ranking of paracetamol against other analgesic agents (firocoxib, flunixin, gabapentin, meloxicam and phenylbutazone) in equine practice, the dose rates used and the practical use of paracetamol. Demographic information (including years qualified and level of specialist qualification) was requested to determine whether the level of experience, the length of time paracetamol has been available, exposure to recent relevant literature or issues around

licensing could have had an impact on paracetamol use. The question types used in the survey included a combination of multiple-choice, ranking and free-text answers. No questions were made compulsory to avoid deterring respondents.

A pilot survey was distributed to five undergraduate veterinary students at the University of Nottingham and 10 equine clinicians from the School of Veterinary Medicine and Science. Following piloting the final survey was distributed on the 13th of October 2020 across several social media groups including the Equine Veterinary Group (UK) and the Equine Clinical Network (International), both email discussion groups, and Facebook groups for Veterinary Voices Equine UK and The Equine Veterinary Group. The aim was for the survey to be redistributed from these groups to reach as many equine practitioners as possible. Follow-up reminder posts were sent out two days before the survey closed to maximise participation. The survey was closed after one week. The final survey can be found in the appendix at the end of the thesis.

Data analysis

Quantitative variables

All responses were exported to Microsoft Excel and respondents were grouped as either specialists or generalists, based on whether they held any post-graduate qualifications. Respondents were also grouped by sex and whether respondents worked in primarily ambulatory or hospital-based practice. Clinical experience (years since graduation) was grouped into categories of 10 year intervals. Group 1 had been graduated for less than 10 years, group 2 for less than 20 years and group 3 for 20 years or more. This grouping has been used in other studies/reports describing veterinary demographics of veterinary surgeons (Golden et al, 2018; Ryan et al, 2022; https://www.rcvs.org.uk/news-and-views/news/rcvs-news-surveys-of-the-professions-2024-repors-published/). The ranked position of paracetamol compared to firocoxib, flunixin, gabapentin, meloxicam and phenylbutazone was determined manually for each of the clinical scenarios. The relative position of the other analgesic agents was not considered in the final analysis. Those who never used paracetamol had other responses omitted from the calculations. The dose of paracetamol used was calculated from the number of tablets respondents said they used for a 500kg horse, where respondents did not answer the specific dose question in mg/kg. The maximum number of tablets clinicians would prescribe was calculated from how many weeks supply they answered they would give, using their dose and dosing frequency answers to standardise the results into numerical answers only.

Statistical Analysis

Descriptive statistics of multiple-choice and free-text questions were calculated. Normality of continuous data (years graduated) was determined by visualization of data followed by a D'Agostino-Pearson test. and discontinuous data (rankings, frequency of use was calculated as median (± interquartile range). Further analysis was only done using non-parametric comparisons as appropriate for categorical data. The frequency of responses was calculated by group and the difference between rankings between specialist veterinary surgeons and generalists, male and female and ambulatory and non-ambulatory and by category of experience were compared using

Mann-Whitney U test and differences between frequency of responses by Chi-Squared or Fischer's Exact tests as appropriate. Statistical significance was accepted with a p-value of <0.05. Statistical analysis was carried out using an online statistical calculator (http://vassarstats.net).

Ethical approval

This study was granted ethical approval from the research and ethics committee of the School of Veterinary Medicine and Science of the University of Nottingham. Participants were asked to consent to the study and were given the opportunity to leave contact details if they wanted a summary of the project. However, data was processed anonymously having removed this data. The data was stored in accordance with General Data Protection Regulations (GDPR).

Results

A total of 116 equine veterinarians completed the survey and the average completion time was 6 minutes and 41 seconds. Respondents were graduated between 1-49 years. This data was found to be not normally distributed (D'Agostino-Pearson test statistic: 17.75) with a median response of 9.5 years (IQR 5-21 years). 50% of respondents were graduated between 5 and 21 years (Figure 1) There were 15 non-responders to the questions asking how many years it had been since they had graduated. There was a significantly higher proportion of female respondents with over double the number of female respondents (77) compared to male (38), with one non-responder to this question. There were almost equal numbers of specialist equine vets (57) compared to generalists (56), with 3 non-responders. Of the responders, 22 worked as non-ambulatory and 94 as ambulatory vets. There were 12/116 (10%) respondents who reported that they never use paracetamol.

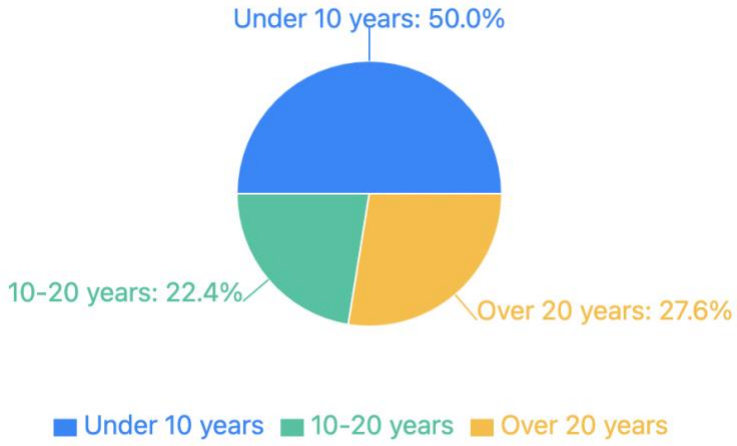

Figure 1: Distribution of Veterinarians by years since graduation (as of 2020). Half of all respondents (50%) have less than 10 years of experience since graduation.

How paracetamol ranks against other analgesics for different clinical scenarios

Phenylbutazone and flunixin were ranked top for all clinical scenarios. Paracetamol was ranked most effective for laminitis (median rank 3; range 2-4; n-83). In cases of cellulitis (median rank 4; range 3-5; n=87), bone spavin (median rank 4; range 4-5; n=99) and palmer heel pain (median rank 4; range 3-5; n=97) paracetamol was ranked similarly. Thoracolumbar pain was ranked as the lowest indication for paracetamol use (median rank 4.5; range 3.25-5; n=100). There was no significant difference in indication by sex of respondent, age group, post-graduate qualification or type of equine practice on how paracetamol was ranked in the different clinical scenarios.

Clinical use of paracetamol

There were 92 respondents who answered which dose of paracetamol they used in horses. The median dose was 20mg/kg, ranging from 10-30mg/kg. There were 97 respondents who answered how frequently they recommend administering paracetamol to horses. The most common answer was to administer twice daily (89.69%), followed by once daily and three times daily, which had equal numbers of respondents (5.15%). There were 104 respondents who answered which formulation of paracetamol they used in horses (Figure 2). Human tables were the most commonly used, followed by special paste formulation. Porcine and canine tablets were used rarely, with intravenous (IV) use only being reported by two respondents.

There were 108 respondents who answered how frequently they use paracetamol in clinical cases. Of these, 20 (18.5%) used paracetamol regularly (daily/at least weekly), 75 (69.4%) used paracetamol occasionally (less than weekly/rarely) and 12 (11.1%) never used paracetamol. There was no significant difference in clinical use by gender ($p=0.96$), degree of specialisation ($p=029$) or how many years graduated ($p=0.13$).

The most frequent reason cited for using paracetamol was where other analgesics had been ineffective, followed by the effectiveness and then the safety of paracetamol (Figure 3). Respondents were able to select multiple options. There were no significant differences found for why respondents chose to use paracetamol between ambulatory and non-ambulatory vets.

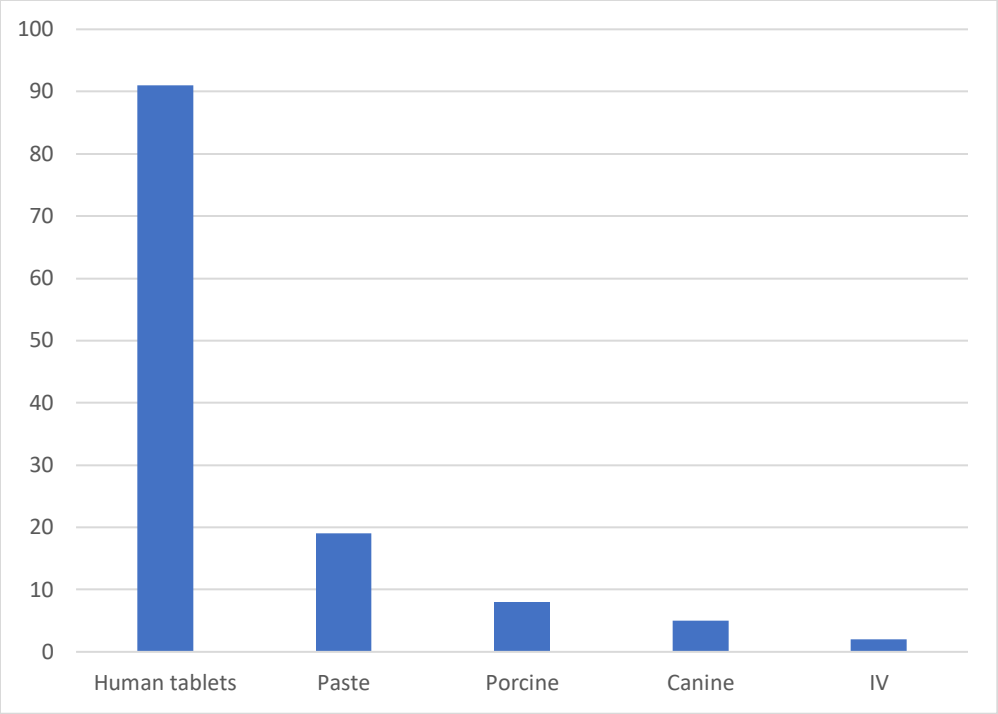

Figure 2: Formulations of paracetamol used by veterinary surgeons in horses (n=104). The x-axis shows the types of formulations with the Y-axis showing the percentage (%) of respondents using each formulation.

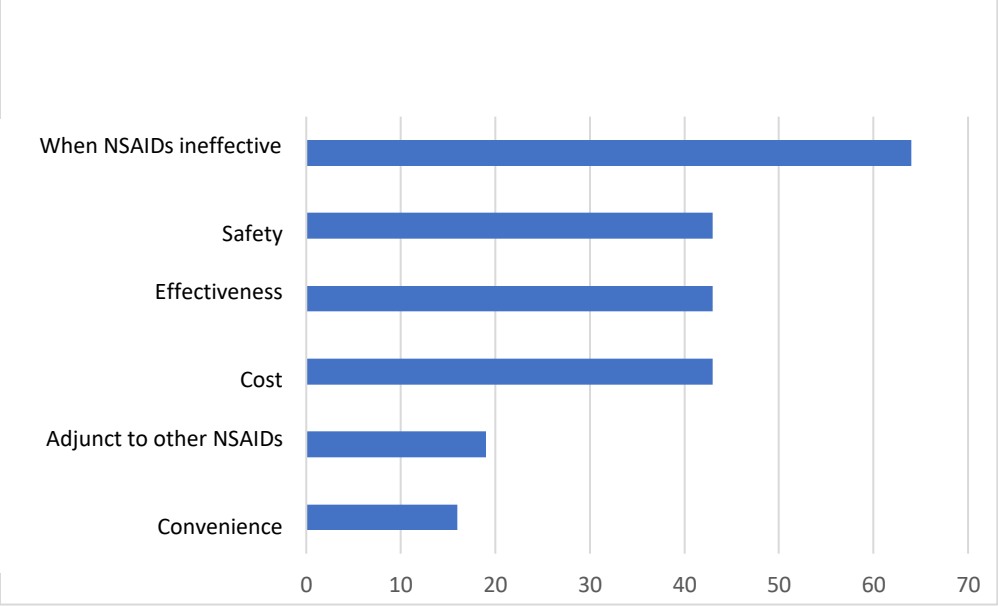

Figure 3: Reasons for paracetamol use (n=97). (Respondents could select multiple options). The Y-axis shows the reasons for paracetamol use. The x-axis shows the percentage of respondents who use paracetamol for this reason.

Discussion

Paracetamol has been available in the UK since the 1960s and is one of the most commonly used analgesics in human medicine (Sheen et al, 2002). Over the past decade, its use in horses has increased significantly and scientific evidence suggests it is an effective analgesic in equine medicine (Bowen et al, 2020). This study demonstrates that paracetamol is currently being used in equine veterinary practice for conditions such as laminitis, cellulitis, bone spavin, palmer heel pain and thoracolumbar pain. In human medicine, paracetamol is considered a first line drug for the management of chronic pain (Freo et al., 2021). In contrast, few equine veterinarians in this study advocated paracetamol as a first-line drug. Although respondents regarded paracetamol as most suitable for the management of pain associated with laminitis, they did not rank it as important as the more traditional analgesic agents, such as phenylbutazone. Despite significant anecdotal evidence of the value of the use of paracetamol alongside traditional NSAIDs and other analgesic agents for the management of acute and chronic pain in horses and growing clinical experience with its use in equine medicine, there are no published clinical comparative studies evaluating its efficacy against other analgesic agents in these conditions. Therefore, the lower ranking of paracetamol might represent uncertainty in its analgesic properties compared to more widely studied analgesic drugs in horses.

Similar responses were found for other clinical scenarios including bone spavin, palmer heel pain and thoracolumbar pain, where paracetamol ranked in a similar way compared to other analgesic agents. Again, this may reflect a lack of experience amongst clinicians in using paracetamol for these conditions together with the lack of supporting literature demonstrating a clinical effect of paracetamol in these specific disease processes. The high rankings of phenylbutazone and flunixin in all the clinical scenarios are supported by data demonstrating that these are the most frequently used analgesic medications in equine practice (Duz et al., 2018)

This study is limited by the relatively small number of responses and most of these responses were from practitioners within the UK. Responses from outside the UK were small, and the discussion of acetaminophen in the title may have encouraged more participation from those who use this term exclusively. The low response rate may mean the study lacked power to document the differences between groups of respondents. Re-posting of the questionnaire on social media sites multiple times so that it appears at the top of the feed may have increased the response rate by reminding potential respondents who may have forgotten. Similarly increasing the duration of data collection may have improved the response rate. Some respondents had no experience with the use of paracetamol, and while these were excluded from the analysis, it is useful in documenting the use of this drug in equine veterinary practice. Questioning around the use of paracetamol in different clinical scenarios was limited to only a few clinical diseases and documenting of use was limited to ranking of paracetamol compared to other analgesics. This did not allow for recording of paracetamol use in a wider array of conditions or of use in conjunction with other drugs (e.g. combined with a NSAID). Respondents may have viewed traditional NSAIDs as more important, but may still commonly use paracetamol in combination for the management of conditions such as laminitis. A greater variety of question types around the clinical use of paracetamol may have allowed us to gain more information around paracetamol use in equine practice.

Further studies are required to further investigate the safety of paracetamol use in horses, particularly around its use in clinical cases. Its inclusion in the BEVA analgesia guidelines (Bowen et al, 2020) after this study may improve uptake amongst veterinary practitioners. A follow-up survey would be required to document the effect of these guidelines on analgesic use in equine clinical practice. Dose optimization studies are required to determine the optimum dose of paracetamol for an analgesic effect whilst minimizing the risk of adverse effects. Finally, the effectiveness of paracetamol in different clinical scenarios needs to be studied to provide improved guidance to equine veterinary practitioners on the clinical use of paracetamol in horses.

The results of this survey provide an overview of how veterinary surgeons are currently using paracetamol in horses. Paracetamol was ranked most effective for laminitis by respondents compared to other conditions such as bone spavin, palmer heel pain and thoracolumbar pain. The survey also indicates that many respondents are using paracetamol in horses, although infrequently. However, the overall response rate was low and, therefore, this may not be representative of the wider equine vet population. The dose of paracetamol used is consistent with published literature, however, there is still insufficient evidence regarding the optimal dose to use in our patients.

Chapter 4: Novel use of paracetamol in horses

Contributions: Adam Redpath was the main researcher with primary responsibility for study design, methodology, data collection and preparation of manuscript. Sarah Freeman and Kate White (University of Nottingham) contributed towards study design and Stuart Paine (University of Nottingham) contributed towards data and statistical analysis. Hannah Wilson (Intern, Oakham Veterinary Hospital) helped with plasma sample collection.

Introduction

Clinical pharmacokinetics is the application of pharmacokinetic principles to the safe and effective therapeutic management of drugs in an individual patient. Although the pharmacological action of a drug is dependent on the drug concentration at specific drug receptors it is often not practical to measure this and, therefore, clinical studies rely on plasma drug concentrations to derive pharmacokinetic information. There are limited data reflecting the clinical pharmacokinetics of paracetamol in horses, although recent studies have started to elucidate these principles (Mercer et al , 2023). It is well established that paracetamol has good oral bioavailability in horses, through its use as a marker of gastric emptying, and recent data suggests that it has favourable pharmacokinetics when administered either via the oral route or intravenously (Doherty et al, 1998; Pesko et al, 2022; Neirinckx et al, 2010). Over the past decade, its use in horses has increased significantly and scientific and anecdotal evidence suggests it is an effective analgesic in equine medicine.

It is considered best practice to adopt a multi-modal approach to providing analgesia in horses undergoing surgery under general anaesthesia. Paracetamol may offer a safe and effective alternative to other more commonly used analgesics in equine anaesthesia. Although the pharmacokinetics of intravenously administered paracetamol have been reported (Neirinckx et al, 2010), there are no studies documenting the pharmacokinetics of paracetamol in horses under general anaesthesia.

There is increasing evidence supporting the use of oral paracetamol as an effective analgesic in horses (Pesko et al, 2022, Mercer et al 2023). However, in the critical care setting, horses are frequently unable to receive oral medications, such as those with postoperative ileus. Post-operative ileus (POI) is common in horses and is defined as the failure of gastrointestinal motility to recover following colic surgery (Merritt et al, 2008). It typically manifests clinically as an increase in net nasogastric reflux (>2L) and has important welfare, cost, and post-operative survival implications (Lefebvre et al, 2016; Lisowski et al, 2018). Although most of the literature around post-operative ileus in horses is centred around gastrointestinal surgery, post-operative ileus is also reported following orthopoedic and gynaecological surgery in human medicine (Lisowski et al, 2018) and, therefore, the potential relevance of alternative routes of administrations may extend beyond our colic patients.

Multi-modal analgesia is important in these patients to improve welfare and typically horses receive a combination of non-steroidal anti-inflammatory drugs (NSAIDs) and opioid analgesia. However, potential adverse effects of these drugs call for additional, safe analgesic drugs to be used in horses in the critical care setting. Intravenously administered paracetamol could be used, but the large volumes required of currently available formulations and the need for intravenous cannulation, make this an impractical method of frequent paracetamol administration in the critical care setting.

Paracetamol has been shown to be well absorbed from the rectum in human patients (Forrest et al, 1982) providing an alternative route for administration of pain relief when the oral route of administration is not possible (e.g., in horses with POI). Given that equine patients undergoing colic surgery commonly develop post-operative ileus in the first 24-48hrs following surgery this would be invaluable in providing a means to provide additional analgesia, improving welfare in these patients.

This study aims were:
To document the pharmacokinetics of intravenous paracetamol in horses undergoing general anaesthesia
To determine the bioavailability of rectal administration of paracetamol in horses compared to oral paracetamol.

Methods

Ethical approval was obtained from the University of Nottingham School of Veterinary Medicine and Science Committee for Animal Research and Ethics. All studies were conducted under an ATC-S certificate awarded by the Veterinary Medicines Directorate (VMD).

There were two components to this study. Phase 1 investigated the pharmacokinetics of intravenous paracetamol administered during anaesthesia in horses undergoing elective orthopaedic surgery under general anaesthesia. The second phase investigated the pharmacokinetics of paracetamol administered per rectum in horses during the post-operative period following colic surgery. As this study was carried out in clinical patients, sampling points were determined by clinical monitoring for both phases of the study.

Phase 1. Pharmacokinetics of intravenous paracetamol under general anaesthesia

Horses
Horses undergoing routine diagnostic arthroscopy with an ASA grade of 1 were recruited into the study. Informed consent was gained from owners prior to recruitment. All horses underwent a full physical examination prior to anaesthesia and any shoes were removed. All horses received non-steroidal anti-inflammatory drugs (phenylbutazone at 4.4mg/kg IV) and antimicrobials (procaine penicillin at 2.2mg/kg) prior to anaesthesia.

Anaesthesia

All horses received 0.03 mg/kg acepromazine IV 20-40 minutes prior to induction. Horses were then brought to the induction box and sedated with 0.02 mg/kg detomidine IV. Once adequate sedation had been achieved, anaesthesia was induced with 0.06 mg/kg midazolam and 2.5mg/kg ketamine. Once recumbent, the horse's trachea was intubated, and the horse hoisted onto the operating table where maintenance of anaesthesia was achieved by connecting the horse to the anaesthetic machine to receive isoflurane in oxygen via a large animal circle. Horses were appropriately positioned on the operating table before connecting to the anaesthetic breathing system to achieve a surgical plane of anaesthesia. All patients were ventilated to normocapnia (40-45mmHg ET CO_2) and a positive inotrope was infused whenever necessary to maintain normotension (MAP >60mmHg). All horses received a balanced electrolyte solution (Hartmann's 5ml/kg/hr for the first hour, then 2ml/kg/hr subsequently). Comprehensive monitoring was carried out throughout anaesthesia to include as per standard protocol, ECG, pulse oximetry, capnography, invasive blood pressure monitoring from a peripheral artery, and blood gases to assess adequacy of ventilation/oxygenation, end-tidal isoflurane, and manual assessment of mucous membrane colour, CRT, and reflexes. All horses had a urinary catheter placed during anaesthesia as per normal practice protocol, to ensure the bladder is empty for recovery and to assess adequacy of IVFT administration.

Paracetamol administration

Immediately prior to the first incision, 20mg/kg paracetamol was administered intravenously as a constant rate infusion through a jugular cannula to augment the analgesia provision. Paracetamol infusions were given using a calibration infusion pump and given over 15 minutes, constituting a rate of 50-100mls/minute. The horses were monitored with continuous ECG throughout the infusion. The infusion was completed before the incision was made.

Sample collection

Residual blood collected during arterial cannula placement and sampling for blood gases (2-3 per case as per usual protocol) was collected and spun down for paracetamol plasma concentration measurements to determine the pharmacokinetics of paracetamol in anaesthetised horses. Urine samples were also collected from the catheter at the same time as blood collection to compare urine paracetamol concentrations. The time of sample collection was recorded in minutes from the start of the infusion.

Phase 2. Pharmacokinetics of rectally administered paracetamol

Horses

Horses admitted to the intensive care facility following exploratory celiotomy for investigation and treatment of colic were given 20 mg/kg of paracetamol per rectum twice daily as part of a multi-modal analgesia plan. All horses received standard clinical care as per practice protocol for horses undergoing exploratory laparotomy for investigation of colic signs.

Paracetamol administration
Horses received 20mg/kg of paracetamol twice daily (8 am and 8 pm). Paracetamol tablets (500mg) were dissolved in water to form a paste and administered per rectum. Horses were appropriately restrained during rectal administration to ensure the safety of the person administering the drug.

Sample Collection
Residual blood collected during routine monitoring of PCV, total protein and lactate (twice daily as per normal ICU standard monitoring), or for any other clinical monitoring, and spun down for paracetamol plasma concentration to determine the bioavailability of paracetamol when administered per rectum. The timing of sample collection was recorded as minutes from last paracetamol administration.

Sample analysis

Preparation of Standards and Quality Controls (QCs)

Standard and QC stocks were prepared from separate accurate weightings of solid paracetamol in DMSO. Standard and QC stocks were serially diluted in DMSO to generate a standard curve (100,000 ng/mL – 50 ng/mL) and QC's (80,000 ng/mL – 200 ng/mL). Calibration and QC spiking solutions were prepared to cover the anticipated concentration range. The standard curve and QCs were prepared by spiking the appropriate calibration or QC spiking solution into the blank matrix (urine and plasma) to produce a standard curve and QCs, followed by protein precipitation with an internal standard working solution in methanol.

Preparation of Samples and Blanks

Plasma samples were taken and DMSO was added, followed by protein precipitation with an internal standard working solution in methanol to give samples that were matrix-matched with the standards and QCs. Samples of blank used for standard and QC preparation were included with each sample analysis batch. All quenched samples were thoroughly mixed, and protein precipitated at -20°C overnight. The samples were centrifuged at 2500 x g for 20 min, 4°C. The supernatants were then transferred and analysed by liquid chromatography-mass spectrometry (LC-MS).

Data and Statistical Analysis

Sample size calculations were based on published data from human medicine (Rawlins et al, 1977) to determine the sample size needed to provide a precision for intravenous pharmacokinetic data of 10%. When comparing intravenous and rectal pharmacokinetic data, precision was established at 20% (80% power) with alpha of 0.05 in a cross over design. Based on the published coefficient of variation from this human study of 11.36%, 5 horses were estimated to be required for the intravenous study, while a cross over study would have required 10 horses in each group to document differences in pharmacokinetic data between routes. Sample size calculations were performed in Python3 using the Matplotlib library. Retrospective sample size calculations were performed on equine data using the same methodology based on the coefficient of variation of the experimental data using 95% confidence intervals of the observed data.

Population parameter estimates were obtained using non-linear mixed effect modelling (NONMEM) using a non-compartmental model using clinPK within the R package. Pharmacokinetic analyses were then conducted using an IV infusion compartmental model for a sample equine population undergoing elective general anaesthesia for orthopaedic surgery using a total dose. Analyses used Phoenix® 64 software (Certara USA, Inc., Princeton, NJ). Compartmental non-linear mixed effects methods (NLME) models were applied using the total dose given to each horse to determine V, Cl, V2, Cl2

The compartmental model used to describe paracetamol concentrations in plasma from IV and rectal administrations is shown in Figure 3 where: Ka and F are the rectal absorption rate constant and bioavailability of paracetamol. CL and CLD are the clearances for paracetamol from the central compartment(C) and distribution between the central comparment (C) and peripheral compartment (C2), respectively. V1, and V2 are the volumes of the central and peripheral compartments, respectively. VUN and kUN are the volume and drug elimination rate constant for the urine of nephrons, respectively. CP and CUN are the plasma and urine concentrations of 2,5-HBSA, respectively.

Pharmacokinetic analyses were conducted using non-linear mixed effects methods (NLME) with Phoenix WinNonlin 8.3 (Certara, Princeton, NJ, USA). Compartmental NLME PK models were applied simultaneously to the plasma and urine concentration data for paracetamol which included censored data below 50 and 100 ng/ml for plasma and urine, respectively. The Laplace engine within Phoenix was used as the inclusion of censored data required a non-gaussian approach. Doses were inputted as the dose of paracetamol per kg bodyweight resulting from either etamsylate or calcium dobesilate monohydrate administration. Residual error was modelled on a proportional error model. An exponential random effect model was chosen to describe inter-individual variability e.g. parameter = typical parameter * exp(eta). A categorical covariate for either etamsylate or CD administration was implemented on the model parameters in a multiplicative exponential way. The model analysis started from the basic compartmental models without the covariate and random effects were added stepwise to the parameters. Next, any contribution of the covariate to the fixed parameters and correlation on the random effects were assessed by a reduction in the objective function (OBF) using stepwise forward inclusion. The OBF was optimised while maintaining shrinkage below 30%. Selection of the best model was based on the lowest value of the Akaike and Bayesian Information Criteria (AIC and BIC), chi-square p-value based on the likelihood ratio test, visual inspection of the population predicted concentration versus the observed concentrations and the resulting conditional weighted residual errors. Finally, the best model was checked for robustness using a bootstrap resampling method.

Results

Pharmacokinetics of intravenous paracetamol under general anaesthesia

A total of 7 horses were included in the study with plasma samples obtained from a total of 18 time points from 0-90 minutes for measurement of plasma paracetamol concentrations following intravenous administration. All horses received a 20mg/kg infusion of paracetamol over 15 minutes. Paracetamol was detected in all 18 samples. The times of sampling (minutes from start of infusion) and corresponding plasma paracetamol concentrations are shown in table 1. No rhythm abnormalities were detected during or after the infusion and there was no evidence of any detectable decrease in mean arterial blood pressure.]

Time (mins from start of infusion)	Concentration (ng/mL)
5	37135
10	70255
16	85529
22	57263
23	66444
25	56389
32	32964
33	60623
40	31980
45	33721
46	33045
56	25047
57	26145
60	27598
65	27316
70	24210
75	21560
90	22790

Table 1: Table showing timepoints for plasma sampling with the respective paracetamol concentrations (ng/ml) measured in each sample.

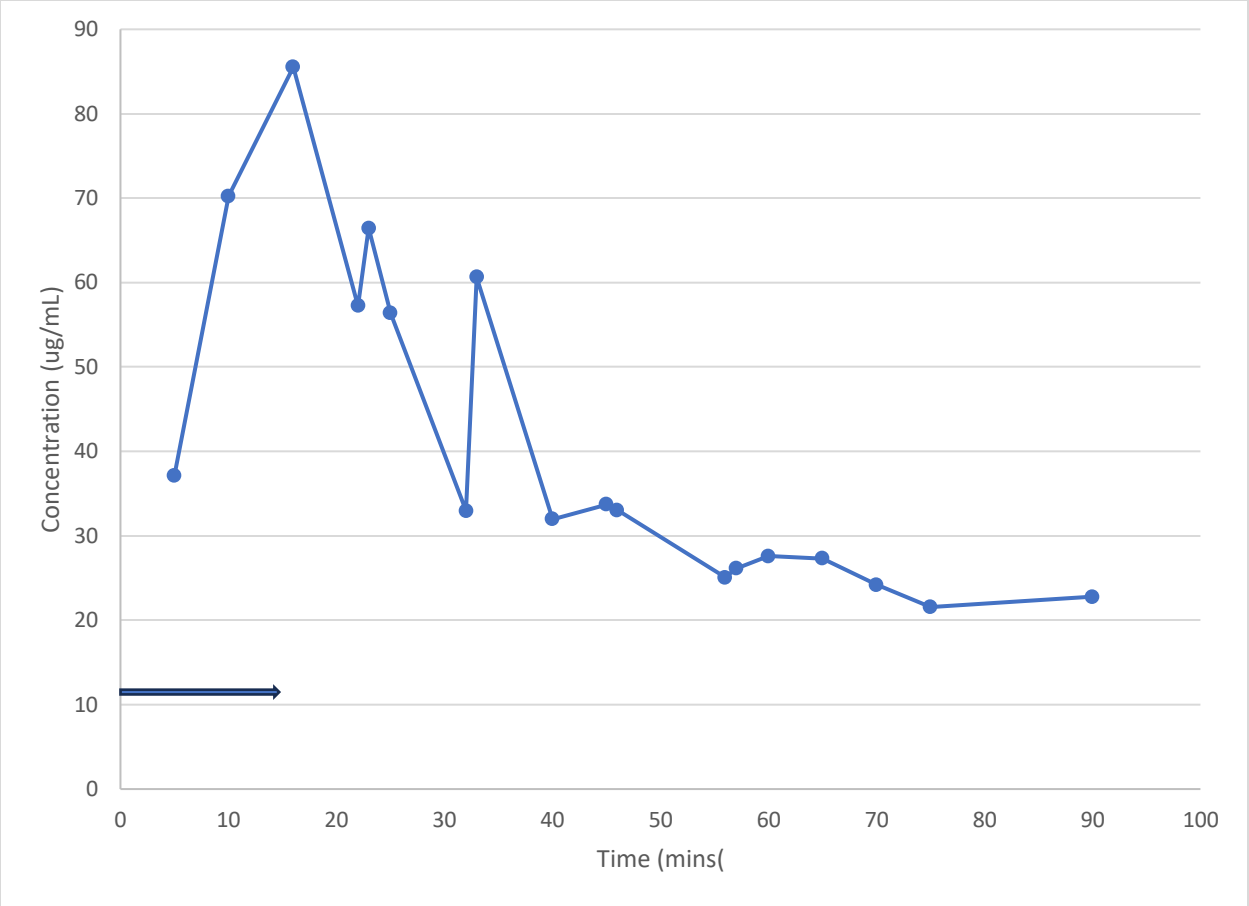

Figure 1: Graph showing plasma concentrations over time following intravenous infusion of paracetamol at 20mg/kg in 7 healthy horses undergoing routine arthroscopy under general anaesthesia. Time is given as minutes from the start of the infusion and shown by the blue arrow. The infusion lasted 15 minutes in all horses.

Pharmacokinetics of rectally administered paracetamol

2 horses receiving care post-operatively for surgical treatment of colic received 20mg/kg of paracetamol per rectum twice daily in addition to non-steroidal anti-inflammatory drugs. Plasma samples were obtained from a total of 16 time points during the period of hospitalisation from 0-11 hours post dosing. Paracetamol was detected in all 16 samples. Sampling time points for plasma paracetamol concentrations following rectal dosing are shown in table 2. Both horses tolerated rectal administration well with no evidence of discomfort or resentment.

Time (mins from last paracetamol admin)	Concentration (ng/mL)
15	3443
35	2807
45	2897
60	3464
78	3040
90	3071
105	2651
120	4408
135	3742
147	2188
180	3746
210	4351
270	3320
341	1424
469	397
647	621

Table 2: Time points sampled post rectal administration of paracetamol with respective plasma concentrations (ng/ml) of paracetamol measured in each sample.

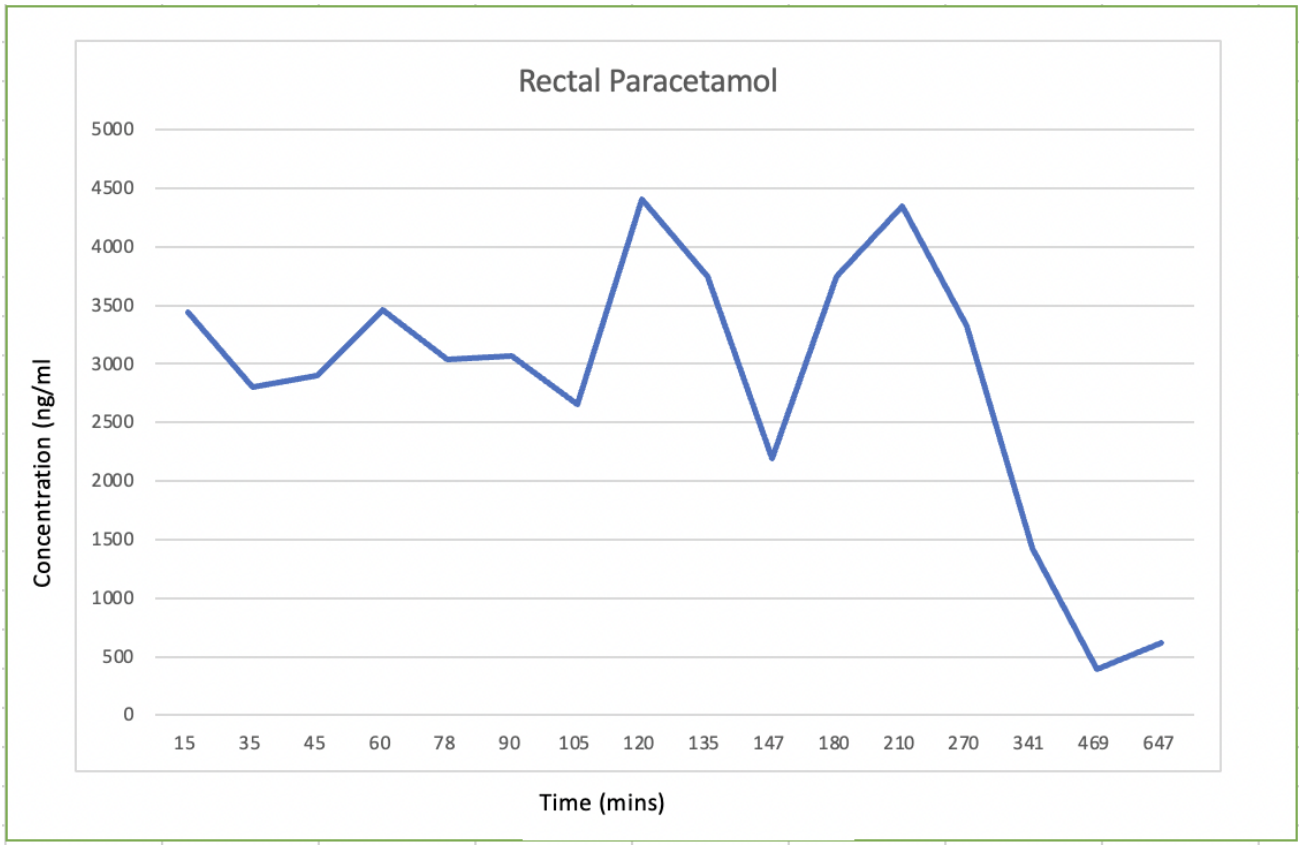

Figure 2: Graph showing plasma concentrations over time following rectal administration of 20mg/kg of paracetamol in two post operative colic patients in a hospital intensive care unit. Time is given as minutes post paracetamol administration.

Population Pharmacokinetics

Population parameter estimates for clearance (Cl), half-life (T1/2), volume of distribution (V), time to maximum concentration (tmax) and maximum concentration (cmax) are shown in table 3 for intravenous administration and table 4 for rectal administration.

Pharmacokinetic parameter	**Value**
kel	0.0181
T1/2	38.3
V	351
Cl	6.34
tmax	16
cmax	87090

Table 3: Population estimates following a single dose of intravenous paracetamol at 20mg/kg under general anaesthesia.

Pharmacokinetic parameter	Value
kel	0.0044
T1/2	158.7
tmax	120
cmax	4451

Table 4: Population estimates following per rectum administration of 20mg/kg of paracetamol in post operative colic patients.

A two compartmental model was shown to have the best fit for this population of 9 horses as shown in figure 3.

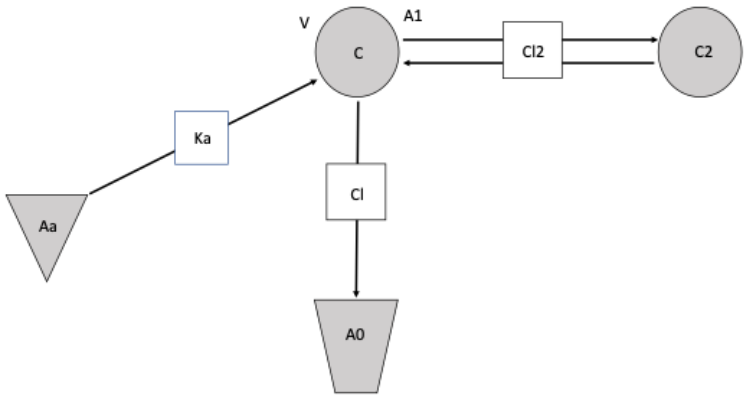

Figure 3: Two compartmental model showing the distribution and clearance of paracetamol in this population of 9 horses following intravenous and rectal administration. C represents the central compartment (plasma and highly perfused tissues including the kidney and liver) and C2 represents the peripheral compartment representing poorly perfused tissues such as muscle. The final population values for V, Cl, V2, Cl2, Ka and F were V=141.6, Cl = 2.5, V2= 304.5, Cl2 = 7.32

The typical values for this population are shown in table 5.

Parameter	Estimate
tvV	142
tvCl	2.52
tvV2	304
tvCl2	7.32
tvKa	0.0132
tvF	0.156
stdev0	0.151

Table 5: Typical values

Values for individual horses are shown in table 6.

Horse	V	Cl	V2	Cl2	Ka	F
1	142	2.78	304	7.32	0.0132	0.156
2	142	2.43	304	7.32	0.0132	0.156
3	142	2.39	304	7.32	0.0132	0.156
4	142	3.69	304	7.32	0.0132	0.156
5	142	2.83	304	7.32	0.0132	0.156
6	142	1.97	304	7.32	0.0132	0.156
7	142	1.79	304	7.32	0.0132	0.156
8	142	2.52	304	7.32	0.0132	0.156
9	142	2.96	304	7.32	0.0132	0.156

Table 6: Individual horse values

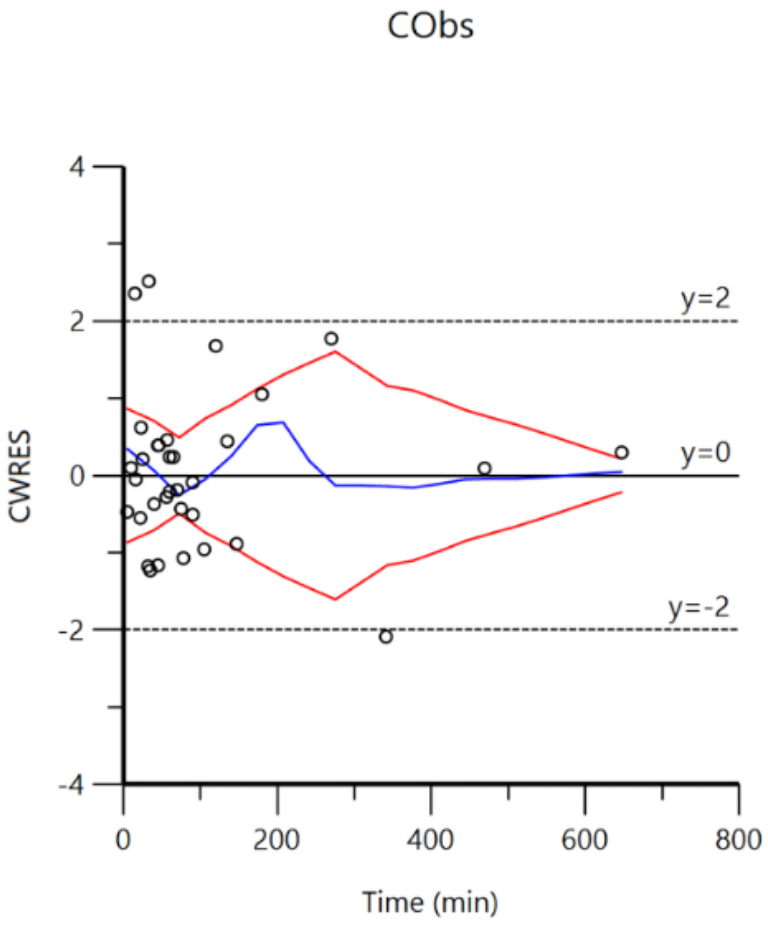

Figure 4: Conditional weighted residuals showing the approximated difference between individual's data and the model prediction for plasma paracetamol concentrations.

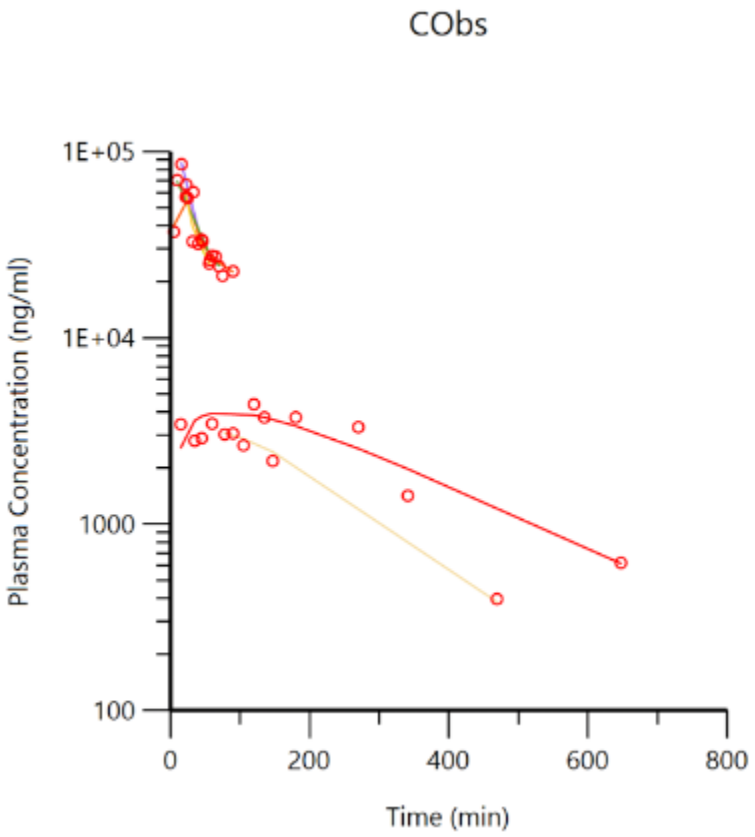

Figure 5: Predicted observed concentrations (Cobs) of paracetamol in plasma over time for this population of horses for intravenous vs per rectum administration.

Precision of these data showed a coefficient of variation of the data of 20.45 %. Retrospective sample size calculations demonstrated that 9 horses provided a precision of ±15% of the intravenous PK parameters, while the sample size calculation showed that 10 horses would be needed to show differences in PK data between IV and rectal administration based on a power of 80%.

Discussion

This study demonstrates that intravenous administration of paracetamol to horses under general anaesthesia results in a rapid increase in plasma concentrations with the highest concentration recorded at the 16-minute sample time. The accepted therapeutic range of paracetamol in human patients is 10-20 ug/ml (Stocker et al 2001). Although the therapeutic range for plasma paracetamol concentrations is not well documented in horses, a recent publication calculated the effective plasma concentration (EPC) to be 8ug/ml following oral dosing with 20mg/kg of paracetamol (Pesko et al, 2021). Previous studies have reported an EPC of 12ug/ml (Ishii et al., 2020) but these calculations were based on 10mg/kg with dosing interval recommendations based on human recommendations. In this population of horses, the plasma concentrations of paracetamol measured above 20 ug/ml in every sample obtained up to 90 minutes post-infusion with maximum concentrations over 4 times greater than the upper end of this range. Assuming that similar therapeutic concentrations are required for a therapeutic effect in horses, this study suggests that intravenous administration of paracetamol at 20mg/kg could provide effective short-term analgesia in horses.

Formulations of paracetamol designed for rectal administration are available in human medicine, with clinically relevant analgesic effects described with this route of administration (Ramsing et al, 2002). In this study, the administration of 20mg/kg of paracetamol tablets as a water-based suspension resulted in plasma concentrations that fall significantly below the accepted therapeutic range in human medicine, with a bioavailability of less than 16% across this population of horses. The absolute bioavailability of rectally administered paracetamol has been described to be 30-40% in healthy adults (Eandi et al, 1984). The significantly lower bioavailability found in this study could be accounted for by several different factors including differences in anatomy, drug formulation or altered perfusion as a result of colic surgery. All horses were considered to be normovolaemic at the time of paracetamol administration, but changes in gastrointestinal blood flow as a result of colic cannot be ruled out, which could, in theory, affect the absorptive capacity of the rectum. The vehicle base of the suppository may influence the absorption of poorly soluble drugs such as paracetamol. A lipophilic base was found to produce higher plasma paracetamol concentrations in human patients compared to a hydrophilic base (Cullen et al 1989), and therefore an oil-based suspension may have improved the bioavailability of rectally administered paracetamol in these two horses. When considering 8 studies that compared rectal administration of paracetamol to placebo in human medicine, only studies using a single dose of 40-60mg/kg or multiple dosing at 14-20mg/kg showed significant analgesic efficacy, whilst single doses at 10-20mg/kg did not demonstrate a significant analgesic effect (Rømsing et al, 2002), suggesting higher doses are required for this route of administration. Further dose optimisation studies are required to determine whether this is a viable route of paracetamol administration in horses.

A two-compartmental model was shown to have the best fit for this population of horses showing that paracetamol was distributed between a central and peripheral compartment. Many drugs administered in veterinary medicine can be described by this model. A two-compartmental model has also been described as being best fit following oral multi-dosing in the thoroughbred horse (Pesko et al, 2021). Non-linear mixed effects (NLME) PK models offer the advantage of having a single model that describes a population and therefore shares data interpretation between animals,

unlike the standard deterministic PK approach. In addition, variations within a population can be described by co-variates that adjust the fixed effects (typical values).

It is difficult to completely rule out a possible drug-drug interaction (DDI) leading to alteration of the paracetamol pharmacokinetics as all horses enrolled in the study were receiving concurrent medications. However, very few drug interactions with paracetamol have been reported in human medicine (Toes et al, 2005) and there is no evidence in the literature describing DDI in horses receiving paracetamol alongside other drugs. Paracetamol is known to be metabolised by at least two pathways (phase 1 and phase 2) (Toes et al, 2005) so if one pathway is inhibited by a coadministered drug there is a second pathway for clearance, so any possible DDI will have less of an impact on the pharmacokinetics. Drug-drug interactions are difficult to predict as they depend on pathways, dose and inhibition potential of coadministered and this has not been studied in the horse. Further equine specific studies would be required to determine whether DDIs would have the potential to impact paracetamol pharmacokinetics in the horse.

Peak plasma cooncentrations were noted to be over four times greater than the perceived therapeutic concentrations in this intravenous study. Consideration should be made to the potential adverse effects of such high concentrations. Although adverse effects were not found in this study, further studies are required to determine the optimum dose and dosing interval of intravenous paracetamol. In this study, samples were obtained for up to 90 minutes post-infusion with plasma paracetamol concentrations remaining above the suggested therapeutic concentrations for the duration of that time. However, further work should be aimed to determine whether effective concentrations are obtained following administration at lower doses and what dosing interval would be required to maintain plasma concentrations within the therapeutic range. This, however, would require a longer sampling period. A longer sampling period in this particular clinical study was not ethically possible as would require delaying the duration of general anaesthesia beyond that required for completion of the arthroscopy.

This study is limited by the small sample size, particularly with those receiving rectal paracetamol. Although the study had sufficient power to document population pharmacokinetics of the intravenous use of paracetamol in horses, although there was much wider variability in the equine data than previously documented in human studies (Rawlins et al, 1977). Although the reasons for the greater variability in horses is not clear, it may represent methodological factors, such as the use of population pharmacokinetics in anaesthetised horses. While blood pressure was monitored through invasive methods, this does not take into account cardiac output and thus hepatic blood flow that may have varied between individuals. While these data provide a useful reference point under general anaesthesia, care must be taken in extrapolating these to the conscious horse. The rectal administration was underpowered to document significant differences in pharmacokinetics by this route of administration. Although population kinetics allows for a smaller sample population, a larger study population of at least 10 horses in an experimental setting would have reduced the influence of individual factors in the horses that received paracetamol per rectum. These horses were also sampled over multiple days whilst receiving twice daily dosing of paracetamol. Although studies describing the pharmacokinetics of oral paracetamol show no significant drug accumulation (Mercer et al., 2020,Pesko et al, 2021), we cannot rule out the effect of multi-dosing in this study. Further work is required to determine the pharmacokinetics of rectal paracetamol following a single dose, but this would have required a large sample size. Baseline

(time zero) bloods were not obtained from any animal as this was a clinical study and was assumed that plasma paracetamol concentrations would be zero. It is acknowledged that this may have underestimated any potential carryover or cumulative effect of the drug.

A population pharmacokinetic model was used to address the collection of clinically relevant samples outside of an experimental model, without the need for multiple observations (rich data) per subject (Mould et al, 2013). Population pharmacokinetics has the advantage of quantifying drug metabolism in the real clinical setting across a number of subjects, and therefore offsets impacts, such as anaesthesia and other drug interactions (Shiener et al, 1992). Processing of such data uses a non-linear mixed-effects model to consider both the non-linear drug concentration, alongside the mixed-effects approach to consider different animals. With small numbers of samples per subject, as with this study, there is a risk of introducing some error in population data, since the impacts of the individual may have a greater impact than the drug metabolism (Mould et al, 2013). Since these samples were all collected on the basis of arterial blood gas analysis during surgery, no baseline concentrations (before dosing) exist, however these horses had no prior treatment, and therefore concentrations are assumed to be zero.

Paracetamol has been shown to follow first-order kinetics in human patients (Bannwarth et al, 2003) and therefore the statistical modelling is relatively simple when using mixed model approaches and thus considered appropriate in this study. It is possible that drug interactions may impact on the pharmacokinetics of paracetamol, and therefore it is important not to draw conclusions beyond the clinical setting described. All horses received alpha-2 agonists, ketamine, midazolam and isoflurane and thus may have impacted drug concentrations. However population pharmacokinetics allows for the modelling of dosing strategies within that clinical scenario, ie under anaesthesia (Ette et al, 2004). It was an assumption of this study that IV paracetamol would be most applicable to peri-operative use, given the challenges of administration of such large volumes in the standing horse, however, different kinetics may occur in such conscious horses. Isoflurane may directly or indirectly impact paracetamol hepatic conjugation. Isoflurane may directly impact hepatic function (Nishiyama et al, 1999), or indirectly by a reduction in cardiac output (Grosenbaugh et al, 1998) that reduces hepatic blood flow, or through hypoxia (Mets et al, 1993). Further evaluation of the impact of hepatic clearance of paracetamol during anaesthesia is warranted.

The study did not aim to determine the analgesic effect of paracetamol in the perioperative period, by documenting a reduction in minimum alveolar concentration (MAC) of isoflurane. Such work is required to demonstrate the clinical value of the use of paracetamol in the perioperative period, but would require an experimental study. The analgesic effect of paracetamol has been evaluated in other species with conflicting outcomes. In experimental animals, no MAC sparing effect was identified in standard experimental models (Chavez et al, 2015; Gonzalez et al, 2020), however anti-nociceptive responses were reported in experimental rats using mechanical stimuli in other studies (Alloui et al, 2002). One study has shown that paracetamol potentiates the MAC sparing effects of opiate analgesia (Benito et al, 2010), suggesting more complex mechanisms of analgesia of paracetamol. The MAC sparing effects of COX inhibiting NSAIDS has also been shown to be inconsistent, with limited MAC sparing effect under anaesthesia in a range of species (Turner et al, 2006; Gomez et al, 1998). Despite the inconsistent MAC sparing effects identified in veterinary species, evidence of sustained post operative analgesia of paracetamol has been established in a

number of studies of human patients (Upadya et al, 2015; Gholami et al, 2016; Gousheh et al, 2013).

While arterial blood samples were obtained for the intravenous study, venous samples were used in the rectal administration study. Data was analysed assuming that drug concentrations would be equivalent. In human patients, capillary blood samples (finger prick) have been shown to differ to venous samples during the absorption phase after oral administration (Mohammed et al, 2010; Rittau et al, 2012), although pharmacokinetic parameters were similar at later time points. There are no studies evaluating pharmacokinetic parameters between arterial and venous samples in any species, although concentrations have been shown to be higher in arterial, compared to venous samples for a range of solutes (Martin et al, 1984; Chen et al, 1982; Lee et al, 1994). These differences may have exacerbated the measured poor bioavailability per rectum. Despite this, the data show that further evaluation is required to determine an appropriate drug dose for per-rectum paracetamol

.

This study suggests that intravenous paracetamol has favourable pharmacokinetics for short-term analgesia in horses under general anaesthesia for routine surgery. The bioavailability of paracetamol when administered per rectum is very low and cannot be recommended for analgesic use using the most commonly used dose and formulation of this drug in horses. Further work is required to determine the optimum dose and dosing interval for intravenous paracetamol and to determine whether higher doses or a different vehicle base for the suppository improve the pharmacokinetics of rectally administered paracetamol.

Chapter 5: Discussion

Summary of findings

Paracetamol has been widely used in human medicine for many years and is one of the most commonly used analgesics in this field. It is considered first line for the management of chronic pain in human patients (Freo et al, 2021; Zambelli, 2022). Paracetamol use in the veterinary industry is increasing and there is increasing evidence of its efficacy as an analgesic in horses, but limited information on how it is currently being used in equine veterinary practice. This study aimed to survey equine practitioners on how they were using paracetamol in horses and to determine the pharmacokinetics of paracetamol in the horse in two different clinical situations.

The first part of the study, the survey of equine veterinarians, showed that many practitioners are using paracetamol in clinical cases such as laminitis, although infrequently. Furthermore, in situations where paracetamol is used, it is still being used secondary to the more commonly used analgesic drugs, such as flunixin and phenylbutazone. This likely reflects reduced awareness of the efficacy of paracetamol as an analgesic in horses and a lack of published data on optimum dose and safety of clinical use. The survey also highlighted that most respondents were using the human tablet formulation of paracetamol in horses, with very few using any other formulation of the drug. Alternative routes of administration may be required in some clinical scenarios, and this provided the impetus for the second study.

In the second part of the study, the pharmacokinetics of intravenous and rectally administered paracetamol were investigated in clinical cases. The administration of an intravenous infusion at 20mg/kg resulted in rapid attainment of high plasma concentrations that remained above predicted therapeutic levels for the duration of the 90-minute study period. The bioavailability of rectally administered paracetamol in this study was lower than 16% suggesting that the dose and method used in this study was not effective.

Study Limitations

Use of paracetamol by equine practitioners

This study had a relatively low number of responses, with only 116 equine veterinarians completing the survey. Response rates for surveys of veterinary surgeons are known to be variable and published rates have been as low as 20-30% (Robinson et al, 2014; Curtis et al, 2015). Whereas studies involving horse owners often have much higher response numbers (Bambra et al, 2020; Murray et al, 2010). The response rate in this study could not be calculated due to the nature in which the questionnaire was distributed to equine vets. The use of social media platforms meant that the total population of vets with access to the questionnaire was unknown. A known total population would provide opportunity to follow up on non-responders which may improve the overall response rate. It would also allow us to determine whether the responders are representative of the study population with consideration of demographic information. In the study by Murray et al, 2010, looking at risk factors for lameness in dressage horses the questionnaire was sent to all

registered members of British Dressage at that time, giving a known total population. In this study there were 1677 responses collected out of 11,205 horses registered with British Dressage in 2018, resulting in a response rate of 15% (Murray et al, 2010). Although the response rate here is still very low, the ability to contact everyone in a known population may have increased the overall number of responses. Although, in that particular study the survey was aimed at horse owners, the UK equine veterinary population could be determined through use of the RCVS website and surveys could be distributed to all registered members.

The low response rate does limit the validity of the conclusions that can be drawn from the study and opens up the study to the effect of bias. Equine practitioners with an interest in clinical research, paracetamol and/or analgesia in general are more likely to participate in the study allowing for selection bias. Self-selection bias may also arise where only participants with access to the online social media platforms used for survey dissemination were able to respond (Fenner et al, 2020). In addition, practitioners are more likely to remember outlier cases such as a difficult or challenging case, or may fail to remember past events accurately (Fenner et al 2020), and therefore responses to questions around their use of paracetamol may be skewed towards these cases and may not accurately reflect more general use. Therefore, the effects of recall bias should be considered when interpreting the results of this study. These types of bias are common when online surveys are used to collect data (Fenner et al, 2020) and are particularly relevant in equine vet surveys where response rates tend to be low and where respondents may not be representative of the total population.

Responses from outside the UK were small, and the discussion of acetaminophen in the title may have encouraged more participation from those who use this term exclusively. The social media platforms utilised in this study were limited to email discussion groups and facebook groups. A study examining the use of Instagram, Facebook and Twitter as survey recruitment strategies showed that Twitter was the most used platform for survey dissemination (Purewal et al, 2021). Therefore, utilisation of a greater variety of social media platforms in this study may have allowed wider distribution and a higher number of responses.

The low response rate may mean the study lacked power to document the differences between groups of respondents. Some respondents had no experience with the use of paracetamol, and while these were excluded from the analysis, it would have been useful to delve deeper into the possible reasons for this, whether that was experience level, or another demographic factor.

Intravenous and transrectal use of paracetamol

The study is limited by the small sample size, particularly with rectal administration of paracetamol. Prospective interventional studies are challenging in veterinary medicine without access to a large population of research animals or clinical cases and this is reflected in the lack of randomised controlled trials in the veterinary literature, particularly those enrolling clinical patients (Girolamo et al, 2016). Clinical studies rely on recruitment of suitable individual cases, which in addition to limiting the study population size, also allows for increased individual animal variation. This variability is just one of the reasons that robust clinical trials are difficult to achieve in veterinary medicine (Girolamo et al, 2016). There is a large amount of variability among individuals around the drug dosages needed for a therapeutic effect (Martinez et al, 2018). The

relationship between the dosage of a drug and its concentration in plasma is affected by that drugs bioavailability, the animals body size and fluid composition, variability in drug distribution within the body and variability in rates of metabolism and excretion. Many of these factors are influenced by genetics, environmental factors, disease state and concurrent administration of other drugs. (Introduction to Clinical Pharmacology, Equine Internal Medicine, 4th Edition, p79). Although the use of population pharmacokinetics allows for a smaller sample population, a larger study population may have reduced the influence of individual factors between horses.

The use of population pharmacokinetics relies on data obtained from a large number of patients with different characteristics and receiving the drug or substance of interest under different conditions. It is able to work with sparse or dense data obtained from patients or animals receiving the drug or substance under different protocols. This allows the identification of factors that can affect the drug pharmacokinetic variability (Hedaya, 2012). Clearly, the primary goal of population pharmacokinetic modelling is to determine population pharmacokinetic parameters, as in this study, and sources of variability in a population, it has the added advantage of reducing the requirement for strict sampling conditions and being able to analyse sparse data sets (Mould et al, 2013). It is these advantages that make this type of modelling particularly useful in equine clinical studies where there is often a large degree of variability in the sample population and sampling conditions.

Main Findings

Use of paracetamol by veterinary practitioners

Both acute and chronic pain can represent major threats to equine welfare with "freedom from pain" representing one of the five freedoms of animal welfare (Botreae et al, 2007). Adequate pain management plays a vital role in recovery from injury or illness in horses and has been shown to result in fewer complications, earlier discharge from the hospital and a reduction in the development of chronic pain syndromes in human medicine (Grass, 2000). We know that pain can have a negative impact on behaviour and performance in horses (Kjaerulff et al, 2022). Therefore, there is clearly a need for increased availability of safe and effective analgesic drugs in horses.

Attitudes towards pain and analgesia in large animals is changing with debates about the need for analgesia in equine castration (Price et al, 2005, Bowen et al, 2020) and the provision of non-steroidal anti-inflammatory drugs in cattle undergoing dehorning and castration playing a key role in the impetus to develop guidelines for the use of analgesia in these species (Bowen et al 2020; BVA and BCVA, 2017). These guidelines should hopefully increase awareness around pain management and encourage the use of analgesic drugs in large animals.

This study surveying the use of paracetamol by veterinary surgeons found that although many practitioners are using paracetamol, its use is still infrequent. This may reflect lack of awareness or experience amongst clinicians using paracetamol in the management of pain associated with common equine musculoskeletal conditions, together with a lack of supporting literature demonstrating a clinical effect of paracetamol in these specific disease processes at the time.

The BEVA primary care guidelines do discuss the use of paracetamol in horses in relation to severe chronic pain that is not controlled by NSAIDs alone, but does highlight the need for further dose optimisation, efficacy and safety studies before further recommendations can be made around its use (Bowen at al, 2020). There is increasing evidence supporting the use of oral paracetamol as an adjunctive analgesic in horses. There are very few analgesic drugs that are suitable for long term use in horses, with a heavy reliance on non-steroidal anti-inflammatory drugs for the management of chronic pain, the adverse effects of which are well documented (Duz et al, 2016). The adverse effects of NSAIDs are discussed in detail earlier in the thesis but include renal necrosis, gastric ulceration and right dorsal colitis (Davis, 2017; Monreal et al, 2004; Adam et al, 2017). Subsequent to this study and the publication of the guidelines, further dose optimisation and safety studies on paracetamol use in horses have been published. Recent evidence suggests an increased analgesic effect at 30mg/kg in a mechanically induced lameness model (Mercer et al, 2023), which is a higher dose than is typically used in equine practice (20mg/kg). In a study using horses with naturally occurring chronic lameness, paracetamol administered at 30mg/kg twice daily for 21 days was found to be safe and provided a transient improvement in subjective and objective lameness evaluation for up to 8 hours post-administration (Mercer et al, 2023). When compared to single-dose pharmacokinetics administered at 20mg/kg, paracetamol administered at 30mg/kg resulted in a more rapid onset and greater improvement in lameness score and heart rate, suggesting this may be a more suitable dose when using paracetamol as a monotherapy (Mercer et al, 2023). More frequent dosing may provide more continuous analgesia and further studies are required to determine the safety and efficacy of paracetamol when administered at this dose more frequently (e.g. 3x daily).

Paracetamol has gained a lot of attention in recent years, and with increasing evidence regarding its effectiveness, consideration must be given to the human safety implications of prescribing large quantities of this drug. The results of the current study suggest that human paracetamol tablets are the most widely prescribed formulation of this drug in equine patients. Paracetamol has been reported to be the most common drug used for self poisoning in people in in the UK, and, if untreated, an overdose of 10-15g (20-30 tablets) can result in fatal hepatotoxicity (Simkin et al, 2012). This quantity represents a single dose in an average sized horse. UK legislation limits pack sizes of paracetamol to 16 x 500mg tablets, and places legal limits on the total quantity of tablets that can be supplied in a single transaction to reduce the chance of deliberate overdose (Bateman et al, 2009). With the potential of ready availability of very large quantities of paracetamol through veterinary prescription, this raises an important ethical dilemma. The use of preparations specifically formulated for equine use, may reduce the likelihood of accidental or deliberate human ingestion. However, whilst there are genuine risks to human health regarding the supply of large quantities of paracetamol, it should also be recognised that deliberate self administration of many oral equine medications could pose a significant risk to human health (Ramsey et al, 1976). Veterinary practitioners have a responsibility as prescribers to provide up to date information about the safe handling, storage and administration of the drugs we prescribe. Client information leaflets are readily available on the British Equine Veterinary Association (BEVA) for a wide variety of different medications (https://www.beva.org.uk/Guidance-and-Resources/Medicines/Cascade/client-information). It would be useful to expand the survey to include questioning about use of and availability of data sheets or client information leaflets by veterinarians prescribing drugs, to explore human safety implications further. Additional questioning may explore specific safety

advice or storage instructions veterinarians give to clients when prescribing large quantities of medication.

The pharmacokinetics of oral paracetamol have been studies and have been shown to be favourable in horses, and superior to that shown in dogs and pigs (Neirinckz et al, 2010), and number of studies supporting its clinical use are increasing (Mercer et al, 2023). However, there is still little to no published literature on the pharmacokinetics of intravenous and rectally administered paracetamol in horses. Intravenous and rectally administered paracetamol would allow increased utilisation in the critical care setting where horses frequently are unable to receive oral medications, for example, due to the presence of post-operative reflux and during general anaesthesia. In addition, such patients may be at an increased risk of non-steoridal anti-inflammatory drug toxicity due to changes in renal or gastrointestinal blood flow associated with hypovolaemia and SIRS, or hypotension under general anaesthesia. Therefore it is important that alternative methods of providing safe additional analgesia are explored.

Intravenous use of paracetamol

The pharmacokinetics of intravenously administered paracetamol appear favourable for short-term analgesia in horses undergoing routine arthroscopy. However, further work is required to document the pharmacodynamic effects of this. In this study, peak plasma concentrations were found to be over four times greater than the perceived therapeutic concentrations and, therefore, consideration should be given to the potential adverse effects of such high concentrations. For an acute overdose, most human adults need to ingest approximately 12g (24 tablets) or more of paracetamol before the risk of serious hepatotoxicity is of concern (Fisher et al, 2019). Although we can't necessarily extrapolate this into equine plasma concentrations, it does suggest that very high doses, that greatly exceed four-times the recommended dose, are required to result in serious adverse effects. Similarly in dogs, single doses of up to 100mg/kg have been shown to result in no clinical adverse effect, with mild and severe toxic effects seen at 200mg/kg and 500mg/kg respectively (Savides et al 1984). The listed dose of paracetamol in dogs in most formularies is 10-15mg/kg twice or three times daily, but the licensed dose in Pardale V™, a canine specific preparation, is 33mg/kg three times daily. In addition, dogs have been shown to tolerate doses of 45mg/kg/day without any adverse effects (Fitzgerald et al, 2006), which supports a relatively wide safety margin in other species. Nevertheless, further dose optimisations studies should be conducted to determine whether lower doses would bring plasma concentrations closer to the perceived therapeutic level, whilst maintaining clinical efficacy as an analgesic. The safety of repeated intravenous dosing of paracetamol should also be evaluated, similar to studies carried out with oral dosing (Mercer et al, 2020).

Transrectal use of paracetamol

The bioavailability of paracetamol administered per rectum in this study is disappointing, and it may be that rectal administration is a not a viable route of administration of paracetamol in horses. We know from previously published literature and from this study that oral and intravenous administration of paracetamol shows favourable pharmacokinetics and likely represent effective route of administration. However, in the hospital or critical care setting many horses may not be able to receive oral medications, such as those with post operative ileus. Whilst intravenous use

may be a viable route of administration pharmacokinetically, the large volumes required may not be practical for frequent or long term use. For those reasons, the rectal route of administration was considered.

Equine post-operative ileus is fairly common in colic surgery cases with the incidence reported to range from 10-47% (Lefebvre et al, 2016) and therefore, represents a frequent challenge for oral drug administration in the critical care setting, highlighting the importance of this study. We know that commonly used non-steroidal anti-inflammatory drugs may have a detrimental effect on intestinal healing, particularly following a small intestinal strangulating obstruction (Naylor et al, 2014), which could perpetuate post-operative ileus. Although the use of more COX-2 preferential NSAIDs did not affect major clinical outcomes in these cases (Naylor et al, 2014), the overuse of NSAIDs in these cases should be avoided. Paracetamol could provide a safe additional analgesic for multimodal pain management in these cases. However, post-operative colic patients, or patients with ileus, represent their own additional challenges in relation to drug administration and absorption beyond the route of administration. Horse can lose large volumes of bilious fluid through gastric reflux (Hellstrom et al, 2021) in addition to the haemodynamic consequences of the systemic inflammatory response syndrome (SIRS), resulting in massive fluid fluxes and redistribution of blood flow. With the prioritisation of blood flow to central critical organs, perfusion to the gastrointestinal system may be reduced. The preliminary work carried out in this study suggests that rectal absorption of paracetamol in normovolaemic post operative colic patients is low. However, further work should be carried out to determine the effects of gastrointestinal blood flow on rectal absorption of paracetamol. The results of such a study would have relevance to other rectally administered medications as well as paracetamol. In addition, as previously discussed, different vehicle bases of the suppository should be explored to determine whether this could improve rectal bioavailability.

If rectal bioavailability could be improved, the pharmacodynamic effects following this route of administration would need to be studied before clinical recommendations could be made. The limitations of pain assessment are discussed below but the challenges of identifying a clinical analgesic effect post drug administration in this population of horses warrants consideration. Changes in behaviour and physiological parameters could be influenced by the presence of SIRS and hypovolaemia or gastrointestinal obstruction in addition to the administration or withdrawal of pain relief. Outcome measures would need to be carefully selected and it is likely that very large numbers of horses would need to be studied.

Future studies/recommendations

A common challenge when conducting clinical research is variability amongst individual animals or patients. The use of population pharmacokinetics allows for this variation, and utilises it to identify factors that may affect drug pharmacokinetics. However, this challenge does identify the need certainly for species specific studies, but also more studies looking at drug pharmacokinetics and dynamics in specific animal populations or different disease states. Our population pharmacokinetic model can help address this by applying it to different horse populations under different clinical or environmental conditions.

This research has shown that intravenous paracetamol shows promise as a short-term analgesic in horses and has favourable pharmacokinetics under general anaesthesia. Clearly, the pharmacodynamic effects of this need to be evaluated. Follow-up studies should be carried out to determine whether the favourable pharmacokinetics have a discernible clinical effect. Such studies may investigate anaesthetic stability, recovery scores and post-operative pain scores. The administration of a single dose of intravenous paracetamol did not significantly reduce sevoflurane minimum alveolar concentration (MAC) in dogs (Gonzalez-Blanco et al, 2020). The authors preliminary work described in this thesis is the first study documenting the pharmacokinetics of intravenous paracetamol under anaesthesia in horses and, therefore, equine specific data regarding the MAC sparing effects of paracetamol is currently still lacking. However, if the same effect, or lack of, was observed in horses as seen in dogs, then it would suggest that improvements in anaesthetic stability are due to an analgesic effect rather than a direct MAC sparing effect. It is likely that such studies would require very large numbers in order to determine a statistical difference, which would be challenging in a clinical setting, and may require an experimental study.

The author is continuing to collect clinical data on anaesthetic stability, anaesthetic recovery scores, post operative pain scoring and the need for interventional analgesia post operatively following the administration of paracetamol to horses undergoing elective surgery. This follow up study would aim to document the clinical benefits of peri-operative use of paracetamol in horses and the author hypothesises that the administration of paracetamol as part of a multi-modal analgesia plan for surgery would improve anaesthetic stability and post operative recovery scores and reduce the need for additional rescue analgesia. An Equine Recovery Quality Scoring System (RQSS) is being used to record recovery scores. The scoring system evaluates ataxia, coordination, attempts to stand, and the risk of self-inflicted injury. A higher score (4-5) indicates a smooth, coordinated recovery, while a lower score (0-1) suggests severe ataxia, violent struggling, or prolonged recovery. The study would use cardiovascular and respiratory parameters, mean alveolar concentration (MAC) and monitor isoflurane use to determine anaesthetic stability and utilise composite pain scores (CPS) post-operatively and the need for additional interventional analgesia as measures of pain in these horses. A significantly larger study population would be required to determine a statistical difference between horses receiving paracetamol and those who do not. For example, a study investigating the influence of a lidocaine continuous rate infusion (CRI) on recovery quality recruited 54 horses that were then randomly allocated to groups and was able to find a statistically significant difference in recovery quality.

Evaluating the analgesic effect of a drug can be difficult with the assessment of pain often criticised as being highly subjective and studies investigating the efficacy of analgesic drugs are often limited by difficulties in perioperative pain assessment (Murrell et al, 2003) A variety of pain scoring systems have been developed to try and improve objectivity in pain assessment, but they still have their limitations, and we know that pain behaviours can be heavily influenced by their environment. Many of the pain behaviours in these rubrics can be difficult to interpret, particularly with mild-moderate pain (Torcivia et al, 2020; Nowak et al, 2024), and physiological parameters lack sensitivity and specificity to differentiate pain from other causes of distress (Arbour et al, 2010; Egan et al 2021). One study showed that owner visiting disrupted behaviours associated with discomfort in equine orthopaedic surgery patients, resulting in under-appreciation of pain (Torcivia et al, 2020). Therefore, we must consider alternative methods of assessing the analgesic

efficacy of drugs. Electroencephalogram (EEG) technology has been used to assess the antinociceptive effects of different anaesthetic agents (Murrell et al, 2003) and in a pilot study for the assessment of stress in horses alongside the horse grimace scale (HGS) (de Camp et al, 2020). Perhaps this could become a valuable tool for the objective monitoring of animal welfare and stress in our hospitalised patients

The results of the survey of veterinary practitioners on the use of paracetamol in equine practice suggested that paracetamol was being used fairly infrequently in comparison to other analgesic drugs. This may have reflected a lack of awareness and availability of robust studies documenting clinical efficacy at the time of the study. Many studies looking into the clinical effect of paracetamol rely on experimental models (Mercer et al, 2024; Mercer et al, 2023) or subjective lameness scores (Mercer et al, 2024). The author is involved in ongoing current work investigating the change in asymmetry data using inertial sensor units with the addition of paracetamol to the analgesia regime in chronically lame horses. Further work is also being carried out looking at changes in locomotor activity associated with the administration of paracetamol to a cohort of 20 geriatric multi-limb lame horses and ponies using poll-mounted triaxial sensors (Actigraph®). By comparing the activities during the pre-treatment, treatment and washout periods (three consecutive periods of seven days) we hope to quantify a positive clinical benefit. Both of these techniques may provide additional objective measures to determine the analgesic efficacy of paracetamol in horses. With increasing objective evidence describing the use of oral paracetamol in clinical cases, it may increase confidence in its use amongst equine practitioners. A study by Maisonpierre et al, 2019, concluded that accelerometery can differentiate standing, grazing and ambulating in horses. Although this study was investigating the influence of pasture size on these activity budgets in relation to obesity management, if these time-budgets could be applied to the painful horse, it may provide an additional tool to objectively assess response to pain medication in horses.

The author is now collaborating with the University of Nottingham and Vet Vision AI to develop algorithms that allow artificial intelligence software to identify behaviours and activity budgets that can be monitored in horses under surveillance. This would allow 24 hour monitoring of pain behaviours, without interrupting the behaviours by approaching the stable to carry out pain assessment. This may allow more accurate pain assessment and earlier interventions as well as overcoming some of the limitations described earlier in determining the clinical effect in analgesia trials. This work is ongoing and still in the preliminary stages of development. Computer AI is able to identify and track key points on an animal (Martvel et al. 2024; Feighelstein et al. 2025), and for equine pain behaviours this would include components of the facial expressions, such as the ear position, eyelid, nostril and lip position (Dalla Costa et al. 2014; Van Loon and Macri 2021). There are a number of areas which need developing to enable this, including establishing what level of high definition cameras are required to capture this detail, and optimal camera positioning. Existing monitoring systems use ceiling or high wall based cameras, but facial expression monitoring may require multiple cameras placed at lower levels to capture the horse's head when standing and lying.

With increasing evidence around the value of paracetamol in managing pain in horses, it is the authors hope that more specific veterinary formulations become available, improving practically of use in large equine patients. Human safety implications have been discussed earlier but would

specific veterinary formulations reduce the risk of deliberate human ingestion compared to human tablets? That question remains but consideration should be given to the fact that accidental ingestion of equine formulations still pose a valid risk. Nevertheless, equine clinicians should prescribe responsibility to reduce the risks of harm as far as possible.

Conclusion

Paracetamol is a promising additional analgesic that is applicable in both primary care and hospital settings. Its use in horses has increased significantly over the past ten years and there is increasing scientific evidence supporting its use as an analgesic drug. Current use of paracetamol in equine practice is mainly limited to oral use, and there are potential concerns around prescribing that should be addressed by regulatory bodies. The authors pharmacological studies show that intravenous paracetamol has potential as a short term analgesic agent in horses, but further research is required before advocating rectal administration as an appropriate method of administration. Further work should focus on documenting the pharmacodynamic effects of intravenous paracetamol around equine anaesthesia. The authors ongoing work with inertial sensors, accelerometry and artificial intelligence software shows promise in improving our ability to objectively monitor response to analgesia. Increased availability of safe and effective analgesics can help to improve the welfare of our equine population.

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Appendix

Appendix 1: Clinical use of Paracetamol Survey Questions

Investigating Veterinary Practitioner's use of paracetamol in equine patients

Consent:
This consent form is a formal way of indicating that you agree to participate in this study and that you understand that any information collected by the researchers:
• will be used for a research study
• may be written in a report for publication
• may be presented at research conferences or meetings
• will be anonymised and treated confidentially
• will only be accessed by research colleagues or examiners
• that you can request to see a copy/summary of the completed study
• that any comments you make will be anonymous so that in the final study write up, it will not be possible to identify you.

This survey should take no more than 10 minutes to complete. No questions are compulsory and you may skip any question.

1. I consent to participate in this study
    a. I consent
    b. I do not consent

2. If you would like a summary of the data collected from this survey please include your email below. Leave blank if not

Demographic information

3. In which country do you practice?

4. At which university did you study Veterinary Medicine?

5. What year did you graduate?

6. In which area of equine practice do you work?
    a. Equine practice
    b. Mixed practice
    c. Equine Hospital

d. Other

7. Do you have any post-graduate qualifications?

    a. No post graduate qualification

    b. Certificate holder/advanced practitioner

    c. FRCVS / RCVS Diploma / American or European Diplomate / Specialist

    d. PhD or DVM

    e. Other

8. What is your gender?

    a. Female

    b. Male

    c. Other

    d. Prefer not to say

Use of different analgesics

When ranking the drugs please drag and drop them in order from best to worst, with the considered best being at the top of the list.

9. How would you rank the analgesic properties of these drugs in the management of severe laminitis?
   BEST ANALGESIC AT THE TOP

    a. Firocoxib

    b. Flunixin
    c. Gabapentin

    d. Meloxicam

    e. Paracetamol

    f. Phenylbutazone

10. How would you rank the analgesic properties of these drugs in the management of severe cellulitis?
    BEST ANALGESIC AT THE TOP

    a. Firocoxib

    b. Flunixin

    c. Gabapentin

    d. Meloxicam

    e. Paracetamol

    f. Phenylbutazone

11. How would you rank the analgesic properties of these drugs in the management of bone spavin?
    BEST ANALGESIC AT THE TOP

    a. Firocoxib

    b. Flunixin

    c. Gabapentin

    d. Meloxicam

    e. Paracetamol

    f. Phenylbutazone

12. How would you rank the analgesic properties of these drugs in the management of severe palmer heel pain/navicular disease?
    BEST ANALGESIC AT THE TOP`

    a. Firocoxib

    b. Flunixin

    c. Gabapentin

    d. Meloxicam

    e. Paracetamol

    f. Phenylbutazone

13. How would you rank the analgesic properties of these drugs in the management of thoracolumbar pain?
    BEST ANALGESIC AT THE TOP

    a. Firocoxib

    b. Flunixin

    c. Gabapentin

    d. Meloxicam

    e. Paracetamol

    f. Phenylbutazone

Paracetamol use

This section is about how you use paracetamol in your equine patients

14. How often do you use paracetamol for equine patients?

    a. Daily

    b. At least weekly

    c. Less than weekly

    d. Rarely

    e. Never

15. Why do you use paracetamol? Please select as many as appropriate.

    a. Cost

    b. Safety profile

    c. Effectiveness

    d. Convenience

    e. Where other analgesics have been ineffective
    f. Other

16. What dose of paracetamol would you normally use in a horse in mg/kg
    If you are unsure of the dose, but know number of tablets please answer the next questions.
    Please write number only (not units)

17. OR – if you don't know the exact dose
    How many 500mg tablets of paracetamol would you give to a 500kg horse?
    Do not include units

18. How often do you recommend administering paracetamol to horses?

    a. Once daily

    b. Twice daily

    c. Three times daily

    d. Other

19. What formulation of paracetamol do you give to equine patients? Please select as many as appropriate.

    a. Intravenous

    b. Human tablets

    c. Canine tablets (Pardale)

    d. Porcine liquid (Pracetam)

    e. Paste formulation (special)

    f. Other

20. How do you obtain consent from your clients in regards to using paracetamol?

    a. I do not obtain specific consent

    b. Signed consent

    c. Verbal consent

    d. Client information sheet

    e. Other

21. If using paracetamol tablets, how many tablets are you prepared to dispense at a time to a client?

22. Do you recommend to clients that they buy paracetamol from supermarket/pharmacies for use in their horse?

    a. Only in emergency situations

    b. Sometimes

    c. Rarely

    d. Never

23. Any other comments?