Ultrasound-guided perineural injection of the tibial nerve in the horse versus a blind technique

Nicholas Alberto Bellitto

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School of Biodiversity, One Health & Veterinary Medicine

College of Medical, Veterinary and Life Science

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Abstract

Background: Proficiency in performing tibial perineural analgesia is an essential skill for clinicians carrying out lameness examinations in horses, allowing accurate localisation of the source of pain. Blind tibial perineural analgesia, however, often fails to provide reliable and prompt onset of analgesia despite the superficial location of the tibial nerve. The most common causes of failure include erroneous subcutaneous injection without penetration of the superficial crural fascia, erroneous intramuscular injection of the lateral deep digital flexor muscle or intravascular injection of the caudal root of the saphenous and caudal femoral veins. To overcome these difficulties ultrasound (US)-guided techniques for tibial perineural analgesia have recently been described and evaluated in cadaver studies but data supporting the use of US-guided tibial perineural analgesia clinically remains.

Objective: To compare US-guided and blind tibial perineural analgesia techniques in lameness investigation.

Materials and Methods: This study describes a randomised, prospective clinical trial. All cases were horses presented for lameness investigation which required tibial perineural analgesia. The cases were randomly assigned to US-guided or blind tibial perineural analgesia. Perineural analgesia was performed at the caudomedial aspect of the distal crus, about 10 cm proximal to the tuber calcanei between the common calcaneal tendon and the lateral deep digital flexor muscle. Injections were performed with the limb bearing weight and using mepivacaine hydrochloride 2% (w/v). Blind tibial perineural injections were performed after the nerve had been palpated with the limb in a non-weightbearing position. US-guided injections were performed using an 8-12 MHz linear transducer which was placed in a transverse orientation; the needle was inserted caudal to the nerve and redirected during injection to allow distribution of the anaesthetic agent around the nerve (single skin penetration). Onset of tibial perineural analgesia was assessed by testing loss of skin sensation at the medial and lateral heel bulbs, which were selected as autonomous zones (dermatomes) of the tibial nerve and following a review of the literature. Loss of skin sensation was assessed by measuring the mechanical nociceptive threshold (MNT) of each skin location using a hand-held algometer with a 1 mm diameter pin. A MNT value of 25 Newton (N) would indicate complete loss

of skin sensation (MNT values for this specific pin had been previously validated). Skin sensation was assessed, prior to injection and then at four intervals post-injection (10–15, 20–25, 30–35 and 40–45 minutes). At each recording, 3 measurements were performed for each skin location and the mean value was used for analysis. The time taken to perform each injection technique and any adverse reactions were recorded (e.g. horse that snatched the limb away or kicked out). Summary statistics were performed to examine differences between groups. The frequency of skin desensitisation was compared between groups using a Fisher's exact test and the length of time taken to perform injections was compared using a Mann–Whitney *U* test.

Results: Sixteen US-guided and 11 blind injections were included in the study. All cases undergoing US-guided injection lost skin sensation, whereas this occurred in only one case receiving the blind injection. The US-guided group had a significantly higher probability of skin sensation loss ($p < 0.001$), although the injection technique took significantly longer to complete compared to the blind group ($p < 0.001$). No adverse reactions were noted with either perineural injection technique.

Conclusions: The US-guided technique described here resulted in a significantly higher percentage of cases with tibial nerve analgesia compared to cases undergoing the blind technique. No differences in patient tolerance and operator safety were observed between the injection techniques. The US-guided technique was straightforward to perform and resulted in complete tibial nerve analgesia within 30-35 minutes in all patients. The findings of this study suggest that the US-guided technique, therefore, should be used instead of the blind technique during lameness investigation when possible.

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Appendix 4: MVLS Interpretation of the University Code of Practice on Alternative Format Theses and Author Declaration & Contribution.

Preface

This is an alternative format thesis (MVLS Interpretation of the University Code of Practice on Alternative Format Theses provided in Appendix 4) which contains a published original research article as the central portion of the document (Chapter 2). The publication is entitled ‘Ultrasound-guided perineural injection of the tibial nerve in the horse versus a blind technique’ (Bellitto, N.A., Voute, L., Reardon, R. and Withers, J.M., 2024, published in Equine Veterinary Education, 36(2), pp.64-73) and contains the introduction, materials and methods, results and discussion sections as published by the journal.

Chapter 1 is a literature review of tibial perineural analgesia and related topics.

Chapter 3 completes the thesis with a general discussion and conclusion section.

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Author’s Declaration

“I declare that, except where explicit reference is made to the contribution of others, this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.”

Printed Name: Nicholas Alberto Bellitto

Signature:

Ethical Approval

The study design was approved by the School of Veterinary Medicine Research Ethics Committee, University of Glasgow (Ref EA28/20).

Consent was given by owners for the use of their horses (Chapter 2). The form used for this purpose is shown in **Appendix 1**.

Definitions/Abbreviations

US: Ultrasound.

MNT: Mechanical nociceptive threshold.

N: Newton.

ALDDFT: Accessory ligament of the deep digital flexor tendon.

PSL: Proximal suspensory ligament

min: minute/minutes

s: second/seconds

h: hour/hours

1 Literature review

1.1 Introduction

Lameness is the most common reason for which horses are presented to the equine clinician (Ross, 2011).

Diagnostic analgesia is routinely performed to aid in the localisation of the source of pain which is causing a horse's lameness. This approach relies on the property of local anaesthetic agents, once injected perineurally to the regional innervation of the affected region or into the affected synovial structure, to interrupt transmission of pain to temporarily reduce the degree of lameness and thus localise the source of lameness. Although important developments in the fields of diagnostic imaging and objective gait analysis to aid the equine clinician in the diagnosis of lameness have been made in recent years, diagnostic analgesia remains the main tool to reliably localise the source of lameness. Recent advances in the field of diagnostic analgesia have related to improvement in the accuracy of injection techniques, the development of novel injection techniques and the use of different local anaesthetic agents (Bassage & Ross, 2010; Baxter et al., 2020).

Perineural analgesia of the tibial nerve (more commonly referred to as tibial nerve block) plays an important role in the investigation of hindlimb lameness as it aids in localisation of musculoskeletal pathology to the distal crus and tarsus (Pilsworth & Dyson, 2015; Plowright & Dyson, 2015). That said, tibial perineural analgesia is reported by many clinicians to be challenging to perform and will therefore often be employed only as a last resort (Bassage & Ross, 2010; van der Laan et al., 2021). The main challenges are a failure to achieve complete nerve desensitisation on the first attempt, requiring the clinician to repeat the procedure (Denoix et al., 2020; Pilsworth & Dyson, 2015; Schumacher & Schramme, 2019), and slow onset of nerve analgesia post-injection of up to 1 hour (h) in many cases (Bassage & Ross, 2010; Denoix et al., 2020).

Ultrasound (US)-guided perineural injection techniques, commonly used in human medicine, have been gaining popularity amongst veterinary surgeons mainly because of their higher accuracy of injection compared to conventional blind perineural injection techniques (techniques that rely only upon standardised

anatomical landmarks and palpation to guide location for perineural injection) (Portela et al., 2018b, 2018a; Weir & Strichartz, 2012).

The need for more accurate perineural injection techniques when investigating lameness in the horse has prompted research studies to develop and validate innovative US-guided injection techniques (Beaumont et al., 2020; Denoix et al., 2020).

Recently, US-guided techniques for tibial perineural analgesia have been described and have also been evaluated in cadaver studies in attempts to overcome the challenges reported when performing blind tibial perineural analgesia (Colla et al., 2023; Denoix et al., 2020; van der Laan et al., 2021).

The lack of in vivo studies supporting the use of US-guided tibial perineural analgesia in lameness investigation has stimulated the research project described in this thesis in which a US-guided injection technique for tibial perineural analgesia was compared with a blind injection technique, using the time of onset of loss of skin sensation in the distal limb as the primary outcome measure.

1.2 The role of perineural analgesia in lameness investigation

Lameness is a clinical sign that can manifest while the horse is static and/or as a gait abnormality, indicating the presence of an underlying structural or functional disorder. When assessing the lame horse, gait alterations due to painful and nonpainful conditions (i.e. conditions causing neuromuscular or mechanical dysfunction) must be differentiated, with the former being more common in horses, as horses with gait alterations due to nonpainful conditions will not be suitable candidates for diagnostic analgesia (Baxter et al., 2020).

Clinical examination findings may assist in the localisation of the source of lameness (e.g. soft tissue swelling) and occasionally pathognomonic gait abnormalities will indicate the precise localisation (e.g. upward fixation of the patella, fibrotic myopathy, stringhalt), allowing the clinician to perform targeted diagnostic imaging of the affected region to identify and characterise the pathology (Ross, 2011). However, clinical examination will frequently not localise the source of lameness and may, at times, even be misleading (e.g. young Thoroughbred racehorses may react to foot palpation using hoof testers or react on firm palpation of the proximal

suspensory ligament without these findings being relevant to the lameness) (Davidson, 2018) and the gait of most lame horses very often is not pathognomonic.

Diagnostic analgesia is important in establishing the relevance of the findings identified on clinical examination and in localising the source of pain in those cases in which no obvious abnormalities are detected on clinical examination prior to performing targeted diagnostic imaging. Diagnostic analgesia includes perineural and intrasynovial analgesia; and requires the clinician to have a good knowledge of musculoskeletal anatomy, peripheral nervous system anatomy and lameness to avoid misinterpretation of the result (i.e. improvement or not in the lameness) (Pilsworth & Dyson, 2015).

Diagnostic imaging in most cases will only be performed after the source of lameness has been localised to an anatomical region. However, in selected instances imaging modalities, such as nuclear gamma scintigraphy or radiography, may be utilised for 'screening' prior to diagnostic analgesia. Diagnostic analgesia may then have a role to play in confirming the clinical significance of any abnormalities visualised when there is uncertainty (Barrett et al., 2020; Quiney et al., 2018).

Perineural analgesia is often performed in a stepwise manner starting in the distal limb and progressing proximally, sequentially desensitising the limb, until a significant improvement in lameness is seen. Starting proximally reduces the accuracy with which the source of pain is localised, and therefore requires time to be allowed for lameness to reappear if further perineural analgesia is to be used for more accurate localisation (Bassage & Ross, 2010; Schumacher & Schramme, 2019).

When interpreting responses to perineural analgesia, it is important to be aware that this diagnostic tool is less specific than formerly thought, with some of the limitations strongly dependent on the particular perineural injection technique (Schumacher & Schramme, 2019).

The clinician should know what structures will be desensitised with the perineural injection technique being performed, as well as being aware of what other structures may inadvertently be desensitised (e.g. following inadvertent penetration and/or diffusion of local anaesthetic agent to structures in close proximity, such as synovial structures or other nerves) to make the technique less than 100% specific (Hinnigan et al., 2014). Proximal diffusion can be reduced by using the lowest volume possible

to achieve nerve desensitisation and making the first assessment within 10 minutes (min) post-injection (Schumacher & Boone, 2021).

Clinicians should also be aware that perineural analgesia more consistently abolishes lameness caused by articular pain than intra-articular analgesia. The difference in response relates to which articular structures are affected by pathology and how those structures are innervated. Pain secondary to synovial membrane pathology, for example, will respond better to intra-articular analgesia than pain secondary to subchondral bone pathology due to the different innervation of these structures (Bassage & Ross, 2010).

The synovial membrane innervation is located with the subintima loose connective tissue which is covered only by a single layer of synoviocytes and therefore is easily reached by the local anaesthetic agent injected intra-articularly; while the innervation of the subchondral bone, which derives from endosteal branches of the peripheral nerve that enter the medullary cavity through the bone's nutrient foramen, is covered by the articular cartilage, and therefore, is not always reached by local anaesthetic agent injected intra-articularly, particularly in those cases in which the articular cartilage is intact (Pujol et al., 2018; van Weeren, 2016).

Diagnostic perineural analgesia should always be interpreted taking into consideration the clinical signs and following careful assessment of diagnostic imaging (Pilsworth & Dyson, 2015). A positive response to perineural analgesia, ideally, would consist in an abolishment of the lameness (100% improvement), or, in those horses bilaterally lame, in a 'switch of lameness' to the contralateral limb. However, this outcome is not always achieved for reasons that remain unknown, and a significant improvement (greater than 70%-80%) in the degree of lameness should be considered a positive response in most horses (Dyson et al., 2021).

1.3 Anatomy of hindlimb innervation in the horse

The tibial nerve, together with the peroneal nerves, originate from the sciatic nerve and are responsible for the motor and sensory innervation of the crus, tarsus, metatarsus and foot with the sole exception of the cutaneous innervation of the medial crus, dorsomedial metatarsus and fetlock region which is provided by the saphenous nerve (branch of the femoral nerve) (Budras et al., 2011; Levine et al., 2007; Singh, 2018).

The sciatic nerve derives from the lumbosacral plexus and emerges through the greater sciatic foramen; after running caudally over the sacrosciatic ligament, the sciatic nerve turns distally caudal to the hip joint to enter the thigh under cover of the biceps femoris muscle. At this level, it branches into the tibial and the common peroneal nerves. Both nerves run together until just proximal to the stifle joint. The common peroneal nerve then runs laterally between the biceps femoris muscle and the lateral head of the gastrocnemius muscle, while the tibial nerve runs between the two heads of the gastrocnemius and crosses the stifle on the surface of the popliteus muscle (Levine et al., 2007; Singh, 2018).

The common peroneal nerve runs under the tendon of the biceps femoris muscle and at the proximo caudolateral margin of the tibia divides into the deep and superficial branches. Before its subdivision, a cutaneous branch is detached (lateral cutaneous sural nerve) that innervates the skin over the lateral aspect of the crus (Singh, 2018).

The superficial peroneal nerve, courses in the groove between long and lateral extensor muscles and innervates the lateral digital extensor muscle and the skin of the lateral tarsus and metatarsus. The deep peroneal nerve courses deeply within the same groove and contributes to the innervation of the lateral and long digital extensor muscles and innervates the cranial tibial and peroneus tertius muscles (Budras et al., 2011; Levine et al., 2007; Singh, 2018). As the deep peroneal nerve courses over the hock, it branches into the dorsal metatarsal nerves (medial and lateral) which are purely sensory and innervate the dorsal aspect of the distal hindlimb.

The extent to which the deep peroneal nerve branches, the medial and lateral dorsal metatarsal nerves, provide sensory innervation to the dorsal aspect of the most distal portion of the hindlimb is unclear from the literature (Levine et al., 2007). Several authors report the hindfoot skin sensation to be exclusively derived from branches of the tibial nerve and others report that the branches of the peroneal nerves may contribute to skin sensation of the dorsal aspect of the hindfoot (Coleridge et al., 2020; Ghoshal, 1966; Singh, 2018).

The tibial nerve runs between the two heads of the gastrocnemius muscle and while still within the thigh, detaches a cutaneous branch (caudal cutaneous sural nerve) which descends in the fascial plane between the calcaneal tendon and lateral digital flexor muscle. This cutaneous branch supplies innervation to the skin

over the plantar-lateral aspect of the hock and metatarsus to the fetlock. The tibial nerve provides innervation to the gastrocnemius, popliteus and superficial digital flexor muscles, as well as the lateral and medial digital flexor muscles (Singh, 2018). At the level of the mid-crus, the tibial nerve emerges at the medial aspect of the crus just cranial to the common calcanean tendon of the biceps femoris and semitendinosus muscles (Denoix et al., 2020). In the mid-distal crus, the tibial nerve is within the superficial caudal crural compartment by the superficial and deep caudal crural fascia. The superficial caudal crural compartment also contains the caudal root of the saphenous and caudal femoral veins, lymphatic vessels and fat (Denoix et al., 2020) (Figures 1 & 2).

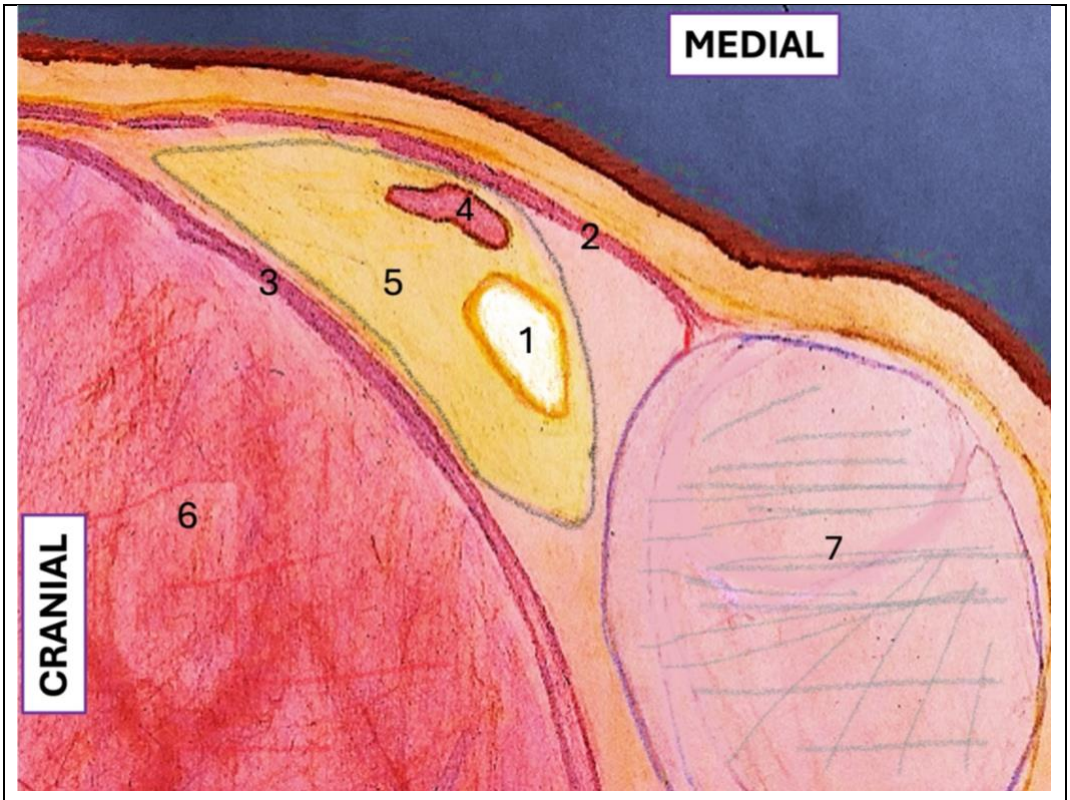


Figure 1: Drawing of a transverse anatomical section of the caudomedial part of the crus approximately 10 cm proximal to the tuber calcanei.

1 = Tibial nerve; 2 = Superficial caudal crural fascia; 3 = Deep caudal crural fascia; 4 = Caudal root of the saphenous vein and caudal femoral vein; 5 = Fat of the caudal crural compartment; 6 = Lateral digital flexor muscle body; 7 = Common calcaneal tendon.

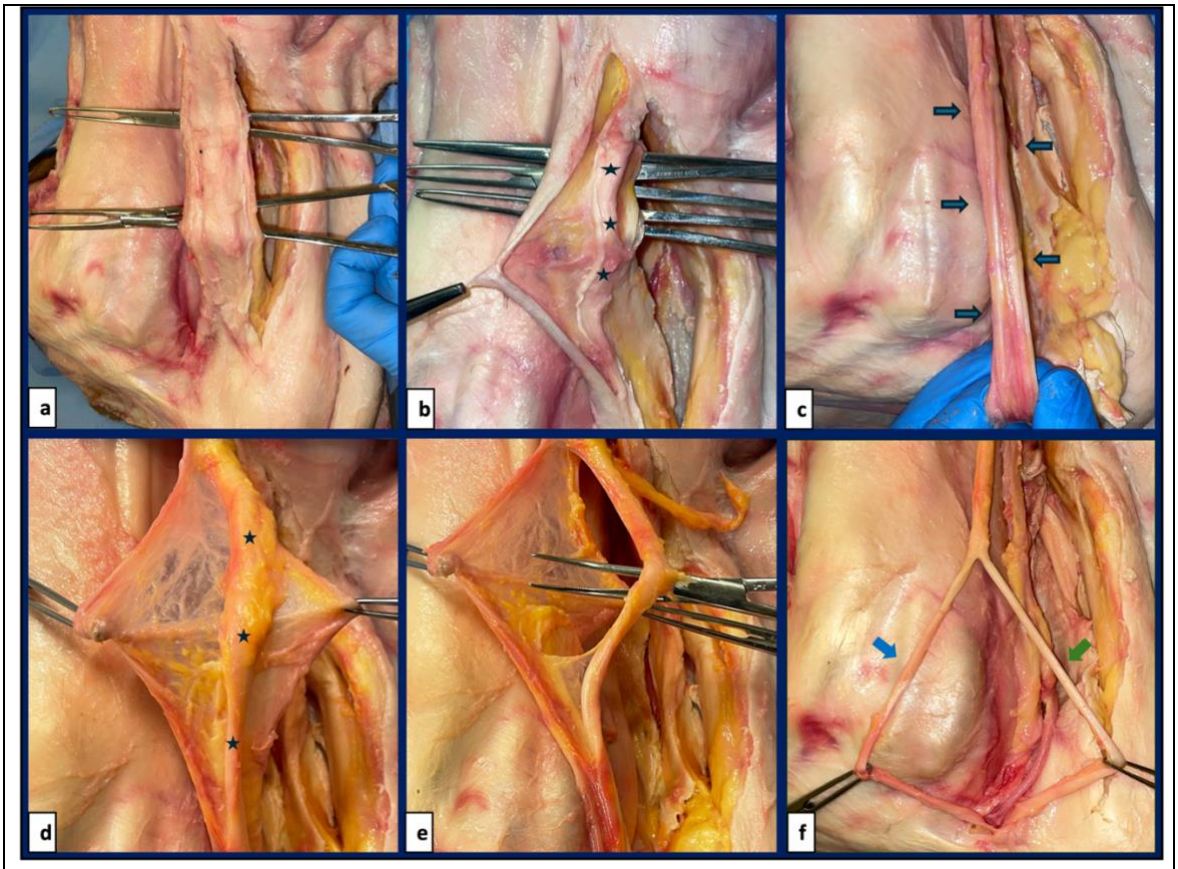


Figure 2: Image series of cadaver limb dissection to show anatomical features of the tibial nerve and associated regional anatomy of the medial aspect of the mid and distal crus (centred approximately 10 cm proximal to the tuber calcanei). Cranial is at the left and proximal at the top of the images.

(a) Superficial caudal crural compartment dissected free from surrounding tissues and elevated by two forceps. (b) The superficial caudal crural fascia has been incised longitudinally and retracted using thumb forceps (left in image) to expose the tibial nerve (blue stars), which is surrounded primarily by fat (c) Tibial nerve within the fascicle sheath held in tension following dissection to remove fat (blue arrows). (d) Tibial nerve (blue stars) exposed by retraction of fascicle sheath using forceps positioned on each dissected margin. (e) Tibial nerve separated from the fascicle sheath; single forceps retracts the fascicle sheath (left in image), double forceps elevate the nerve (right in image). (f) Tibial nerve division into the lateral and medial plantar nerves, forceps isolate each division: medial plantar nerve left in image (blue arrow) and lateral plantar nerve right in image (green arrow).

The tibial nerve, proximally to the calcaneus, divides into the lateral and medial plantar nerves, which run over the sustentaculum tali in proximity to the deep digital flexor tendon. The level of the division most commonly occurs 3.5-4 cm proximal to

the tuber calcanei but may vary from the level of the tuber to 9 cm proximal Ghoshal (1966) (Figure 2).

The lateral plantar nerve gives off a deep branch at approximately 2–4 cm proximal to the head of the fourth metatarsal bone, which provides innervation to the proximal aspect of the suspensory ligament and gives rise to the medial and lateral plantar metatarsal nerves (Pezzanite et al., 2020). The lateral and medial plantar and plantar metatarsal nerves are responsible for the sensory innervation of the distal limb (metatarsus, fetlock and foot) (Budras et al., 2011; Levine et al., 2007; Singh, 2018).

1.4 Local anaesthetic agents used for perineural analgesia

In 1884, Karl Koller, an Austrian human ophthalmologist, used cocaine to perform the first surgery under local anaesthesia. Soon after, cocaine became widely used as a local anaesthetic agent in clinical procedures across Europe and North America. Cocaine, in 1885, was also used by a Pennsylvania veterinarian to desensitise a horse's limbs. However, the administration of cocaine to patients had to be interrupted a few years later when its toxic effects, manifested as numerous deaths among both patients and addicted medical staff, became evident. In the decades following, local anaesthetic agents with lower toxicity and a longer duration of action were developed and were used in place of cocaine, most of which are still currently in use (Ruetsch et al., 2001; Schumacher & Boone, 2021).

Local anaesthetic agents prevent propagation of the nerve action potential along the axon by inhibiting the influx of sodium ions through channels within neuronal membranes resulting in blockage of nerve conduction (Becker & Reed, 2012).

The molecular structure of a local anaesthetic is composed of three portions: lipophilic aromatic ring, intermediate ester or amide linkage, and terminal amine. These components contribute to the distinct clinical properties of each local anaesthetic agent (Becker & Reed, 2012).

The molecular composition of the aromatic ring is the main determinant of the differences in lipid solubility present between local anaesthetic agents. A greater lipid solubility enhances diffusion through nerve sheaths and neural membranes and

therefore determines local anaesthetic agent potency and rapidity of onset of analgesia (Becker & Reed, 2012).

The molecular composition of the intermediate linkage, amide or ester molecule, determines the pattern of elimination of the local anaesthetic agents and provides a practical basis for classification. Amide-type local anaesthetic agents are bio-transformed in the liver while ester-type local anaesthetic agents are hydrolysed in the bloodstream by plasma esterases (Taylor & McLeod, 2020).

The terminal amine, instead, determines the ability of the local anaesthetic to penetrate the lipid cell nerve membrane because of its ability to convert from a tertiary form (lipid-soluble form) into a quaternary form (water-soluble form) and vice versa. When the terminal amine is in a tertiary form, the local anaesthetic agent is able to enter the lipid cell nerve membrane, while this will not be possible when the terminal amine is in a quaternary form (Becker & Reed, 2012).

The pKa of local anaesthetic solutions significantly influences their action kinetics. The pKa represents the pH at which 50% of the drug is in a quaternary form (water-soluble form) and 50% is in a tertiary form (lipid-soluble form). Local anaesthetics naturally exist in equilibrium between these two forms. When the pKa of a local anaesthetic is closer to physiological pH, a larger proportion of the drug is in its tertiary form (lipid-soluble form) accelerating onset of analgesia (Becker & Reed, 2012).

Local anaesthetic agents used in veterinary and human medicine are typically formulated as water-soluble hydrochloride salts, where the terminal amine is in quaternary form, with a pH usually ranging between 5 and 6. Upon injection into tissues, the local anaesthetic solution encounters tissue buffers, which raise its pH. This alkalization facilitates the conversion of the terminal amine into a lipid-soluble tertiary form, allowing the local anaesthetic agent to transfer through the lipid cell nerve membrane. Once inside neuronal cells, the tertiary amine reverts to the quaternary form, enabling the local anaesthetic to block sodium channels and interrupt nerve conduction (Taylor & McLeod, 2020).

Local tissue toxicity can result from injections of local anaesthetics, due to the solutions' irritating nature and the pressure exerted by large injectate volumes, potentially leading to ischemic tissue necrosis. The severity of local toxicity is both concentration-dependent and specific to the type of local anaesthetic agent used. Hence, the selection of agents with low toxicity profiles, such as mepivacaine, and

the use of low-concentration solutions (typically 2%) are crucial for ensuring patient safety. Solutions with higher concentrations pose a greater risk of causing ischemic necrosis and direct neurotoxic effects on nerves (Becker & Reed, 2012; Taylor & McLeod, 2020).

In veterinary and human medicine, local anaesthetic agents belonging to the amide-type sub-category are predominantly used. Common examples include lidocaine, mepivacaine, bupivacaine, ropivacaine and levobupivacaine, all of which are widely utilized for their efficacy (i.e. ability to desensitise nerve fibres) and safety profiles (low toxicity) in clinical practice (Becker & Reed, 2012).

The local anaesthetic agents most commonly used for diagnostic analgesia in horses are lidocaine hydrochloride 2% (w/v) and mepivacaine hydrochloride 2% (w/v). Many experts have suggested that mepivacaine hydrochloride 2% (w/v) should be the local anaesthetic agent of choice, when performing lameness investigations, because of its higher efficacy, lower tissue inflammatory properties and longer duration of action compared to lidocaine (Baxter et al., 2020). This suggestion is substantiated by a study that compared the use of lidocaine hydrochloride 2% (w/v) and mepivacaine hydrochloride 2% (w/v) for palmar digital perineural analgesia in horses with an induced lameness, which found that mepivacaine reliably eliminated lameness while lidocaine did not (Hoerdemann et al., 2017). On the other hand, when lidocaine hydrochloride 2% (w/v) is combined with epinephrine the efficacy seems to be comparable to mepivacaine hydrochloride 2% (w/v) (Boorman et al., 2022). Boorman et al., (2022) compared the use of lidocaine hydrochloride 2% (w/v) containing 5µg/mL (1:200 000) epinephrine and mepivacaine hydrochloride 2% (w/v) for median and ulnar perineural analgesia and did not find any difference in efficacy.

The addition of epinephrine results in prolonged and intensified analgesia by counteracting the vasodilatory effect on neural vasculature to result in an increased uptake and slower local clearance of lidocaine (Alvarez et al., 2018). For these reasons, lidocaine hydrochloride 2% (w/v) containing 5µg/mL (1:200 000) epinephrine is commonly preferred to lidocaine hydrochloride 2% (w/v) alone for equine perineural analgesia (Schumacher & Schramme, 2019).

Sodium bicarbonate is another additive used to enhance the potency and speed of onset of local anaesthetics for perineural analgesia. Its addition creates a buffered solution, increasing the pH and thus the proportion of the fat-soluble form of the

anaesthetic agent. This results in a more intense and rapid onset of analgesia (Boone et al., 2019).

Despite these benefits, its use remains uncommon among equine practitioners due to the limited research and clinical evidence supporting its efficacy and safety in horses. However, a recent study by Boone et al. (2019) reported that combining sodium bicarbonate with mepivacaine hydrochloride 2% (w/v) led to earlier onset and more profound analgesia compared to mepivacaine hydrochloride 2% (w/v) alone for perineural analgesia of the median and ulnar nerves. These encouraging results may prompt more equine practitioners to use sodium bicarbonate as an additive to local anaesthetics, particularly for perineural analgesia of large nerves (Boone et al., 2019).

Mepivacaine hydrochloride 2% (w/v) is considered relatively fast-acting (less than 5 min for palmar digital nerve perineural analgesia) and has a duration of action reported to range from 90 min to 3 h (Harcourt et al., 2021). However, the rapidity of onset of perineural analgesia is not only dependent on the innate properties of the local anaesthetic and on the use of additives, but also on the proximity of injection to the nerve, size of the nerve, physiological conditions of the tissue at the site of injection and the dose of the local anaesthetic (determined by concentration and volume) (Schumacher & Boone, 2021)

The larger nerves are usually reported to have a slower onset of analgesia, likely because of the longer time required for the local anaesthetic to penetrate to the core nerve fibres (the more peripheral fibres are anaesthetised earlier) (Schumacher & Schramme, 2019). The onset of perineural analgesia is reported to range from 5-10 min for the distal limb nerves up to over 1 h for the larger nerves of the proximal limb (e.g. median, ulnar, peroneal and tibial nerves).

For the larger nerves of the proximal limb, the pattern of onset of analgesia in the limb could also be affected by the fibre arrangement because those innervating the most distal structures run centrally, while nerve fibres innervating the more proximal structures are more peripheral (Schumacher & Boone, 2021).

The onset and duration of perineural analgesia are influenced by the presence of inflammation in the tissues surrounding the nerve at the injection site. Inflamed tissues have a lower pH which results in a lower proportion of the lipid-soluble form being available (Schumacher & Boone, 2021). Additionally, peroxynitrite, an

oxidising agent produced by inflamed tissues, can interact with local anaesthetics to modify their pharmacological activities to reduce efficacy (Ueno et al., 2008).

Recommendations for volumes of local anaesthetics to be used for specific perineural analgesia techniques for lameness diagnosis vary in the literature and appear to be based on clinical experience rather than evidence (Schumacher & Schramme, 2019). However, using the lowest volume possible for effective desensitisation is an appropriate guiding principle.

1.5 How is onset of perineural analgesia assessed and the role of skin sensation?

The importance of assessing whether perineural analgesia has resulted in successful nerve desensitisation, before re-evaluating the horse's degree of lameness, cannot be underestimated, as the conclusions drawn will significantly affect the decision-making process of the clinician, including decisions on further diagnostics and/or treatments required (Pilsworth & Dyson, 2015).

Testing for reduction in skin sensation, by applying pressure with a ballpoint pen or other blunt object, within the dermatome corresponding to the nerve being blocked, is often used in a clinical setting as an indicator of successful perineural analgesia (Schumacher & Schramme, 2019).

Horses' response to skin sensation testing can differ between individuals and assessment of the horses' response prior to performing perineural analgesia (baseline skin sensation response) or testing skin sensation of the contralateral limb can be a useful guide for whether onset of perineural analgesia has occurred. Practical advice, when testing skin sensation, is to cover the horses' visual field (e.g. eye covered with a hand or using blinkers) to avoid pre-emption of skin contact and therefore avoid an anticipatory response. For the same reason, it is also advisable that the operator testing skin sensation is positioned on the side of the opposite limb (Bassage & Ross, 2010).

If the clinician should be unsure if skin sensation is still present or not, the horse's lameness should be reassessed and if only partially ameliorated or unchanged, perineural injection should be repeated to minimize the potential for misinterpretation (Pilsworth & Dyson, 2015).

In research environments, and less commonly in a clinical setting, subjective assessment of skin sensation by means of a ballpoint pen has recently been replaced by algometers (Gozalo-Marcilla et al., 2020). Algometers are devices which allow an objective, controlled and safe measurement of the maximum force, measured in Newton (N; unit of force), being applied to the skin prior to the horse reacting to the noxious mechanical stimulus (Schambourg & Taylor, 2020). The minimal intensity of the noxious mechanical stimulus that causes an aversive response, as a result of the activation of the nociceptive pathway to the spinal cord, is defined mechanical nociceptive threshold (MNT) (Taylor et al., 2016).

An increase in the amount of force applied to the skin by means of an algometer, compared to baseline values (i.e. increase in the MNT), required to induce an adverse reaction is an indicator of a reduction in skin sensation (partial loss of skin sensation). If no reaction is induced, this indicates a complete loss of skin sensation.

Algometers suitable for testing skin sensation in horses are available commercially or can be created by customising generic devices; they are either designed to be hand-held or fixed to the horse's limb (and operated remotely) and have a range of probe tips of different shapes and sizes (Jordana et al., 2014; Miagkoff & Bonilla, 2021; Schambourg & Taylor, 2020).

The size and shape of the probe tip have a considerable impact on the MNT values recorded with probe tips with 1 mm diameter providing less variability (i.e. more reliability) in the MNT measurements compared to larger diameter tips (Taylor et al., 2016).

When testing skin sensation, clinicians should be aware that noxious mechanical stimuli do not exclusively activate fibres responsible for pain (A-delta and C nociceptive fibres) but also activate non-nociceptive fibres responsible for mechanosensation (A-beta fibres). This makes discrimination between true MNTs and stimulation of mechanoceptors (known as the 'touch-on' response) not possible when assessing deep skin sensation (Gozalo-Marcilla et al., 2020).

The 'touch-on' response may occur when the horse feels a blunt object (e.g., ballpoint pen or probe tip) contacting the skin. This can result in low and fictitious values recorded by the algometer, which are not genuine responses to a nociceptive mechanical stimulus and therefore should not be considered true MNT values. However, this inability to distinguish between true MNT and the 'touch-on' response does not have practical repercussions when MNT measurements are used to

assess the onset of perineural analgesia, as all nerve fibres are expected to be desensitised in this context (i.e. complete loss of sensation) (Schambourg & Taylor, 2020).

Alternative methods used to assess nociception in horses include electrical and thermal stimulation. Mechanical, thermal and electrical stimulation of the limb have all been observed to be reliable, sensitive and specific, indicating their validity for use in research. However, mechanical stimulation has so far been observed to provide the best results and is also the most practical method for the assessment of deep sensation/pain following perineural analgesia in horses (Luna et al., 2015).

1.6 Operators' safety and patient adverse reactions to perineural analgesia

Ensuring the safety of both the operator and the patient is paramount during perineural analgesia injections in horses.

An equine practitioner faces considerable personal injury risks, with incidents occurring approximately once every 43 months during routine clinical tasks like foot lameness examinations (11.3%), dental checks (6.8%), female reproductive assessments (6%), and distal limb diagnostic analgesia (5.3%). The most common causes of injuries are hindlimb kicks (49.1%) and forelimb strikes (11.8%) (Parkin et al., 2018).

Implementing appropriate restraint techniques is crucial for safeguarding practitioners and horses when performing perineural analgesia injections (Pearson et al., 2021). Sedation and effective handling techniques help minimise sudden movements of the horse that could lead to injuries, particularly in fractious or needle-shy horses, which pose heightened occupational risks (Termansen & Meehan, 2021). Recent studies have found that sedation, using low doses of alpha-2 agonists, does not alter baseline lameness levels, affirming its value in enhancing safety during perineural analgesia injection procedures, particularly in challenging patients (Termansen & Meehan, 2021).

Regarding patient safety, adverse reactions directly related to local anaesthetic agents are largely limited to transient soft tissue swelling post-injection (Rubio-Martinez & Hendrickson, 2021).

Complications associated with perineural analgesia injection technique errors mainly involve vascular puncture and nerve injury. Although vessel puncture may cause bleeding, systemic toxicity due to intravascular injection is rare due to the low volumes of local anaesthetics used in equine lameness investigations (Rubio-Martinez & Hendrickson, 2021).

Reports of serious nerve injuries from direct needle puncture or intraneural injection are also exceedingly rare in horses, in alignment with the low incidence observed in human studies (Sondekoppam & Tsui, 2017).

In recent years, there has been a growing advocacy for the use of US-guided perineural injections to enhance the safety of both operators and patients. US guidance ensures more precise needle placement compared to blind injections, thereby reducing the likelihood of inadvertent needle-related incidents, such as horses suddenly reacting violently due to nerve puncture. Moreover, US-guided techniques diminish the risks of inadvertent vascular puncture and nerve injury in the patient (Rubio-Martinez & Hendrickson, 2021).

1.7 Ultrasound-guided perineural analgesia in lameness investigation

US-guided techniques for perineural analgesia are the accepted gold standard in human medicine (Kruisselbrink & Chin, 2015) and are increasingly being used in veterinary medicine, with no additional risks reported compared to blind techniques (Portela et al., 2018a, 2018b).

Deposition of the local anaesthetic agent in close proximity to the nerve but not within the fascicle sheath of the neurovascular bundle has been reported to potentially cause a delay in onset and/or decrease in the level of analgesia and may even result in a failure to achieve complete nerve analgesia (Nagy et al., 2010).

Injection under US guidance allows visualisation of the target nerve thereby increasing the accuracy of needle placement, facilitating deposition of local anaesthetic within the fascicle sheath and achieving an increased success rate as well as a reduced time for onset of nerve desensitisation in comparison to blind injection techniques (Souto et al., 2020).

When performing US-guided injections, operators may employ two different levels of guidance: US-assisted injections and real-time US-guided injections. In US-assisted injections, the ultrasound is used exclusively to identify anatomical landmarks and the target nerve prior to performing the injection (van der Laan et al., 2021). In contrast, real-time US-guided injections involve the use of US imaging while positioning the needle, allowing visualisation of the needle while being advanced into the proximity of the nerve and allowing visualisation of deposition of local anaesthetic solution around the nerve (Denoix et al., 2020).

It is generally advisable to scan the injection site with ultrasound before commencing the procedure. This preliminary scan allows the operator to precisely locate the target nerve, select an appropriate needle path to avoid injury to adjacent structures and measure the depth of the target to ensure the selection of a suitable needle length (Estrada, 2024a).

The operator should know in advance where the needle is expected to appear on the screen of the US machine and the needle must be in alignment with the longitudinal axis and the centre of the transducer's footprint for ease of visualisation (Vaughan et al. 2009).

Linear, microconvex or macroconvex transducers may be suitable for US-guided injections, with selection determined by operator preference, depth of target structure and features of injection site (e.g. access, presence of a flat or concave, deformable or not, injection surface) (Estrada, 2024a).

US-guided perineural injection techniques for the median nerve, ulnar nerve and tibial nerve in equine clinical cases have been described (Beaumont et al., 2020; Denoix et al., 2020; Marolf et al., 2016; Souto et al., 2020). Ex vivo studies have assessed and compared US-guided perineural injections and blind perineural injections respectively for the tibial and peroneal nerves, but a comparison of the techniques in clinical cases has not been documented (Colla et al., 2023; van der Laan et al., 2021).

US guidance is not advantageous for all perineural injections. Key considerations for its use include the ability to clearly visualise the nerve (Estrada, 2024b) and whether the efficacy of blind perineural injection is already sufficiently high, such that the benefits of US guidance do not outweigh the practical disadvantages (Nagy & Malton, 2015). Notably, efficacy data for blind perineural injection is often lacking, underscoring the need for further studies in this area.

The primary disadvantages of US-guided injections include the necessity of having access to an ultrasound machine and the requirement for proficiency in its use for this specific application. While the principles of US guidance are straightforward, novice operators must perform several supervised procedures to achieve sufficient competence (Barrington et al., 2012; Sites et al., 2007).

1.8 Tibial perineural analgesia

1.8.1 Perineural techniques

Perineural analgesia of the tibial nerve is most commonly performed at the caudomedial aspect of the distal crus, about 10 cm proximal to the tuber calcanei between the common calcaneal tendon and the lateral deep digital flexor muscle. The nerve can be palpated as a firm cordlike structure at this level guiding the selection of the site of injection. The limb may be positioned bearing weight or flexed according to the preference of the clinician, with the latter allowing easier identification of the nerve (Bassage & Ross, 2010; Dyson, 1984). The needle is inserted over the caudal surface of the lateral deep digital flexor muscle, and following penetration of the skin and the superficial caudal crural fascia enters the superficial caudal crural compartment and the local anaesthetic agent is injected in close proximity to the nerve (Figure 1).

A lateral approach injecting from the caudolateral aspect of the distal crus instead of the caudomedial aspect has also been described (Bassage & Ross, 2010; Carpenter & Byron, 2017). However, the lateral approach is not commonly used probably because the greater depth of needle insertion increases the likelihood of injection inaccuracy (Moyer et al., 2011).

The volumes of local anaesthetic used for tibial perineural analgesia are reported to range from 10-20 mL, with most clinicians preferring to use higher volumes (Baxter et al., 2020).

For tibial perineural analgesia 2.5-3.8 cm, 21-23 G needles are commonly used (Bassage & Ross, 2010; Carpenter & Byron, 2017; Moyer et al., 2011).

When performing tibial perineural analgesia, particularly the blind injection techniques, clinicians should be aware of the most common pitfalls in order to prevent adverse outcomes.

Contact of the needle with the nerve should be avoided to prevent the horse from reacting violently, such as kicking out (Moyer et al., 2011; Schumacher & Schramme, 2019).

Subcutaneous injection and intramuscular injection into the lateral deep digital flexor muscle, located deep to the deep caudal crural fascia, must be avoided, as these will result in poor distribution of the local anaesthetic agent to the tibial nerve (poor diffusion through the superficial and deep crural fascia) leading to incomplete desensitisation of the nerve (Denoix et al., 2020; Moyer et al., 2011; Schumacher & Schramme, 2019).

Intravenous injection of the caudal root of the saphenous and caudal femoral veins should also be avoided, as this would result in failure to achieve perineural distribution of the local anaesthetic and thus unsuccessful desensitisation of the nerve (Denoix et al., 2020).

Due to these numerous issues encountered with blind tibial perineural analgesia, recent studies have focused on developing and validating US-guided tibial perineural injection techniques.

Denoix et al., (2020) described a US-guided technique for tibial nerve perineural analgesia in clinical cases. The tibial nerve is imaged using a 6-10 MHz microconvex probe positioned 10 cm proximal to the tuber calcanei, on the medial aspect of the crus at the cranial aspect of the common calcanean tendon and caudal to the caudal root of the medial saphenous and caudal femoral veins (Figure 1). The nerve is injected under US guidance from two separate injection sites (one slightly cranial and the other caudal to the probe) in order to completely surround the nerve with the local anaesthetic agent. The volume of local anaesthetic used ranged between 10-16 mL in total. According to Denoix et al., (2020), using this US-guided technique, a significant number of horses showed amelioration of the lameness starting 5-10 min post-injection.

More recently, two studies have compared US-guided tibial perineural analgesia with conventional blind injection techniques using cadaveric limbs (Colla et al., 2023; Van der Laan et al., 2021).

Van der Laan et al., (2021) used a low volume of dye (1 mL methylene blue) to assess the accuracy of both injection techniques. The US-guided injection was performed using a single injection site, with a 1-inch, 21 gauge needle inserted cranial to the nerve, and a linear US transducer (7.5 MHz) placed along the

transverse axis of the limb. US imaging, however, was only used to assist in tibial nerve localisation prior to needle insertion and not to guide the insertion of the needle in real time. Tibial nerve staining was found to occur in 85.7% of limbs injected with the US-guided technique and in 47.6% of the limbs injected with the blind technique.

Colla et al., (2023) used a mixed radiopaque contrast agent and dye solution to compare the accuracy and assess the diffusion of injectate between a US-guided technique and a conventional blind injection technique. Staining immediately adjacent to the tibial nerve occurred in all cadaver limbs for both injection techniques. For the tibial US-guided injection, a 10-14 MHz linear ultrasound transducer was positioned 10 cm proximal to the tuber calcanei on the lateral aspect of the limb and used to guide the needle to the nerve from an entry point proximal to the transducer (real-time US-guided injection). The volume of injectate US-guided injection was 3 mL, compared to 10 mL used for the blind injection, and a 1.5-inch, 22 gauge needle was used.

The description provided by Colla et al., (2023) (“the transducer was placed medially, 10 cm proximal to the tuber calcanei, to visualise the tibial nerve, a needle was guided to the nerve from placement proximal to the transducer”), is unclear, making the injection technique difficult to reproduce due to the limited information provided. The placement of the needle is described as being proximal to the transducer, suggesting that the US transducer and needle were placed along the longitudinal axis of the limb, in contrast to the techniques described by Van der Laan et al. (2021) and Denoix et al. (2020), where the US transducer was positioned transversely.

Volumes of injectate markedly differed between the two ex vivo studies, with Colla et al. (2023) using 3 mL of solution and Van der Laan et al. (2021) using 1 mL of solution. In the study by Colla et al. (2023), the presence of dye immediately adjacent to the nerve was classified as a successful perineural injection, while in the study by Van der Laan et al. (2021), the perineural injection was considered successful only if the nerve was found to be coloured with dye. Therefore, the different criteria used to define the success of perineural injection and the different volumes of injectate could explain the differences in results observed between these two ex vivo studies.

All the US-guided tibial perineural injections so far described in cadaver limbs and live horses have substantial differences in their injection techniques. These differences prevent direct comparisons between studies highlighting the need for further research to validate the use of US-guided tibial perineural analgesia in horses.

1.8.2 When and how to assess if perineural analgesia of the tibial nerve has been achieved?

In the literature, it is reported that the onset of tibial perineural analgesia following the blind technique should be assessed starting from 10 min until up to 1 h after injection (Bassage & Ross, 2010; Denoix et al., 2020). Precise locations recommended for testing skin sensation to determine if nerve analgesia has been achieved vary between authors, although the plantar metatarsal region and the heel bulbs are the most commonly reported locations (Table 1).

Skin location for testing tibial perineural analgesia	**References**
Heel bulbs and medial metatarsus.	Labens et al., 2012; Moyer et al., 2011; Prange, 2019.
Plantar-medial metatarsus.	Budras et al., 2011; Levine et al., 2007; Prange, 2019.
All plantar aspect of distal limb (including plantar-medial metatarsus and heel bulbs).	Carpenter & Byron, 2017; Skarda et al., 2009.

Table 1: Skin testing locations for tibial perineural analgesia based on the current literature.

Loss of skin sensation, following tibial perineural analgesia, has been reported by some experts not to occur in all horses, with lameness resolving without loss of skin sensation (Bassage & Ross, 2010; Dyson, 1984). It is possible, in these cases that complete nerve desensitisation did not occur or that skin was not tested at an appropriate location. Although this finding has not been rigorously investigated and appears to be anecdotal, the recommendation is that lameness should be assessed at regular intervals post-injection (every 10 min until up to 1h) to observe for any

change, irrespective of whether no or partial skin desensitisation is obtained (Bassage & Ross, 2010; Denoix et al., 2020; Moyer et al., 2011).

1.8.3 Tibial perineural analgesia during lameness assessment

Tibial perineural analgesia is indicated when more distal diagnostic analgesia, such as a high plantar nerve block, has either failed to improve or has only partially improved hindlimb lameness. Tibial perineural analgesia can be performed simultaneously with perineural analgesia of the superficial and deep peroneal nerves or separately, typically depending on the clinician's preference (Bassage & Ross, 2010; Schumacher & Schramme, 2019).

Performing tibial perineural analgesia separately can be particularly useful for more precise identification of the site of pain. For instance, it helps differentiate between lameness due to proximal suspensory ligament (PSL) desmitis and lameness caused by osteoarthritis of the distal tarsal joints. These are common conditions in horses that can be difficult to distinguish and diagnose reliably, especially when the pathology is mild (Dyson et al., 2021; Schumacher & Schramme, 2019).

Tarsometatarsal joint analgesia and perineural analgesia of the deep branch of the lateral plantar nerve, which is derived from the tibial nerve and innervates the PSL, may yield false positive results due to the proximity of the injection sites and the risk of local anaesthetic agent diffusion between the two structures. Inadvertent penetration of the tarsometatarsal joint while performing perineural injection of the deep branch lateral plantar nerve is also a concern (Leelamankong et al., 2018).

Therefore, as innervation of the PSL is exclusively derived from the tibial nerve, while the tarsus is also innervated by the superficial and deep peroneal nerves, performing tibial perineural analgesia separately from the superficial and deep peroneal nerves can assist in differentiating between these two conditions (Dyson et al., 2021; Schumacher & Schramme, 2019).

Perineural analgesia of the tibial and the superficial and deep peroneal nerves is often more effective than intra-articular analgesia in eliminating pain from the hock joints. This is because, as previously discussed in Section 1.2, perineural analgesia more consistently abolishes lameness caused by articular lesions and subchondral bone lesions compared to intra-articular analgesia (Bassage & Ross, 2010). Consequently, improvement in lameness following perineural analgesia of these nerves, despite a poor response to intra-articular analgesia of the hock and after

excluding a more distal source of lameness, should not rule out the presence of articular pathology, including subchondral bone lesions.

Perineural analgesia of the tibial and the superficial and deep peroneal nerves also serves as an alternative to intra-articular analgesia of the distal tarsal joints, particularly for patients not compliant with intra-articular injections, reducing risks to both the operator and the patient. Additionally, perineural analgesia of these nerves can be performed when wanting to avoid injection of the centrodistal joint due to the technical challenges associated with this procedure (Hoaglund et al., 2019).

For horses with clinical findings suggestive of stifle joint pathology, clinicians should consider performing perineural analgesia of the tibial and the superficial and deep peroneal nerves to exclude the distal limb as a source of lameness, either before or after performing intra-articular analgesia of the stifle joints. This approach is supported by the observation that lameness in 30% of horses with distal limb pain improved by up to 50% within 30 min following stifle intra-articular analgesia, highlighting the risk of misdiagnosis if relying solely on the results of intra-articular analgesia of the stifle (Radtke et al., 2020). The most likely explanation for this phenomenon is the extra-articular diffusion of the local anaesthetic agent to the tibial and common peroneal nerves, which are in close proximity to the stifle joints.

Examples of causes of lameness that respond primarily to tibial, superficial and deep peroneal perineural analgesia include:

- Talocalcaneal joint osteoarthritis: Smith et al. (2005) found that in horses affected by talocalcaneal osteoarthritis, tarsocrural joint analgesia produced an improvement in lameness only in 6 out of 11 horses (55%) while perineural analgesia of the tibial and superficial and deep peroneal nerves produced an improvement in lameness 10 out of 11 of horses (93%).
- Injury to the proximal portion of the accessory ligament of the deep digital flexor tendon (ALDDFT) or entheses pathology of the third metatarsal bone: Plowright & Dyson (2015) reported that horses with concurrent injuries of the PSL and of the ALDDFT or entheses pathology of the third metatarsal bone (at the origin of the PSL), unresponsive to perineural analgesia of the deep branch of the lateral plantar nerve, require tibial perineural analgesia to resolve the lameness.
- Injury to the distal aspect of the common calcaneal tendon: Dyson & Kidd (1992) reported that in horses with gastrocnemius tendinitis, perineural

analgesia of the tibial and the superficial and deep peroneal nerves resulted in a substantial improvement in lameness.

- Distally located tibial stress fractures: Perineural analgesia of the tibial and the superficial and deep peroneal nerves results in resolution of lameness in horses with mid-diaphyseal and distal metaphyseal stress fractures of the tibia (Pilsworth & Dyson, 2015).

1.9 Aim of this study

The overall aim of this study was to compare US-guided tibial perineural analgesia with blind tibial perineural analgesia in horses. The hypotheses tested and objectives were:

1st Hypothesis

The hypothesis was that US-guided injection would result in a higher proportion of horses having loss of skin sensation compared to horses undergoing blind injection and that horses undergoing US-guided injection would have loss of skin sensation at an earlier time than horses undergoing blind injection.

The objective was to compare US-guided and blind tibial perineural analgesia by assessing the onset of loss of skin sensation at the medial and lateral heel bulb.

2nd Hypothesis

The hypothesis was that US-guided injection would take longer to perform compared to the blind injection technique.

The objective was to compare the time required to perform US-guided and blind tibial perineural injections.

3rd Hypothesis

The hypothesis was that US-guided injection would result in fewer adverse behavioural reactions by horses compared to blind injection technique.

The objective was to compare horses' tolerance and operators' safety between US-guided and blind tibial perineural analgesia.

2 Ultrasound-guided perineural injection of the tibial nerve in the horse versus a blind technique

2.1 Introduction

Tibial perineural analgesia is a valuable aid in the diagnosis of musculoskeletal pathology of the hindlimb during lameness examination of the horse, allowing the clinician to identify sources of lameness originating from the distal crus, plantar tarsus, or the more distal limb (Bassage & Ross, 2010; Kawcak et al., 2020).

Perineural analgesia of the tibial nerve is achieved by injecting a local anaesthetic agent into the caudomedial aspect of the distal crus, approximately 10 cm proximal to the calcaneus between the common calcaneal tendon and the lateral digital flexor muscle (Bassage & Ross, 2010; Dyson, 1984; Moyer et al., 2011).

At this level the tibial nerve is located within the superficial caudal crural compartment (delimitated by the superficial and deep caudal crural fasciae), caudomedial to the lateral digital flexor muscle and cranial to the common calcaneal tendon (Denoix et al., 2020) (Figure 3).

Assessment of the response to tibial perineural analgesia has been recommended between 10 min and 1 h following injection (Bassage & Ross, 2010; Denoix et al., 2020). The potentially prolonged time required for adequate tibial perineural analgesia has been attributed anecdotally to the topography and large size of this nerve which may require a longer period of time for diffusion of the local anaesthetic agent (Denoix et al., 2020; Kawcak et al., 2020). Tibial perineural analgesia can fail because of erroneous subcutaneous injection without penetration of the superficial crural fascia, erroneous intramuscular injection of the lateral digital flexor muscle or intravascular injection of the caudal root of the saphenous or caudal femoral veins (also contained in the superficial caudal crural compartment), requiring the clinician to repeat the perineural injection (Denoix et al., 2020; Pilsworth & Dyson, 2015; Schumacher & Schramme, 2019). Inadvertent contact with the tibial nerve during placement of the needle can result in a violent reaction of the horse (e.g. kicking out, bucking, etc.) and therefore places the clinician at risk of injury (Moyer et al., 2011; Schumacher & Schramme, 2019).

Ultrasound (US)-guided technique is the accepted gold standard for perineural analgesia in human medicine (Kruisselbrink & Chin, 2015) and is increasingly used in veterinary medicine (Beaumont et al., 2020; Denoix et al., 2020; Portela et al., 2018a, 2018b). Injection under US guidance is reported to increase the accuracy of needle placement compared to blind injection techniques, potentially reducing complications associated with inaccurate deposition of injectate or inadvertent damage to surrounding structures (Jarosinski et al., 2020; Schneeweiss et al., 2012). Therefore, the use of US guidance for perineural analgesia in lameness investigation of the horse could result in an increased success rate of injection, more prompt onset of analgesia and increased operator and patient safety (Beaumont et al., 2020; Denoix et al., 2020; Kruisselbrink & Chin, 2015).

More recently, US-guided techniques for tibial perineural analgesia have been described and evaluated in cadaver studies (Denoix et al., 2020; van der Laan et al., 2021), but in vivo studies supporting the use of US-guided tibial perineural analgesia in lameness investigation are still lacking.

Subjective evaluation of skin sensation by applying firm pressure with a blunt object (e.g. ballpoint pen) is often used to assess if perineural analgesia has been adequately performed (Bassage & Ross, 2010; Schumacher & Schramme, 2019). More recently, algometers, instruments that allow measurement of the pressure applied, have been used to test skin sensation in the research setting (Gozalo-Marcilla et al., 2020; Hinnigan et al., 2014; Hoerdemann et al., 2017; Jordana et al., 2014).

The principal aim of this study was to compare a US-guided tibial perineural analgesia technique with a blind technique in lameness investigation in the horse by assessing the onset of loss of skin sensation in the tibial nerve's autonomous zones using an algometer. A further aim of this study was to compare horses' tolerance of the procedure and operator safety between US-guided and blind tibial perineural analgesia.

We hypothesised that the US-guided technique would result in a quicker and more consistent onset of loss of skin sensation of the distal limb compared to the blind technique. Also, we hypothesized that the US-guided technique would take longer to complete but be better tolerated by the horse compared to the blind technique.

2.2 Materials and methods

2.2.1 Animals

Horses were recruited from clinical cases presented for lameness examination to two equine referral hospitals over an 18-month period (2020–2022). All horses included in the study required tibial perineural analgesia for diagnostic purposes as part of a lameness investigation. Ethical approval for the study was granted by the lead institution (School Research Ethics Committee, School of Veterinary Medicine, University of Glasgow, Ref EA28/20) and horse owners gave written consent for participation. Horses were included in the study if no diagnostic analgesia procedures were performed within 6 h preceding tibial perineural analgesia on the limb being investigated, except for perineural analgesia of the superficial and deep peroneal nerves. None of the horses in the study received any sedatives or tranquillisers prior to or during tibial perineural injection.

2.2.2 Study Design

It was estimated that 10 cases of US-guided and 10 cases of blind tibial perineural injection would be sufficient to investigate the difference in the time required for loss of skin sensation at the heel bulbs. Sample size calculations were not performed as no pre-existing data were available.

Recruitment of 20 clinical cases was anticipated and these were randomly pre-assigned to either the US-guided or blind tibial perineural injection groups using a random-number generator (Excel, Microsoft Corporation) with an allocation ratio of 1:1. Cases were assigned based on chronological presentation (e.g. case number one was preassigned to the blind injection group). After completion of 20 cases, additional cases were sequentially randomised using a web-based programme (random.org, Randomness and Integrity Services Ltd).

Skin sensation was assessed prior to performing tibial perineural analgesia and at four subsequent time points following injection: 10-15, 20-25, 30-35, and 40-45 min. One investigator assessed skin sensation in all cases, while four operators, with a similar level of experience, performed the tibial perineural injections (three ECVS-certified surgeons and one surgical resident).

The time taken to complete the tibial perineural injection procedure, whether by US-guided or blind technique, was recorded for each clinical case, as well as any

complications that arose from the procedure, including reactions from the horse at the time of perineural injection that might endanger operator safety.

Effect of tibial perineural analgesia on lameness was purposely not reported as this was beyond the scope of this study.

2.2.3 Tibial perineural analgesia injection techniques

The anatomic site for tibial perineural injection was prepared by clipping the hair using a No. 40 clipper blade, followed by cleaning using a dilute chlorhexidine solution then alcoholic spirit (95% ethanol and 5% methanol)**.** In all cases, in both groups, 2 mL mepivacaine hydrochloride 2% (w/v) (Intra-Epicaine, Dechra Veterinary Prod) was deposited subcutaneously using a 25 gauge 5/8-inch needle prior to performing tibial perineural injection. Tibial perineural analgesia was performed by injecting 20 mL of mepivacaine hydrochloride 2% (w/v) into the caudomedial aspect of the distal crus, with the limb weightbearing and in a slightly retracted position, 10 cm proximal to the tuber calcanei, between the common calcaneal tendon and the lateral digital flexor muscle. The syringes containing the local anaesthetic agent were connected to the needle via a 200 cm long, 2 mm diameter extension line (Lectrocath, Vygon) in all cases.

US-guided perineural injections were performed using an 8-12 MHz linear transducer (Vivid S60N, GE Healthcare) and a 21 gauge, 1.5-inch needle. The transducer was placed in transverse plane at the level of the injection site allowing identification of the tibial nerve. The sonographic appearance of the tibial nerve has previously been described by others (Denoix et al., 2020). Briefly, the tibial nerve is oval in outline and echogenic, and lies superficial to the deep caudal crural fascia, caudal to the saphenous and femoral veins and cranial to the common calcaneal tendon (Figure 3). Following identification of the nerve, the transducer was moved cranially to create space for needle insertion caudal to the nerve (i.e. a caudal approach was used).

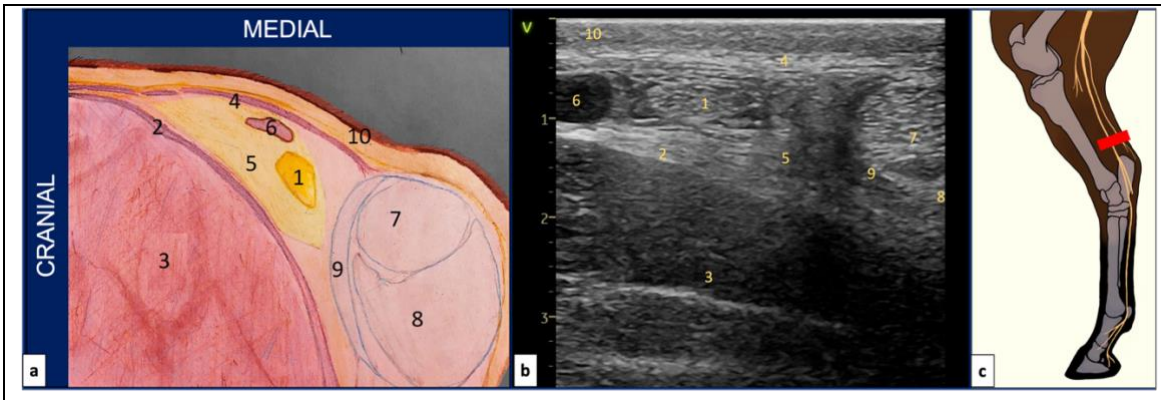


Figure 3: (a) & (b) Drawing of a transverse anatomical section of the caudomedial part of the crus and ultrasonographic image obtained at the injection site for tibial perineural analgesia.

1 = Tibial nerve; 2 = Deep caudal crural fascia; 3 = Lateral digital flexor muscle body; 4 = Superficial caudal crural fascia; 5 = Fat of the caudal crural compartment; 6 = Caudal root of the saphenous vein and caudal femoral vein; 7 = Superficial digital flexor tendon; 8 = Gastrocnemius tendon; 9 = Tendon of the caudal femoral muscles; 10 = Skin. (c) Drawing shows the site of a transverse anatomical section and transverse ultrasonographic image with the broad red line indicating the positioning of the ultrasound transducer.

The needle penetrated the limb at a 20-30° angle to the skin on the caudomedial aspect of the distal crus and was visualised along the long axis of the transducer. Following penetration of the superficial crural fascia, the needle was advanced in the caudal crural compartment until its tip was immediately adjacent to the tibial nerve. Local anaesthetic solution was first injected around the caudal aspect (10 mL) of the nerve and then the needle was redirected, under ultrasound guidance, at a 15-20° angle and advanced superficially in a cranial direction, to enable distribution of the local anaesthetic agent around the cranial aspect of the nerve (10 mL).

Alcoholic spirit (95% ethanol and 5% methanol) was used to provide contact between the ultrasound transducer and the skin.

Two operators were required for the ultrasound-guided technique; one held the transducer in one hand and the needle in the other (Operator A), while the second (operator B) held the syringe (extension set connecting syringe and needle) and injected under the instruction of operator A (Figure 4). Operator B was also

responsible for maintaining the safety of the transducer cable and for moving the ultrasound machine away from the horse if needed.

Operator A stood lateral to the limb being injected. Operator B and the ultrasound machine were positioned on the contralateral side of the horse, such that Operator A had a good view of the ultrasound machine monitor (Figure 4).

Blind perineural injections were performed using a 23 gauge, 1-inch needle. The nerve was first identified by palpation (firm cord-like structure) caudal to the lateral digital flexor muscle and cranial to the common calcaneal tendon with the limb in a flexed position. Then, with the limb in a weightbearing position, the needle was inserted up to the hub over the caudal surface of the lateral digital flexor muscle to position its tip close to the nerve. The needle was then redirected four times in a fan shape (45°, 75°, 105° and 135° angle to the skin) with 5 mL local anaesthetic agent deposited in each plane to allow distribution around the nerve. The operator performing the injection stood lateral to the limb being injected.

In addition to operator/s involved in the perineural injection, one person was required to restrain the horse.

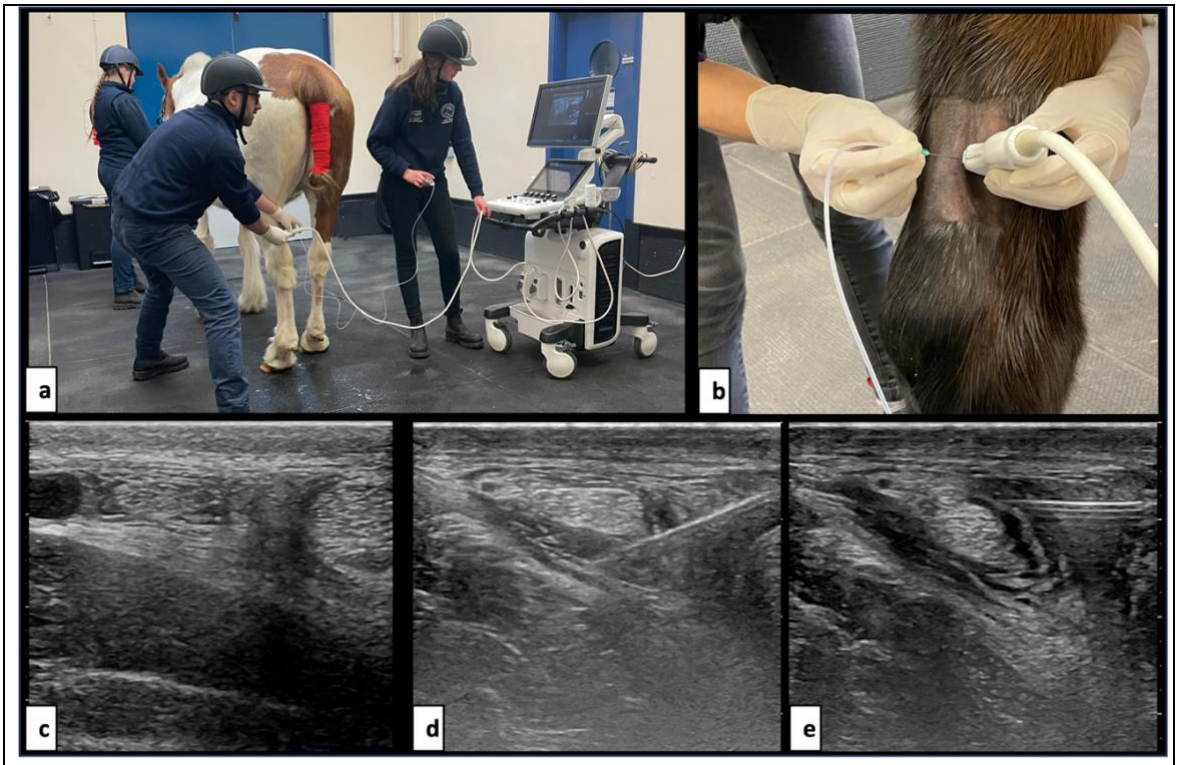


Figure 4: Images showing the US-guided technique being performed.

(a) Set-up and positioning of operators when performing perineural injection using the US-guided technique; the transducer is placed on the caudomedial aspect of the distal crus. (b) The image shows the operator handling the linear transducer and the needle attached to the extension line simultaneously; the tip of the needle penetrates the skin on the caudomedial aspect of the distal crus, just caudal to the transducer. (c–e) Sequence of ultrasonographic images showing US-guided perineural injection (caudal is to the right). (c) The tibial nerve is identified in a transverse plane just cranial to the superficial digital flexor tendon. (d) The tip of the needle is then inserted adjacent to the caudal margin of the tibial nerve. (e) Following injection of local anaesthetic around the caudal margin of the nerve the tip of the needle is redirected at the superficial (medial) margin of the nerve to allow further advancement and injection of local anaesthetic around the cranial margin of the nerve.

2.2.4 Skin sensation testing

Skin sensation was assessed by the maximum force that could be applied to the skin prior to inducing a horse's reaction. Application and measurement of force was

by a hand-held digital algometer (Prod, TopCat Metrology) attached to a long custom-made handle (Figure 5). An increase in the force, measured in newton (N), reflects an increased mechanical nociceptive threshold (MNT) and a reduction in skin sensation.

The algometer features a silent, pneumatic actuator with a 1 mm diameter flat-ended pin (Figure 5) and was manually applied against the limb's skin. The horses' eyes were covered by the operator holding the horse or by blinkers. The force applied to the skin was progressively increased at a rate of 1–2 N/s. The force rate increase was monitored using LEDs on the algometer, which guided the operator when testing skin sensation: green too slow, red too fast; LEDs are not illuminated when the rate is correct. The algometer was removed as soon as the horse reacted (limb lift or stamp, shoulder muscle contraction, shifting weight to the non-tested limb), with the MNT displayed being recorded, or applied until a value ≥ 25 N was reached. The MNT value of 25 N achieved using a 1 mm diameter pin indicated a complete loss of skin sensation (Schambourg & Taylor, 2020). Skin sensation measurements were performed prior to performing tibial perineural analgesia and at four time points following injection (10-15, 20-25, 30-35, 40-45 min after injection). A 5-min window was allowed for each testing time point to allow the operator to complete the task.

Three measurements at a minimum of 30-s intervals were carried out at each time point to ensure the reliability of the readings. If a horse reacted or moved for reasons unrelated to the test, that measurement was discarded and then repeated. The readings displayed were recorded for data analysis.

When a value ≥25 N was recorded at a skin location, no further measurements were made at that location at the remaining time points.

The locations used for testing were the lateral and medial heel bulbs (1-2 cm above the coronary band) (Figure 5), with measurements completed at the lateral heel bulb at each time point before proceeding to the medial. The heel bulbs were selected following a review of the available literature (Carpenter & Byron, 2017; Labens et al., 2012; Moyer et al., 2011; Prange, 2019; Skarda et al., 2009).

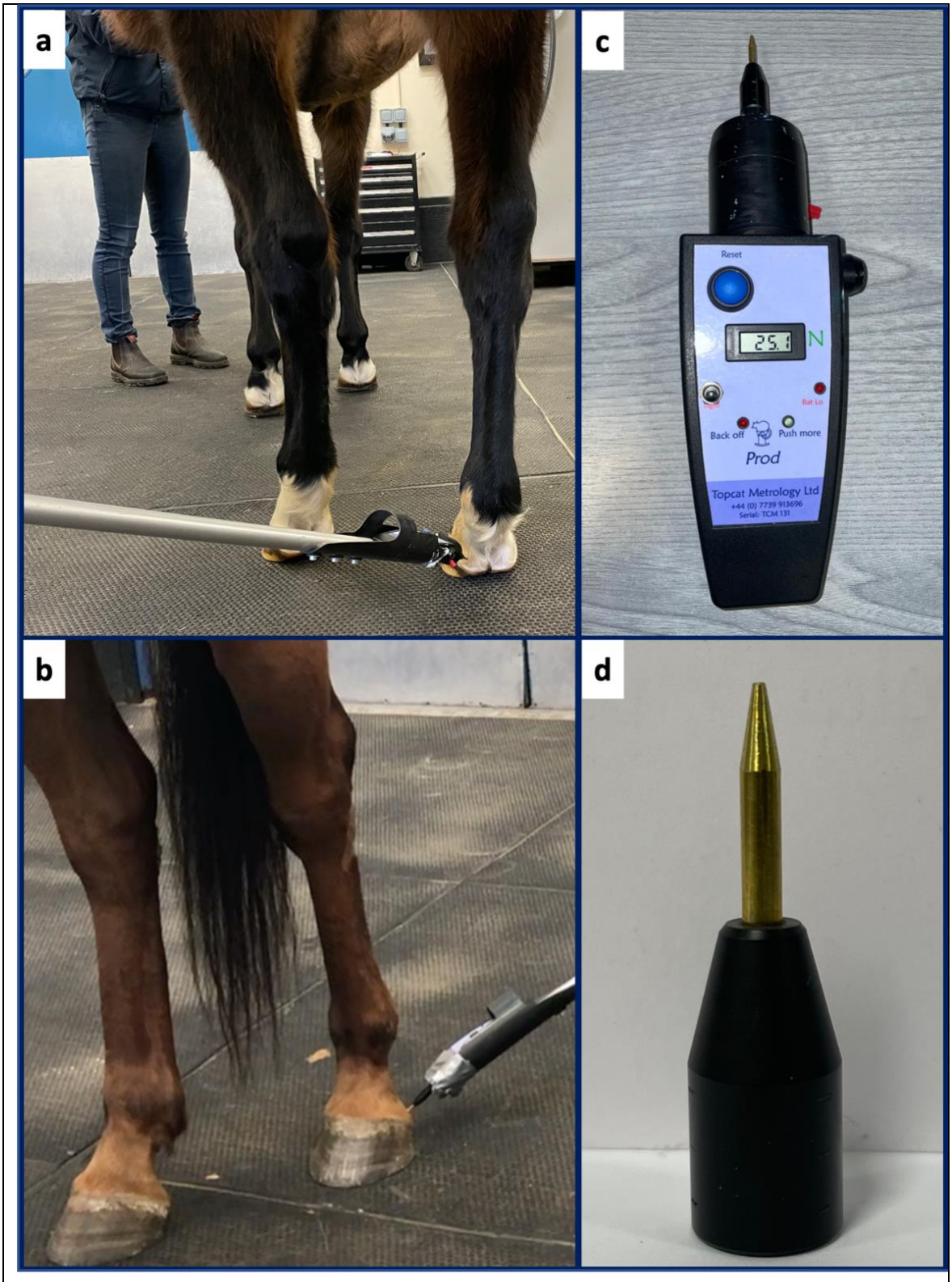


Figure 5: Images showing skin sensation testing and the digital algometer.

(a, b) images show the medial and lateral heel bulbs being tested using the digital algometer attached to a custom-made handle. (c) Digital algometer. (d) Close-up of the 1 mm diameter tip that was used for testing skin sensation.

2.2.5 Time required to complete injections

Time required to complete each tibial perineural injection was recorded in seconds using a stopwatch. The time for subcutaneous placement of 2 mL local anaesthetic was not recorded for either technique. For US-guided perineural injections, the stopwatch was started as soon as the transducer contacted the skin. For blind injections, the stopwatch was started at the time of palpation of the nerve with the limb in a flexed position. The stopwatch was stopped for both injection techniques when injection of the total volume was completed.

2.2.6 Complications and adverse reactions to perineural injections

Any complications of the injection techniques were recorded as well as any adverse reaction of the horse with implications for horse or operator safety; these included: horses kicking out at the time of injection, sudden foot stamping of the horse, horse moving abruptly, injury to the operators and/or horses.

2.2.7 Statistical analysis

Summary statistics were performed to examine differences between groups (US-guided and blind).

The dichotomous outcome ‘desensitisation at 40-45 min’ ‘yes’ or ‘no’ was defined as loss of skin sensation (no response to ≥25 N pressure) at the medial and lateral heel bulbs. The frequency of this outcome was compared between US-guided and blind groups using a Fisher’s Exact test.

The speed of onset of medial and lateral heel bulb desensitisation (≥25 N) was evaluated between US-guided and blind groups graphically.

The lengths of time taken to complete the nerve blocks were compared between groups US-guided and blind groups using a Mann-Whitney U test.

2.3 Results

A total of 27 cases were collected in this study, with 27 tibial perineural injections being performed on 22 horses (8 mares, 14 geldings); breeds included 10 Cob-type horses, 9 warmblood crossbreed horses and 3 thoroughbred crossbreed horses. The horses ranged in age from 5 to 20 years of age (mean ± SD, 10 ± 4 years). Sixteen cases were assigned to the US-guided group and 11 cases were assigned to the blind group.

One horse underwent three blind tibial perineural injections at different times (2 left hindlimb and 1 right hindlimb), one horse underwent 1 US-guided injection and 1 blind injection (both right hindlimb), and one horse underwent 2 blind injections (1 left hindlimb and 1 right hindlimb).

Nine out of 16 cases that underwent US-guided injection were cob types, six were warmblood crossbreeds and one was a Thoroughbred crossbreed. Five out of the 11 cases that underwent blind injections were warmblood crossbreeds, four were Thoroughbred crossbreeds and two were cob types.

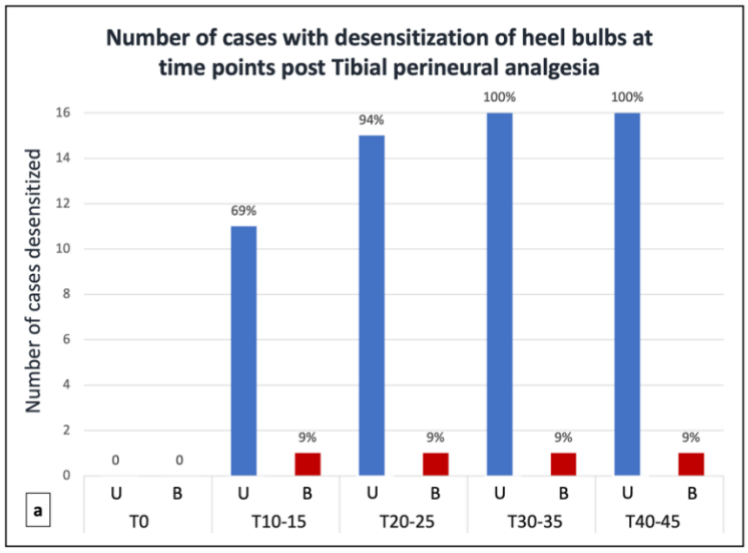
Four operators performed the tibial perineural injections [three boarded surgeons (JW, MM and CB) and one surgical resident (NB)]. NB performed six out of 11 blind injections and 10 out of 16 US-guided injections. The boarded surgeons performed the remainder: JW one blind injection and six US-guided injections, MM two blind injections, CB two blind injections.

Eleven cases had superficial and deep peroneal perineural analgesia performed at the time of tibial perineural analgesia (6 out of 16 US-guided cases and 5 out of 11 blind cases).

2.3.1 Desensitisation at heel bulbs

There was no difference in timing of desensitisation between lateral and medial heel bulbs. All 16 US-guided injection cases lost skin sensation at the heel bulbs by 30-35 min post-injection. One out of the 11 blind injection cases lost skin sensation (this occurred by 10 min post-injection). Timing of desensitisation for the groups is shown in Figure 6a. Significantly more ($p<0.001$) cases had lost skin sensation at medial and lateral heel bulbs at 40-45 min post-injection in the US-guided group than the blind group as shown in Figure 6b.

The mechanical nociceptive threshold values recorded for both groups are shown in Figure 7.



	'Blind'	US-guided
Desensitisation at 40-45 minutes (%)	1 (9)	16 (100)
No desensitisation at 40-45 minutes (%)	10 (91)	0 (0)

b

Figure 6: (a) Histogram showing the number(and percentage) of cases with desensitisation (no response to ≥25 N pressure) of the heel bulbs at time points post (T [min]) tibial perineural analgesia using a 'Blind' (B, columns in red) or a US-guided (U, columns in blue) technique. (b) Table shows the number (and percentage) of cases that had desensitisation (loss of skin sensation [no response to ≥25 N pressure] at medial and lateral heel bulbs) or no desensitisation at 40–45 min post-injection, subdivided between injection technique ('Blind' or US-guided).

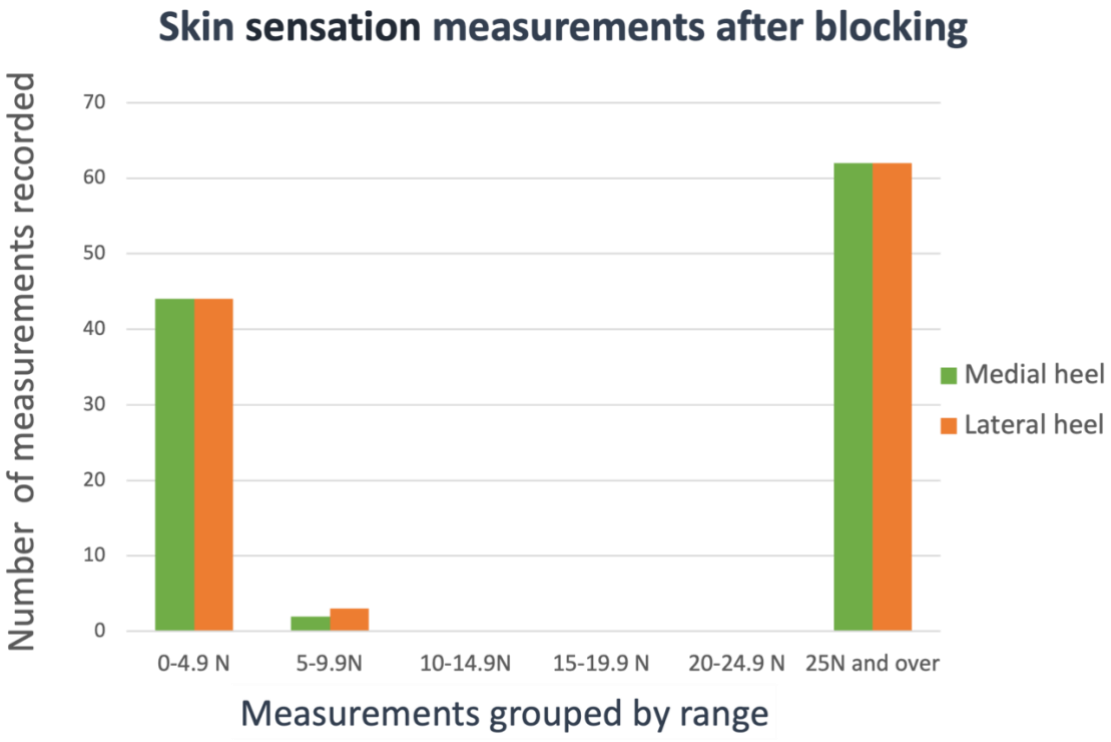


Figure 7: Histogram showing mechanical nociceptive threshold (MNT) measurements in Newton (N) for the medial and lateral heel bulb recorded after performing tibial perineural analgesia grouped in ranges.

2.3.2 Time to complete perineural injections

The mean injection time for the US-guided group (275.5 s, range:90-485) was significantly longer than for the blind group (115.7 s, range:40-310), $Z=-3.53$, $p<0.001$, as shown in Figure 8.

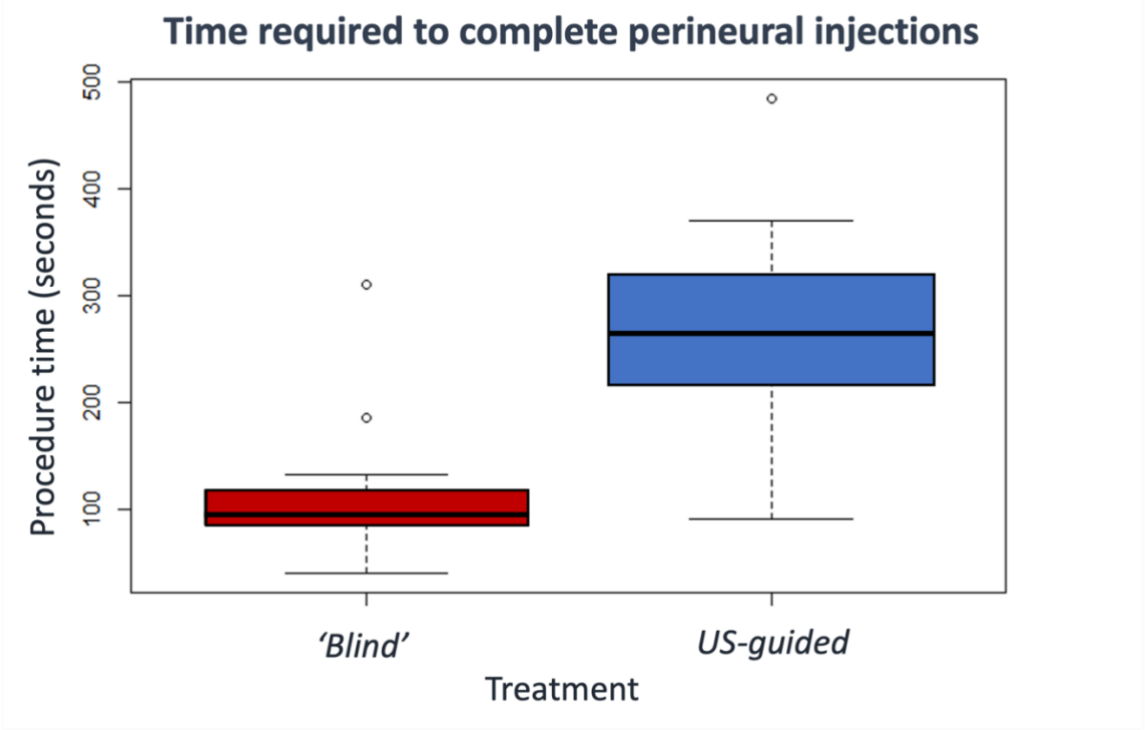


Figure 8: Box plot showing procedure (injection) times (s) between the 'Blind' (red) and US-guided (blue) techniques.

Lower and upper box lines = 25th and 75th percentiles, respectively; middle box line in bold = median; lower and upper whiskers = lower and upper adjacent values, respectively; open circles = outliers.

2.3.3 Complications and adverse reactions to perineural injections

The only complication reported was an inadvertent intravenous puncture in one case in the blind injection group. No adverse reactions to perineural injection were observed with either injection technique.

2.4 Discussion

This study demonstrated that a US-guided tibial perineural analgesia technique resulted in a greatly increased probability of achieving loss of skin sensation at the heel bulbs compared to an equivalent blind technique.

Skin sensation, which was measured prior to and after performing tibial perineural analgesia, was used to determine the onset of nerve blockade following injection of a local anaesthetic agent. As well as being used clinically, loss of skin sensation has been used commonly in research to verify the onset and duration of perineural analgesia (McCracken et al., 2020; Schambourg & Taylor, 2020) and to investigate the diffusion of local anaesthetic agents to nerves in the proximity of injection sites (Hinnigan et al., 2014; Jordana et al., 2014; Miagkoff & Bonilla, 2021). The lateral and medial heel bulbs are autonomous zones (i.e. where testing of the skin sensation provides information on the function of a specific nerve) of the tibial nerve as they are innervated exclusively by the lateral and medial plantar digital nerves respectively, which are ramifications of the tibial nerve (Labens et al., 2012; Moyer et al., 2011; Prange, 2019; Singh, 2018). Therefore, testing of skin sensation at the heel bulbs was an appropriate assessment method for the tibial perineural injection techniques investigated in this study.

All skin sensation testing was performed by the same operator using a hand-held digital algometer, allowing objective quantification of the effect of tibial perineural analgesia on skin sensation. The operator testing skin sensation was not blinded to the injection techniques being performed.

Algometers are instruments that provide reliable, objective, controlled and safe measurements of mechanical nociception (Gozalo-Marcilla et al., 2020; Luna et al., 2015; Schambourg & Taylor, 2020). The pressures applied using hand-held algometers are manually generated by the operator and are comparable to the pressures that are applied by clinicians when testing skin sensation using a blunt object (e.g. ballpoint pen) in clinical practice.

Previous studies have reported good intra-observer repeatability, interobserver reproducibility and reliability of measurements from algometers, indicating that the use of a single and non-blinded operator would have had minimal effect on the validity of results (Luna et al., 2015).

A binary outcome was observed following tibial perineural analgesia with skin sensation being either present or lost (Figure 7). This pattern of outcome for

diagnostic analgesia has been reported by others (Hoerdemann et al., 2017; Schambourg & Taylor, 2020) but partial loss of skin sensation has also been described (Jordana et al., 2014; Miagkoff & Bonilla, 2021). It is possible that the nature of the probe tip used (size and shape) may have played a role in determining the binary outcome observed in this study (Taylor et al., 2016), or that the timing of sensation testing missed cases that had partial loss of sensation.

In 11 out of 27 cases in this study, superficial and deep peroneal perineural analgesia was performed at the same time of tibial perineural analgesia. This is not considered a limitation of this study as the heel bulbs are an autonomous zone of the tibial nerve and therefore skin sensation at this site is unaffected by perineural analgesia of the peroneal nerves.

This study used a US-guided injection technique that differed in a number of respects from the descriptions in the literature (Denoix et al., 2020; van der Laan et al., 2021), although the location of the injection sites was similar. Denoix et al. (2020) described a US-guided technique using a 25 gauge, 5/8-inch needle and a 6-10 MHz microconvex transducer, rather than the linear transducer used in this study. Use of a shorter needle necessitated perineural injection to be performed from two sites (one slightly cranial and the other caudal to the nerve), rather than one. Additionally, 4-10 mL less local anaesthetic agent were infiltrated around the nerve. Van der Laan et al. (2021) compared the accuracy of a conventional blind technique and a US-guided technique for perineural injection of the tibial nerve, using cadaveric limbs and a low volume of dye (1 mL methylene blue) in the place of local anaesthetic agent. Similarly, to this study, the US-guided technique was performed using a single injection site, a 21 gauge needle inserted cranially to the nerve and a linear transducer (7.5 MHz). Ultrasonography, however, was only used to assist in tibial nerve localisation prior to needle insertion and not to guide the insertion of the needle in real time.

The blind injection technique for tibial perineural analgesia selected for this study is one of a number described in the literature. For the majority, the horse is weightbearing on the limb and needle insertion is from the medial aspect. Potentially significant variations include performing the injection with the limb in a flexed position and a lateral approach with the injection being performed from the lateral aspect of the crus (Bassage & Ross, 2010; Carpenter & Byron, 2017).

In their study, Van der Laan et al. (2021) found that perineural injection of methylene blue resulted in successful tibial nerve staining in 85.7% of limbs with the US-guided technique and 47.6% with the blind technique, while 100% of US-guided injections and only 9% of ‘blind’ injections resulted in successful perineural analgesia in our study. The difference in results suggests that the greater precision and accuracy of needle placement achieved through US guidance is an important factor in successful tibial perineural analgesia, potentially because perineural fat is a barrier to the diffusion of local anaesthetic agent deposited external to this layer (Denoix et al., 2020; van der Laan et al., 2021). The results presented here indicate that the use of 20 mL local anaesthetic agent and allowing up to 45 min for effect are not sufficient by themselves (blind technique) for adequate diffusion. It seems possible, however, that US guidance might permit the use of a lower volume without impact on the success rate. The use of a lower volume has been described but there are no objective supporting data in relation to success (Denoix et al., 2020).

A longer needle (1.5 inches) was selected for the US-guided injection compared to the needle used for the blind injection (1 inch) and to the needles used by Denoix et al. (2020) and Van der Lann et al. (2021). The length facilitated repositioning of the needle for injection of local anaesthetic agent around the nerve without a second skin penetration, as well as the shallow angle of tissue penetration helpful to maintaining separation of transducer and needle and to needle visualisation.

An 8-12 MHz linear transducer was used to perform US-guided injection in our study while Denoix et al. (2020) used a 6-10 MHz microconvex transducer for the technique. The linear transducer was easy to handle and provided good visualisation of the tibial nerve and needle insertion in all cases, including those horses with thick skin (Cob-type breeds). An advantage of the linear transducer is that it may be more readily available in equine practice.

Performing US-guided tibial perineural analgesia safely in the live horse has been regarded as particularly challenging because of the number of personnel required (van der Laan et al., 2021). Denoix et al. (2020) recommended that two operators restrain the horse, with additional operators responsible for ultrasonographic imaging and for injection of local anaesthetic agent. Despite only one person restraining horses in this study, however, no safety concerns were reported. Nevertheless, the technique requires additional operators (in common with those described in the literature) compared to the blind technique, and the availability of

assistance may therefore be a limiting factor for equine practitioners wishing to perform the US-guided technique in the field.

The operators participating in this study, who were experienced in the use of both tibial perineural analgesic techniques, took significantly longer to perform the US-guided technique than the 'blind' technique (275.7 ± 20.6 s versus 115.7± 24.9 s; $p<0.001$). The US-guided injection however was completed in less than five minutes in the majority of cases. Any disadvantage of increased time required to complete the US-guided technique is arguably outweighed by the 100% success rate compared to the 9% success rate for the blind technique given that the need for the injection to be repeated when part of a lameness investigation would be rare, in contrast to the blind technique.

In this study, there were no differences in patient tolerance and operator safety between the two injection techniques, contrary to the expectation that the US-guided technique would be superior in these regards. The good tolerance observed in our study for both techniques may be explained by subcutaneous infiltration with local anaesthetic agent prior to performing tibial perineural injection in all cases. Although not reflected in these results, US guidance reduces the risk of needle puncture of the nerve and the horse suddenly kicking out (Denoix et al., 2020; Rubio-Martinez & Hendrickson, 2021). Whether this reduced risk, together with the decreased requirement for injections to be repeated, outweighs the greater duration of exposure to risk because the US technique takes longer to perform, is not possible with the information available currently. Conclusions about the relative safety of the techniques therefore await further studies.

The study's main limitations are that four different operators performed the perineural injection techniques, that cases were not equally distributed between the two techniques and that the operator testing skin sensation was not blinded. Although no difference in results between operators for the two injection techniques was apparent, case numbers were insufficient and their distribution between techniques was inappropriate to explore intra-operator variability further. A study design with the four operators assigned an equal number of cases for each injection technique may have been preferable.

The absence of pre-existing data meant that sample size calculations were not performed as part of the study design and 20 cases were arbitrarily set as the target. Although additional cases were recruited, the total number remained relatively low

(16 US-guided, 11 blind injection cases). The use of different horse breeds did not seem to influence the results between the two injection techniques; however, no statistical analysis was performed to test the effect of breed, or other independent variables such as age and sex, due to the small sample size.

In conclusion, the US-guided perineural injection technique for the tibial nerve described in this study was straightforward to perform, well tolerated and resulted in complete tibial nerve analgesia within 30-35 min in all patients.

These results suggest that US-guided tibial perineural analgesia should be used during lameness investigation in preference to blind tibial perineural analgesia when possible. The considerable and significant difference in results observed between the two injection techniques is unlikely to have been greatly impacted by the limitations of the study.

3 General discussion

This thesis provides an important and original contribution to the field of equine lameness, reporting a novel US-guided injection technique for tibial perineural analgesia and presenting evidence of the benefits of adopting US-guided tibial perineural analgesia injection techniques, in replacement of the established blind techniques.

The results of the study presented here show a US-guided equine tibial perineural local analgesia injection technique to result reliably in complete analgesia in clinical cases (all 16 of cases in which it was used) while a blind injection technique infrequently resulted in complete analgesia (one of 11 cases).

The US-guided tibial perineural injection technique described in this work is novel as it differs in a number of important aspects from the only previously published description of a US-guided tibial perineural injection technique used in clinical cases (Denoix et al., 2020). The main differences are the use of an 8-12 MHz linear US transducer instead of a 6-10 MHz microconvex transducer, a single skin needle penetration instead of two, and the use of three operators instead of four.

The US-guided technique described in this study has the advantage of being practicable and should be easy to perform in ambulatory field practice, requiring only one operator to be competent with US-guided injections in order to complete the procedure (Estrada, 2024b). The same operator handles simultaneously the US transducer and the needle, while in the technique reported by Denoix et al., (2020), one operator is responsible for US image acquisition, and another is responsible for performing the injection.

Simultaneous handling of the US transducer and needle by one operator is likely to facilitate needle detection in the US image and the required adjustments in the position of the transducer and needle. For the technique described by Denoix et al., (2020), good movement coordination between the two operators is required to successfully perform US-guided tibial perineural injections (Estrada, 2024b).

Another likely advantage of the US-guided technique described in this study is the lower probability of the operator being exposed to an adverse patient response due to single skin needle penetration, instead of the two separate skin penetrations of the technique described by Denoix et al., (2020).

Despite the differences outlined here, both US-guided injection techniques ensure deposition and distribution of the local anaesthetic agent in close proximity and entirely surrounding the tibial nerve, rather than depositing the local anaesthetic agent elsewhere within the superficial caudal crural compartment (fascial compartment which contains the nerve- see Section 1.3). Accurate injection of the local anaesthetic agent avoids any potential barrier effect to local anaesthetic distribution from the perineural fat present within the superficial caudal crural compartment and from the fascicle sheath that surrounds the tibial nerve (Nagy et al., 2010).

In this study, the blind injection technique for tibial perineural analgesia required the limb to be in a weightbearing position following identification of the tibial nerve by palpation (firm cord-like structure) with the limb flexed. The medial approach with the limb in a weightbearing position was selected as it is commonly used in clinical practice and would allow a more direct comparison with the US-guided injection, as limb positioning and the injection site were the same for both techniques.

Reported variations of the blind injection technique include having the limb in a flexed position while injecting or using a lateral approach with the injection being performed from the caudolateral aspect of the crus (Bassage & Ross, 2010; Carpenter & Byron, 2017).

In this study, the blind tibial perineural injection technique resulted in complete onset of tibial nerve analgesia in one case at 10-15 min following injection but in the remaining 10 cases blind tibial perineural injection failed to achieve onset of tibial perineural analgesia.

The results reported for the blind injection technique differ from those obtained in ex vivo studies, which used tibial nerve staining following perineural injection of dye as the proxy for analgesia. Colla et al., (2023) in their study reported a success rate of 100% for the blind injection technique while Van der Laan et al., (2021) in their study reported a success rate of 47.6%. Notably, these ex-vivo studies reported a higher success rate compared to the results of this study.

However, a direct comparison between the findings of these ex vivo studies and the result of this study is not possible. Studies evaluating perineural injections in cadaver limbs using tissue dye staining should not be considered fully representative of the distribution of local anaesthetic agents in vivo. This discrepancy arises mainly due to differences in density and viscosity between the

dye and the local anaesthetic solutions, which likely result in different tissue diffusion patterns (de Miguel Garcia et al., 2020). Additionally, ex vivo injections do not account for the unique challenges of injecting into the limb of a live horse (Claunch et al., 2014).

For example, inadvertent intravenous injections of the caudal root of the saphenous and caudal femoral veins, a known complication during tibial perineural analgesia injections in live horses, are less likely to occur in cadaver limbs due to the veins being typically collapsed post-mortem (Denoix et al., 2020; Egger et al., 2023).

The following sections discuss important aspects of the study design and results, highlighting implications for current clinical practice and the directions for further research.

3.1 Choice of injection site, volume of injectate and local anaesthetic agent

The injection site selected for both the US-guided and blind tibial perineural analgesia injections investigated in this study was the same, with injections being performed 10 cm proximal to the tuber calcanei. This injection site was selected because the location of the division of the tibial nerve into the lateral and medial plantar nerves has been observed to range from the level of the tuber calcanei to 9 cm proximal to the tuber calcanei, and therefore injections performed 10 cm proximal to the tuber calcanei would avoid the risk of only injecting one of the branches instead of the tibial nerve (Ghoshal, 1966).

In this study, mepivacaine hydrochloride 2% (w/v), at a fixed volume of 20 mL, was selected to perform all tibial perineural injections as this is the most commonly used and the most reliable local anaesthetic agent for diagnostic analgesia in lameness investigations in the horse (Schumacher & Boone, 2021).

The volumes of local anaesthetic used for tibial perineural analgesia are reported to range from 15-20 mL, with some clinicians opting for higher volumes to increase the likelihood of achieving complete nerve desensitisation (Bassage & Ross, 2010; Carpenter & Byron, 2017; Moyer et al., 2011). The higher end of this range was chosen for both injection techniques to facilitate comparison with published results and to maximise injection success rates.

Concerns regarding proximal diffusion of the local anaesthetic solution with higher volumes were not considered significant, given the regional anatomy of the tibial nerve injection site means the risk of diffusion to other nerves or synovial structures is low.

3.2 Loss of skin sensation as an indicator of successful tibial perineural analgesia

In this study, loss of skin sensation at the level of the medial and lateral heel bulbs, autonomous zones of the tibial nerve, was used as an indicator for successful tibial perineural analgesia.

3.2.1 Skin locations

Other skin locations in which the onset of tibial nerve analgesia could have alternatively been tested (i.e. alternative autonomous zones) include the plantar-medial and plantar-lateral surface of the metatarsus, fetlock, and pastern (Carpenter & Byron, 2017; Skarda et al., 2009).

However, since perineural administration of local anaesthetics is believed to affect the more distal anatomical structures last, as fibres relating to the most distal anatomical structures are positioned within the nerve's core, the heel bulbs were determined to be the most appropriate site for testing of skin sensation as an indicator of complete tibial nerve desensitisation (Bidwell et al., 2004; Schumacher & Boone, 2021)**.**

Testing skin sensation at the heel bulbs, instead of using more proximal skin locations (e.g. proximal plantar medial metatarsus), which are closer to the injection site for tibial perineural analgesia, also had the advantage of avoiding potential false positive results due to analgesia of the cutaneous innervation prior to achieving or without tibial nerve desensitisation (Hoerdemann et al., 2017).

In this study, no difference was observed between timing of loss of skin sensation at the medial and lateral heel bulbs, with loss of skin sensation occurring simultaneously in both locations.

These findings suggest that clinicians may test either the medial or lateral heel bulb, rather than both. Simultaneous loss of skin sensation at the medial and lateral heel bulbs could be explained by the presence of a ramus communicans between the

plantar nerves, despite this having been reported anecdotally to be rudimental or not present in the horses' hindlimbs (Coleridge et al., 2020), or by local anaesthetic distribution around the tibial nerve which avoids selective analgesia of fibres giving rise to the lateral or medial plantar nerves. However, as no clear explanation can be given at this time, testing of both heels seems prudent when assessing the onset of complete tibial nerve desensitisation.

3.2.2 Reliability of loss of skin sensation as an indicator of onset of nerve desensitisation

Loss of skin sensation is commonly utilised in research to evaluate the onset and duration of perineural analgesia (McCracken et al., 2020; Schambourg & Taylor, 2020). However, studies have shown that loss of skin sensation does not consistently correlate with alleviation of pain (i.e. loss of deep pain sensation). There are instances where horses experience improved lameness following perineural analgesia without concurrent loss of skin sensation in the corresponding nerve's dermatome (respective nerve's autonomous zone) (Schumacher & Boone, 2021).

Local anaesthetic agents affect various classes of nerve fibres differently, starting with the loss of deep pain, followed by thermosensation, mechanosensation (i.e., skin sensation), and finally motor function. This differential susceptibility to local anaesthetics among nerve fibre types may explain why the resolution of lameness does not always correlate with the loss of skin sensation in the respective dermatome following perineural analgesia (Schumacher & Boone, 2021).

It's important to note that loss of skin sensation occurs only after deep pain is lost, indicating that loss of skin sensation implies loss of deep pain but not necessarily vice versa. Therefore, any potential lack of correlation between loss of skin sensation and improvement in lameness would not diminish the validity of the findings from this research project.

3.2.3 Alternatives to skin sensation testing

Amelioration of horses' lameness is another indicator that is often used in research studies, instead of loss of skin sensation, to determine if the onset of perineural analgesia has occurred (Alvarez et al., 2018; Boorman et al., 2022; McGlinchey et al., 2019).

Amelioration of lameness was not considered an appropriate method to determine the onset of tibial perineural analgesia for this study, as the population of horses available were animals with a naturally occurring lameness presented for diagnostic investigation, in which the exact cause of lameness had not been determined. Therefore, this population potentially included horses with a source of lameness located proximal to the distal crus region which, therefore, would not have shown an amelioration of lameness following successful analgesia of the tibial nerve (false negative cases).

In order to use amelioration of lameness as an indicator of onset of tibial nerve analgesia to compare the US-guided and blind tibial perineural injection techniques, a population of horses in which a reversible lameness could be induced or horses with a definitive diagnosis for their lameness would be required.

Inducing lameness in a sound horse is difficult to justify ethically (Marr, 2015) and recruiting horses with the appropriate diagnoses entails additional logistical challenges.

Therefore, skin sensation testing was considered to be the appropriate and practical outcome measure pertaining to the main objective of this research project, which was to compare the onset of complete tibial nerve analgesia for the US-guided and blind tibial perineural injection techniques.

3.2.4 Algometer

In this study, a hand-held digital algometer was selected to quantify objectively the effect of tibial perineural analgesia on skin sensation at the heel bulbs.

The algometer used in this study was attached to a custom-designed handle, allowing the operator to assess hindlimb skin sensation from a safe distance. Alternatively, a limb-mounted algometer, fixed to the horse's limb and operated remotely, could have been used (Schambourg & Taylor, 2020). Limb-mounted algometers potentially offer more precise measurements because the operator's remote position reduces the likelihood of anticipatory responses from the horse. However, direct comparisons between hand-held and limb-mounted algometers in horses have not been conducted, and thus evidence on the implications for testing skin sensation using hand-held or limb-mounted algometers in horses is currently lacking.

In this study, a hand-held algometer was selected for its perceived practicality and comparability to common equine clinical practices, such as testing skin sensation by applying pressure with a ballpoint pen (Taylor et al., 2016). Additionally, the horses' peripheral vision was obstructed, either by the operator who was holding the horse or by using blinkers, to prevent the horses from anticipating the noxious mechanical stimulus as the operator approached the limb with the hand-held algometer.

3.3 Operators' safety and patient adverse reactions to perineural analgesia

In this study, complications associated with injection techniques and any adverse reactions of the horses, which have implications for the safety of both horses and operators, were evaluated. The only complication reported was an inadvertent venous puncture in one case within the blind injection group. No adverse reactions concerning horse and operator safety were observed with either injection technique, contrary to the expectation that the US-guided technique would be superior in these regards.

To avoid confounding skin sensation testing recordings, sedation or tranquillisation was not used to restrain horses during tibial perineural analgesia injections. However, it has been reported that low doses of sedatives and tranquillisers may be used without the concern of potential lameness-masking effects for hindlimb lameness and should be considered in clinical settings to enhance the safety of both horses and operators, particularly during perineural analgesia injections in fractious or needle-shy horses (Rettig et al., 2016; Taintor et al., 2016; Termansen & Meehan, 2021).

The good tolerance observed for both injection techniques in this study may be attributed to the subcutaneous infiltration with a local anaesthetic agent prior to performing the tibial perineural injection in all cases. This practice should be considered by equine practitioners performing tibial perineural injections.

Although no adverse reactions concerning horse and operator safety were observed with either injection technique in this study, it is likely that US guidance could prevent the needle from contacting the nerve, thereby enhancing operator safety (Denoix et al., 2020; Rubio-Martinez & Hendrickson, 2021).

Therefore, despite the findings of this study, given the need to reduce occupational risks to veterinary surgeons (Parkin et al., 2018), consideration should be given to replacing conventional blind tibial perineural injections with US-guided tibial perineural injections.

3.4 Limitations

The small sample size and unequal distribution of cases between the US-guided and blind injection groups may initially appear to be significant limitations of this research project. However, the impact of these limitations is mitigated by the contrasting results for the groups. Sample sizes ranging from 6-20 cases (3-10 per group), assuming successful perineural analgesia percentages between 5-20% for the blind technique and 80-95% for the US-guided technique, would be sufficient to identify significant differences between the two groups with 80% statistical power and 95% confidence.

On the other hand, larger sample sizes would facilitate statistical analysis to determine if horse demographics affect the outcomes of blind and US-guided tibial perineural injections. Additionally, larger samples would enable the exploration of intra-operator variability for each injection technique.

3.4.1 Time interval in which the onset of tibial perineural analgesia was assessed

In this study, onset tibial perineural analgesia was assessed until 45 min post-injection. Most commonly, testing of onset tibial perineural analgesia is recommended up to 30 min post-injection (Denoix et al., 2020; Schumacher & Schramme, 2019), however, some authors have reported that waiting up to 1 h or more may be required for onset of tibial perineural analgesia to occur (Bassage & Ross, 2010). Therefore, extending the assessment of tibial nerve analgesia onset up to 1 h post-injection may have increased the observed success rate of blind tibial perineural injections. However, this approach would have had some practical disadvantages, including increased time commitment, additional resource allocation, and ethical concerns due to the requirement of further skin sensation testing and the potential for additional, likely unnecessary, discomfort for the horses.

3.4.2 Operators performing tibial perineural injection techniques

In this study, four operators with a relatively similar level of experience performed the perineural injection techniques. The level of proficiency of each operator for either perineural injection technique, however, was not assessed prior to collecting the data. Proficiency of the operators could have been assessed, prior to starting this study, by evaluating the outcome of blind and US-guided tibial perineural injections with colour dye on cadaver limbs.

To explore the level of difficulty of the US-guided injection technique, operators with different levels of experience could have been recruited. A study in which the operators performing the injections had no previous experience with tibial perineural analgesia and in general, with US-guided injections, may provide information on the learning curves of the respective tibial perineural injection techniques. In such a study, a dedicated theoretical and practical session to train all operators on both tibial perineural injection techniques, prior to starting data collection, would be necessary.

3.4.3 Operator testing skin sensation

In this study, all skin sensation testing was conducted by a single operator using a hand-held digital algometer, enabling objective quantification of the effect of tibial perineural analgesia on skin sensation. However, it is important to note that the operator was not blinded to the injection techniques being performed.

Previous studies have demonstrated good intra-observer repeatability, inter-observer reproducibility, and overall reliability of measurements obtained from algometers (Luna et al., 2015). The nature of the probe tip has been shown to significantly affect MNT measurements (Taylor et al., 2016). Nevertheless, various external factors—including the operator, environmental conditions, anatomical site, rate of stimulus application, and tissue characteristics—have all been reported as potentially influencing MNT measurements (Taylor et al., 2016). Given that the operator was unblinded to the injection procedure, there is a risk of bias, particularly for measurements where MNT values did not reach the 25N threshold. This lack of blinding could have influenced the consistency of the measurements, representing a limitation of the study. However, the results clearly showed a binary distribution, with the two groups being very distinct and demonstrating a large effect size, which mitigates against the influence of bias and supports the validity of the study.

3.5 Future studies

3.5.1 Comparison of US-guided tibial perineural injection techniques

Future studies making a comparison between the US-guided tibial perineural injection described in this study and the US-guided injection technique described by Denoix et al., (2020) should be considered. These would highlight whether the two US-guided techniques result in a different proportion of cases with successful onset of tibial nerve analgesia and if the time required for the onset of analgesia differs between the techniques. Differences in the time required to perform the injections and in patient tolerance and operator safety could also be assessed.

3.5.2 Alternative skin locations for testing

Studies validating skin locations more proximal to the heel bulbs for testing sensation following tibial perineural analgesia should be considered as these would be of potential benefit to equine practitioners. Validating additional skin testing sites could enable practitioners to reliably test the onset of tibial nerve analgesia even in cases where perineural analgesia of the distal limb, such as heel bulb desensitisation from an abaxial sesamoid nerve block or a low four-point nerve block, had already been performed.

3.5.3 Return of skin sensation following tibial perineural analgesia

The return of skin sensation following tibial perineural analgesia was not assessed in this study, as it was beyond its scope. However, future research investigating the time of return of skin sensation and its relation to the return of deep pain sensation would be highly valuable. Such studies would guide equine practitioners on the appropriate waiting times required before any additional diagnostic analgesia in the hock region or distal limb (e.g. intrasynovial analgesia of the distal hock joints) could be performed for more accurate localisation of the source of pain.

3.5.4 Level of difficulty of US-guided tibial perineural analgesia injections

The US-guided technique described in this study was reported to be practical and easy to perform. To validate this further and allow comparison with the other US-guided and blind injection techniques, future studies that objectively evaluate the level of difficulty of the procedure should be considered. As already mentioned in Section 3.4.2, these studies could recruit operators with different levels of experience or only operators with no prior experience with tibial perineural analgesia injections in order to allow evaluation of the difficulty level of each injection technique and assessment of the operators' learning curves for the techniques (Estrada, 2024a).

3.5.5 Anatomical studies

Future anatomical studies should consider assessing variations in tibial nerve branching and regional anatomy between different breeds. This would facilitate breed-specific modifications of blind tibial perineural injection techniques to potentially improve injection success rates, especially given that breed-specific differences in foot innervation have already been documented (Silveira et al., 2020).

Anatomical studies could highlight any variations in the branching of the caudal cutaneous sural nerve (cutaneous branch of the tibial nerve - see Section 1.3) which could potentially help establish additional breed-specific sites for testing skin sensation after tibial perineural analgesia.

Furthermore, anatomical studies could help verify whether the site 10 cm proximal to the tuber calcanei, a level at which the tibial nerve has not yet branched into the lateral and medial plantar nerves according to the latest literature, remains the most appropriate injection site for tibial perineural analgesia (Singh, 2018).

This information would be particularly valuable in situations where US-guided injections are not available.

3.5.6 When tibial perineural analgesia is enough and when perineural analgesia of the superficial and deep peroneal nerves should be used

In horses presented for lameness investigation in which pain is suspected in the tarsus, tibial perineural analgesia is often performed in combination with perineural

analgesia of the superficial and deep peroneal nerves (Bassage & Ross, 2010; Kawcak et al., 2020). However, there is a lack of clear guidelines indicating when superficial and deep peroneal perineural analgesia should be performed alongside tibial perineural analgesia.

Currently, few specific orthopaedic conditions have been reported to exclusively improve with tibial perineural analgesia, solely with superficial and deep peroneal perineural analgesia, or to require both perineural injections (Pilsworth & Dyson, 2015; Plowright & Dyson, 2015). This information would significantly aid equine practitioners in narrowing differential diagnoses, thereby facilitating more precise diagnostic and therapeutic strategies.

3.5.7 Ultrasound transducers

In this study, an 8-12 MHz linear transducer and a high-quality console-based US machine were found to provide good visualisation of the tibial nerve and needle in all cases, including those horses with thick skin (Cob-type breeds).

A wide range of US machines are available, these can vary significantly in purchase cost and image quality, but low-cost small portable US machines are more often available to the equine practitioner.

Future studies evaluating how US machine quality can affect the success rate of US-guided tibial perineural injections should be considered.

Hand-held ultrasound machines, some of which are part of wireless systems, have recently gained popularity in human medicine, and in certain circumstances, have been reported to be suitable alternatives to the more expensive US machines (Carvalho et al., 2019; Yamaguchi et al., 2023). The fact that some of these transducers are wireless and have been found to be appropriate for musculoskeletal use (Zardi et al., 2019), could be of potential interest to equine practitioners. In particular, the use of a linear wireless hand-held transducer could be of benefit when performing US-guided tibial perineural analgesia and potentially have an impact on operator and equipment safety, mainly as no transducer cables would be in proximity of the horse’s hindlimbs during the procedure. Therefore, future studies that investigate the use of wireless hand-held US transducers for US-guided tibial perineural analgesia would be of great interest.

3.5.8 Local anaesthetic distribution

In this study, US-guided tibial perineural injection of local anaesthetic agent resulted in complete analgesia by 30-35 min of injection in all cases. In the majority of cases (11 of 16 cases) complete analgesia was detected at 10-15 min (first testing time point following injection), in four cases analgesia was detected at 20-25 min and in one case at 30-35 min.

The cause of the differences in the timing of onset of nerve analgesia observed for the US-guided tibial perineural injection, is unclear but is likely to be related to variations in the accuracy of deposition of local anaesthetic agent around the tibial nerve, with the perineural fat and the thick fascicle sheath of the tibial nerve likely acting as a barrier to diffusion of the local anaesthetic agent around the nerve (Denoix et al., 2020; Nagy et al., 2010).

Therefore, future studies assessing the effect of placing the local anaesthetic agent within the fascicle sheath and/or around the entirety of the tibial nerve over just placing local anaesthetic within the superficial caudal crural compartment would be beneficial.

For such purpose, studies in live horses in which the regional distribution of local anaesthetic agent following injection is assessed in combination with skin sensation testing, as the means to verify the onset of tibial nerve desensitisation, should be considered. In these studies, local anaesthetic distribution could be assessed by adding a contrast agent to the injectate and then tracking its diffusion with the aid of advanced three-dimensional imaging modalities such as standing computed tomography (Claunch et al., 2014; Mageed, 2022).

3.5.9 Choice of local anaesthetic agent

In this study, mepivacaine hydrochloride 2% (w/v) was the local anaesthetic used to perform all tibial perineural injections.

The use of other local anaesthetic agents in lameness investigations for perineural analgesia of median and ulnar nerve (proximal nerves of the forelimb) has been focus of interest of recent studies with evidence of more rapid onset of nerve analgesia occurring with chloroprocaine hydrochloride 3% (w/v) and mepivacaine hydrochloride 2% (w/v) buffered with sodium bicarbonate compared to mepivacaine hydrochloride 2% (w/v) (Boone et al., 2019; Boone et al., 2020).

Future studies investigating how these different local anaesthetic agents can affect the onset of analgesia following tibial perineural injections, both US-guided and blind injection techniques, should be considered. Extrapolation from results reported for perineural analgesia of the median and ulnar nerve ignores the significant differences in regional anatomy between these nerves and the tibial nerve, such as the lack of thick fascial compartment surrounding the median and ulnar nerves (Beaumont et al., 2020; Denoix et al., 2020).

3.6 Conclusions

The first hypothesis of this research project proposed that the US-guided injection technique would result in a higher proportion of horses experiencing loss of skin sensation compared to those receiving the blind injection technique and that the onset of skin sensation loss would occur earlier in horses undergoing the US-guided injection. The study confirmed this hypothesis, finding that a significantly higher number of horses, all 16, experienced loss of skin sensation following US-guided tibial perineural analgesia, compared to just one out of 11 horses undergoing blind injection.

The second hypothesis of this research project proposed that the US-guided injection technique would take longer to be performed than the blind injection technique. The study confirmed this hypothesis, finding that the time required for US-guided injections was significantly longer than for blind injections. However, the additional time required to perform the US-guided injection, which was less than three minutes in most cases, was arguably outweighed by its significantly higher success rate. This higher success rate means that the need to repeat US-guided tibial perineural analgesia injection during a lameness investigation is rare, unlike the blind injection technique.

The third hypothesis of this research project proposed that the US-guided injection would result in fewer adverse behavioural reactions in horses compared to the blind injection technique. This hypothesis was rejected, as no adverse behavioural reactions affecting the safety of the horses or operators were reported for either injection technique. This outcome was contrary to the expectation that the US-guided technique would be superior in minimizing adverse behavioural reactions.

In conclusion, this research project provided evidence that blind tibial perineural analgesia is unlikely to result in the onset of nerve analgesia, supporting the need

for novel injection techniques to improve the success rate following perineural analgesia, such as the US-guided tibial perineural injection technique described and validated in this study.

The novel US-guided tibial perineural injection reported in this research project resulted in the successful onset of nerve analgesia in a very high proportion of cases. The technique was also found to be straightforward and safe to perform, could be performed in a timely manner and resulted in prompt onset of nerve analgesia in most cases.

Therefore, when performing tibial perineural analgesia, clinicians should consider the low success rate that was observed for blind tibial perineural injection in this study and when possible, US-guided tibial perineural analgesia should be used instead during hindlimb lameness investigation in the horse.

Appendices

Appendix 1: Information sheet and consent form provided to owners of the horses enrolled in the study.

University of Glasgow

Pool House Equine Clinic
Putting your horse first

Clinical Research Project
Information Sheet and Client Consent Form

Study title: Proximal forelimb (median and ulnar nerves) and hindlimb (tibial, superficial and deep peroneal nerves) ultrasound guided perineural analgesia in lameness examination of the horse.

Title in plain language: The use of ultrasound guidance for nerve blocks, above the carpus and hock, in lameness investigation of the horse.

Name of Principal Investigator:
Lance Voute BVSc PhD CertES(Orth) DiplECVS MRCVS
Senior University Clinician in Equine Surgery
Weipers Centre Equine Hospital
School of Veterinary Medicine
University of Glasgow G61 1QH
Tel: 0141 330 5999; Email: Lance.Voute@glasgow.ac.uk

You are invited to participate in this project by enrolling your animal in a clinical research study. The definition of "research" is: "the systematic investigation into and study of materials and sources in order to establish facts and reach new conclusions". Participation is entirely voluntary, and you may withdraw your animal from the study at any time by notifying the principal investigator. There is no penalty if you choose not to participate.
Please take your time to read the following information if you wish to consider for participation in this project.

Project Summary
Nerve blocks are an important tool and are routinely used by veterinarians for localising the source of lameness in the horse. Nerve blocks require local anaesthetic agent to be injected immediately adjacent to a nerve and can therefore fail to due to inaccurate needle placement. This would require the clinician to repeat the procedure.
Ultrasound-guidance of needle placement is an established clinical technique used when accurate placement is required for medication or sampling. This technique therefore has the potential to increase the success rate of nerve blocks, particularly when the nerve is deep-lying and not readily palpated (felt). Ultrasound guided techniques and potential related benefits have been reported but not investigated for nerve blocks in the proximal forelimb (above the carpus- median and ulnar nerves) and hindlimb (above the hock- tibial, superficial and deep peroneal nerves) of the horse.
Assessment of skin desensitisation is used by veterinarians to verify efficacy of nerve blocks. Skin sensitivity will usually be tested using a blunted object (for example, a ballpoint pen). In this project the skin sensitivity will be tested with the aid of an instrument (algometer), specifically designed for this purpose and validated by other studies, which will allow a controlled and safe force to be applied.
The aim of this study is to assess and validate the use of ultrasound guidance in the nerve blocks of the proximal limb by comparison with the standard techniques (not ultrasound guided) in use.
Please be aware that you have been invited to participate to this study as your horse is being presented for lameness /poor performance and may require nerve blocks of the proximal limb as part of the lameness examination. For this project we aim to recruit approximately 40 horses in total. By participating, your horse will be randomly assigned to either the technique with or without ultrasound guidance.
The results of this study will be used as part of a postgraduate research program at the University of Glasgow (Residency with Combined Masters of Veterinary Medicine).
Enrolling your horse in this clinical research study does not imply any additional health risks nor any additional financial expenses.

University of Glasgow

Pool House Equine Clinic
Putting your horse first

Confidentiality

All sensitive personal data collected will be kept strictly confidential. You and your horse will be identified by a case number, and any information about you will have your name and address removed so that you cannot be recognised from it. All data will be digitally stored on secure password–protected online storage and will be retained for at least 10 years post collection for research and integrity purpose.

All the personal information will be collected, stored and processed in accordance with The General Data Protection Regulation (2018). This project has been reviewed by the College of Medical, Veterinary & Life Sciences Ethics Committee, University of Glasgow. If any of the information given above is not clear or if you would like more information, please ask prior to giving consent.

Thank you for the time spent reading the above information sheet.

Statement of Consent

In giving my consent by signing this form:

1. I acknowledge that I have been informed and understood the purpose and nature of this study.
2. I acknowledge that I will not receive any direct benefit taking part to this study.
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected.
4. I confirm that I agree to the way my data will be collected and processed and that data will be stored for up to ten years in University archiving facilities in accordance with relevant Data Protection policies and regulations.
5. I agree that my name, contact details and data described in the information sheet will be kept for the purposes of this research project.
6. I understand that all sensitive personal data I provide will be kept confidential and will be seen only by study researchers and regulators whose job it is to check the work of researchers.
7. I understand that if I withdraw from the study, my data collected up to that point will be retained and used for the remainder of the study.
8. I agree to take part in the study.
9. I further certify that I am the owner (or duly authorized agent of the owner) of:

Animal's name: ..... Species: EQUINE

Breed: ..... Age: ..... Sex: ..... Neutered: Yes ❑ No ❑

I, ...(Owner/Agent) confirm that I have read and understand the Plain Language Statement for the above study and have had the opportunity to ask questions. Additionally, I give my permission for any further information required to be requested from my primary veterinary surgeon.

Signature: ..... Date: .....

Name (printed) and signature of veterinary surgeon obtaining consent: .....

Please make sure you take a copy of this information sheet and the signed consent form.

If you have any questions or concerns about this study may rise at a later stage, please don't hesitate to contact me.

Nicholas Bellitto, DVM(Hons), MRCVS
Project Investigator
Weipers Centre Equine Hospital
School of Veterinary Medicine
University of Glasgow G61 1QH
Tel: 0141 330 5999; Email: n.bellitto.1@research.gla.ac.uk

WEIPERS CENTRE EQUINE HOSPITAL
School of Veterinary Medicine
University of Glasgow
Bearsden Road, Glasgow, G61 1QH
Telephone: 0141-330 5999 *Fax:* 0141-330 6025 *Email:* equine@vet.gla.ac.uk
Twitter: @WeipersEquine
University of Glasgow Charity Number: SC004401

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Appendix 2: Mechanical nociceptive threshold values at the heel bulbs for each case recorded at the predetermined time points, prior and post tibial perineural analgesia (US-guided and blind injection technique).

Assigned case number	Treatment (A=Blind; B=US)	Time point (min)	Medial heel bulb mean value (N)	Lateral Heel bulb mean value (N)
1	**A**	T0	2.5	5.2
		T10	3.4	8.2
		T20	2.5	4.0
		T30	5.5	3.1
		T40	2.9	2.8
2	**A**	T0	0.5	0.4
		T10	0.5	0.4
		T20	0.5	0.4
		T30	0.5	0.4
		T40	0.7	0.4
3	**A**	T0	2.1	3.0
		T10	1.1	1.4
		T20	2.7	1.7
		T30	2.9	1.4
		T40	2.7	1.5
4	**A**	T0	2.5	5.2
		T10	3.4	8.2
		T20	2.5	4.0

		T30	5.5	3.1
		T40	2.9	2.8
5	**A**	T0	0.7	0.5
		T10	0.6	0.6
		T20	0.7	0.7
		T30	0.6	0.5
		T40	0.7	0.7
6	**A**	T0	2.4	3.6
		T10	2.8	2.6
		T20	3.0	2.5
		T30	1.4	1.1
		T40	1.9	1.4
7	**A**	T0	0.3	1.2
		T10	0.4	0.3
		T20	0.4	0.3
		T30	0.5	0.3
		T40	0.3	0.5
8	**A**	T0	0.3	0.3
		T10	25.0	25.0
		T20	25.0	25.0
		T30	25.0	25.0
		T40	25.0	25.0

9	**A**	T0	0.5	0.5
		T10	0.5	0.3
		T20	0.4	0.3
		T30	0.3	0.3
		T40	0.4	0.4
10	**A**	T0	0.5	0.5
		T10	0.6	0.5
		T20	0.3	0.3
		T30	0.3	0.3
		T40	0.3	0.4
11	**A**	T0	2.4	3.2
		T10	3.0	3.4
		T20	2.4	2.4
		T30	2.6	1.6
		T40	3.0	2.7
1	**B**	T0	0.5	2.5
		T10	25.0	25.0
		T20	25.0	25.0
		T30	25.0	25.0
		T40	25.0	25.0
2	**B**	T0	3.0	4.2
		T10	25.0	25.0

		T20	25.0	25.0
		T30	25.0	25.0
		T40	25.0	25.0
3	**B**	T0	1.7	0.8
		T10	25.0	25.0
		T20	25.0	25.0
		T30	25.0	25.0
		T40	25.0	25.0
4	**B**	T0	8.2	9.9
		T10	25.0	25.0
		T20	25.0	25.0
		T30	25.0	25.0
		T40	25.0	25.0
5	**B**	T0	0.7	2.3
		T10	25.0	25.0
		T20	25.0	25.0
		T30	25.0	25.0
		T40	25.0	25.0
6	**B**	T0	0.7	0.5
		T10	0.8	0.8
		T20	0.7	0.8
		T30	25.0	25.0

		T40	25.0	25.0
7	**B**	T0	0.6	0.7
		T10	0.7	0.3
		T20	25.0	25.0
		T30	25.0	25.0
		T40	25.0	25.0
8	**B**	T0	2.9	4.2
		T10	25.0	25.0
		T20	25.0	25.0
		T30	25.0	25.0
		T40	25.0	25.0
9	**B**	T0	1.0	0.8
		T10	0.8	1.0
		T20	25.0	25.0
		T30	25.0	25.0
		T40	25.0	25.0
10	**B**	T0	0.7	0.6
		T10	25.0	25.0
		T20	25.0	25.0
		T30	25.0	25.0
		T40	25.0	25.0
11	**B**	T0	0.7	0.7

		T10	25.0	25.0
		T20	25.0	25.0
		T30	25.0	25.0
		T40	25.0	25.0
12	**B**	T0	1.0	0.9
		T10	25.0	25.0
		T20	25.0	25.0
		T30	25.0	25.0
		T40	25.0	25.0
13	**B**	T0	2.6	2.1
		T10	25.0	25.0
		T20	25.0	25.0
		T30	25.0	25.0
		T40	25.0	25.0
14	**B**	T0	0.5	0.7
		T10	25.0	25.0
		T20	25.0	25.0
		T30	25.0	25.0
		T40	25.0	25.0
15	**B**	T0	0.7	0.6
		T10	0.5	0.5
		T20	25.0	25.0

		T30	25.0	25.0
		T40	25.0	25.0
16	**B**	T0	0.9	0.3
		T10	0.7	0.4
		T20	25.0	25.0
		T30	25.0	25.0
		T40	25.0	25.0

Appendix 3: Additional data collected in this study: time required to perform tibial perineural injection, operator who performed the injection, signalment of the horse, if perineural analgesia of the peroneal nerve was performed contemporaneously and if any complications occurred while performing tibial perineural injection. (Operators: NB= Nicholas Bellitto; JW= Jonathan Withers; CB= Christian Byrne; MM= Mattie McMaster).

Assigned case number	Treatment (A=Blind; B=US)	Time required for procedure (secs)	Operator	Limb blocked	Breed	Age (years)	Sex	Peroneal block	Complications
1	**A**	310	JW	RH	Cob	20	mare	yes	venipuncture
2	**A**	186	NB	LH	Irish sport horse	5	mare	no	no
3	**A**	132	NB	RH	Welsh sec. A	10	gelding	no	no
4	**A**	40	MM	RH	Warmblood	9	gelding	no	no
5	**A**	45	MM	LH	Warmblood	9	gelding	yes	no
6	**A**	88	CB	RH	Thoroughbred	17	gelding	yes	no
7	**A**	84	CB	RH	Warmblood	7	gelding	yes	no
8	**A**	105	NB	LH	Anglo-arab	17	mare	no	no
9	**A**	95	NB	LH	Anglo-arab	17	mare	no	no
10	**A**	93	NB	RH	Anglo-arab	17	mare	no	no
11	**A**	95	NB	LH	Irish sport horse	10	mare	yes	no
1	**B**	265	JW	RH	Connemara	7	mare	no	no
2	**B**	220	NB	RH	Warmblood	5	gelding	yes	no
3	**B**	250	JW	LH	Irish sport horse	12	gelding	no	no
4	**B**	260	NB	LH	Highland	8	gelding	yes	no
5	**B**	338	JW	RH	Irish sport horse	8	gelding	yes	no
6	**B**	485	JW	RH	Cob cross	14	gelding	no	no

7	**B**	320	NB	RH	Welsh cross	8	gelding	yes	no
8	**B**	320	NB	LH	PRE	7	gelding	yes	no
9	**B**	268	NB	RH	Welsh sec. A	10	gelding	no	no
10	**B**	370	NB	LH	Welsh sec. A	12	gelding	yes	no
11	**B**	215	JW	RH	Cob	8	gelding	no	no
12	**B**	320	JW	RH	Irish sport horse	12	mare	no	no
13	**B**	210	JW	RH	Cob cross	10	gelding	no	no
14	**B**	268	NB	LH	Warmblood	7	gelding	no	no
15	**B**	212	NB	RH	Cob	6	mare	no	no
16	**B**	90	NB	RH	Thoroughbred	8	mare	no	no

Appendix 4: MVLS Interpretation of the University Code of Practice on Alternative Format Theses and Author Declaration & Contribution**.**

MVLS Interpretation of the University Code of Practice on Alternative Format Theses

Submission by alternative format is allowed under the University Code of Practice. This applies to PhD, MD, MVM and MSc(research) degrees.

A thesis in an alternative format may only be requested by the PGR student. There is no requirement to publish research in journals for the award of a postgraduate research degree from the University of Glasgow and this should never be stated as a requirement by a supervisor. Submission in an alternative format must never be required by a supervisor, nor should the practice of a supervisor lead to pressure on a PGR student to submit in this format.

The PGR student and their supervisor must read the University Code of Practice, together with documentation from the College Graduate School and their School or Institute on how the code should be interpreted. As per the Code of Practice the consideration and decision on whether to request to submit a thesis by alternative format should be made at the earliest opportunity during the research degree when it becomes clear that both the student and supervisor wish to consider this option. It is recognised that the appropriate timepoint at which a decision to proceed to preparation and submission of thesis by alternative format may vary depending on the discipline and specific project. However, it is important that the decision is made in a timely manner to ensure that the requirements below can be met and documented and that the request can be submitted to the Graduate School for review and approval in advance of thesis preparation and submission. The approval procedure is that students must explicitly confirm in writing that they have read and understand these documents and agree to adhere to them. Students may ask for approval for this style of thesis at any time during their studies. However, final confirmation of the format for submission will be required when the student submits their Intention to Submit notification (in future the TAP system will be amended to include this confirmatory step); we strongly encourage that this is done as early as possible (i.e. 6 months prior to intended submission). Outline permission to write a thesis by alternative format will be granted by a School's PGR Convenor, who in turn will inform HDC for noting only, and hence final approval on behalf of the Graduate School. Like any other PGR thesis, an alternative format thesis can be embargoed following completion of the examination process and submission of the final approved thesis to the library.

- To apply for approval students are requested to email their PGC directly at any time, copying in their primary supervisor, to note their intention to submit a thesis by alternative format, including a statement indicating that they are aware of the guidelines for this approach. However, as outlined above, confirmation must be sought at the Intention to Submit phase.
- When PGC approve (by reply) to the student's request, they should copy in the Graduate School to ensure notification for HDC.

To satisfy the requirements for an alternate format thesis, a minimum of 1 chapter (paper) must be fully published, i.e. akin to a journal manuscript format. Should there be no published papers available, a student must write their thesis in the 'traditional' standard format. Papers included in an alternative format thesis must either be published, have been accepted for publication, or be written in such a manner that they are ready for submission for publication. A combination of published paper chapters and traditional format chapters is acceptable. Published papers for inclusion should be papers containing original data/results, although in some subject areas meta-analysis or systematic reviews are commonplace and highly appropriate, and hence are also acceptable. A paper that is essentially a review of the literature would not meet the criteria of an original published paper. In some research students' studies clinical case reports are often published. It may be acceptable to include these types of publications in the alternative format thesis alongside more 'typical' scientific publications, so long as there is a clear link between these publications.

Confirming Authorship

A thesis must be the candidate's own work. However, this can present problems with multi-author papers, hence the student must:

- be first author on any paper included in the thesis.
- include, separate to the thesis, a declaration document (see end of this file) confirming in writing their contribution according to relevant categories of the Contributor Roles Taxonomy (CRediT) system, which will include amongst other things their contribution to the writing of the results and discussion sections. A declaration document should be filled out for each published paper and submitted as one overall document in addition to, but separate to, the thesis. In some research groups, the PGR student provides the data and figures and the supervisor writes the text. This would not fulfil the authorship criteria for a research thesis. The PGR student must have made a major contribution to writing the text, for example by providing a reasonable quality first full draft of the Results and Discussion sections. The declaration document for each paper must be signed off by the primary supervisor and/or corresponding author (if different).
- explicitly confirm that the research they contributed to in the papers was carried out while they have been registered as a PGR student at the University of Glasgow or were registered as a PGR student at another institution (in cases where they have transferred to Glasgow during the course of their PGR studies).

As co-signatory of the authorship declaration documents the primary supervisor (and corresponding author if different) are confirming in writing that:

- the PGR student's assessment of their contribution to the papers is correct as stated.
- they acknowledge that, as the contribution by the student is deemed significant enough for the paper to be included in the thesis, then no other UofG student can use the paper in an alternative format thesis.
- the supervisor supports the writing up in an alternative format style.

Guidelines on the structure and content of theses with an alternative format

Requirements for the introductory and summary chapters

As with all theses, an alternative format thesis must have Introductory and Summary Chapters (with references). The introductory chapter should set out the aims of the body of work as a whole in the context of the relevant literature. It is recognised that some of this introduction will be repeated in the introductions of the chapters that are the texts of papers. It is noted that introductions in papers are constrained by the requirements of publishers and referees. On the other hand, the introduction of a thesis includes fuller discussion and more comprehensive overviews of the relevant literature and the fundamental concepts underlying the work. Therefore, for an alternative format thesis, which comprises of possibly a number of published papers, there is likely to be a greater requirement for a fuller, more detailed introductory section in order to outline how these papers/chapters form a comprehensive body of work. The summary chapter should be akin to a General Discussion/Conclusion chapter in a standard-format thesis, and should summarise and bring together the findings of the thesis as a whole and propose future work where relevant.

Requirements for Chapters comprised of papers

- Each paper should be a separate chapter with the chapter title corresponding to the paper title.
- There should be a statement within the declaration document that accompanies each chapter detailing where the work is published and including all authors. For papers not yet submitted for publication there should be a statement indicating the intended journal of publication.
- The text of the chapter should be identical to the text of the paper, except for numbering of Figures, Tables etc, which should follow the sequence of the thesis as a whole as in a standard-format thesis.

- The text should be formatted as is standard for theses and should be the same as the introductory and general discussion chapters.
- There are often size constraints on Figures in papers. The Figures in the theses should be optimal for reading, which may mean that they are enlarged and at higher resolution. Otherwise, their appearance should be identical to their published form.
- Where there is a supplementary figure(s) referred to in the text, the figure should be placed close to the text that refers to it. The reader should not be required to look elsewhere in the thesis to follow the discussion.
- As in all theses, the contributions of others must be acknowledged. Acknowledgment at the beginning of the Chapter is not sufficient. Where, for example, data have been acquired or interpreted by others, this must be explicitly stated and the individuals named at the relevant place in the thesis, e.g. in the legend of a figure and the text that discusses it. Failure to do this would be considered plagiarism.

Requirements for full information and readability

- There should be sufficient detail in the thesis to provide the necessary evidence for the claims made and for the work to be reproduced. Methods, data and analyses that would normally be presented in theses of that discipline must be presented in a thesis submitted in an alternative format.
- The incorporation of a paper as part of the thesis should not adversely affect the readability of the thesis (see comments above on siting of supplementary figures).
- For the above reasons, preliminary communications or papers with extensive supplementary information may not be suitable for alternative format theses. However, some supplementary data/tables may be appropriate to include as an appendix at the end of the thesis.
- Examiners can require changes to the arrangement of material in any thesis where the structure impedes understanding of the work presented, and this may include material presented as an already published paper. In rare instances, the examiners may require the chapter to be re-written as a traditional format chapter, or indeed the overall thesis to be re-written as a traditional format thesis.
- In a standard-format thesis, linking/bridging between chapters is important. The alternate thesis model may make this more challenging. Therefore, it is a permissible option to include in the thesis a 1-2 page section as a preface to each chapter with the intention of maintaining the narrative and readability of the thesis.

Author Declaration & Contribution

Full paper citation, incl. authors and their affiliation (or link/ref to preprint server for papers not published)	Bellitto, N.A., Voute, L., Reardon, R. and Withers, J.M., 2024. Ultrasound-guided perineural injection of the tibial nerve in the horse versus a 'blind'technique. *Equine Veterinary Education*, *36*(2), pp.64-73. **Nicholas A. Bellitto;** Glasgow Equine Hospital & Practice, School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Glasgow, UK; **Lance Voute;** Glasgow Equine Hospital & Practice, School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow, Glasgow, UK. **Richard Reardon;** The Equine Dental Clinic Ltd., Edinburgh, Scotland. **Jonathan M. Withers;** Pool House Equine Clinic, Lichfield, Staffordshire, UK

Please state your author contribution below for each category in accordance with the principles of the Contributor Roles Taxonomy system (CRediT). Please refer to the descriptors of each category before completion (https://credit.niso.org/)

Conceptualisation	Nicholas Bellitto, Jonathan Withers
Data Curation	Nicholas Bellitto
Formal Analysis	Nicholas Bellitto,Richard Reardon
Investigation	Nicholas Bellitto
Methodology	Nicholas Bellitto
Project Administration	Nicholas Bellitto
Visualisation	Nicholas Bellitto
Writing – original draft	Nicholas Bellitto

Writing – review & editing	Nicholas Bellitto, Lance Voute
Software (optional)	
Validation (optional)	

We declare that the author contribution statements, in accordance with the CRediT system, are an accurate representation for this chapter/paper:

Author signature	
Primary supervisor signature (or corresponding author if different)	

List of references

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