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ORIGINAL ARTICLE



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

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Summary

Background: Tibial perineural analgesia has often been reported to fail to achieve nerve desensitisation in horses. Ultrasound-guided (US-guided) techniques have recently been described to improve tibial perineural desensitisation.

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

Study design: Randomised clinical trial.

Methods: Horses presenting for lameness investigation, which required tibial perineural analgesia, were randomly assigned either to a US-guided or blind injection group. The efficacy of perineural analgesia was assessed by testing the loss of skin sensation at the medial and lateral heel bulbs. Skin sensation was assessed, prior to injection and then at four intervals post-injection (10–15, 20–25, 30–35 and 40–45 min) using a hand-held digital algometer with a 1 mm diameter pin; a value of 25 N was defined as indicative of skin desensitisation. The time taken to perform each injection technique and any adverse reactions were recorded. 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.

Main limitations: Limited number of cases for each injection group.

Conclusions: These findings suggest that US-guided tibial perineural injection is more likely to result in adequate and prompt tibial perineural analgesia compared to the blind injection technique, although it takes longer to complete.

KEYWORDS

horse, lameness investigation, loss of skin sensation, tibial perineural analgesia, ultrasound-guided injection

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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 10cm 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 1).

Assessment of the response to tibial perineural analgesia has been recommended between 10 min and 1h 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, the 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



FIGURE 1 (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.

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 hypothesised that the US-guided technique would take longer to complete but be better tolerated by the horse compared to the blind technique.

MATERIALS AND METHODS

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 6h 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 tranquilisers prior to or during tibial perineural injection.

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 preexisting 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 pre-assigned 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.

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

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 and then alcoholic spirit (95% ethanol and 5% methanol). In all cases, in both groups, 2mL mepivacaine hydrochloride 2% (w/v) (Intra-Epicaine, Dechra Veterinary Products) was deposited subcutaneously using a 25 gauge 5/8-inch needle prior to performing the tibial perineural injection. Tibial perineural analgesia was performed by injecting 20mL 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 a 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 1). 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).

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 the 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. The 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



FIGURE 2 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.

instruction of operator A (Figure 2). 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 2).

Blind perineural injections were performed using a 23 gauge, 1inch needle. The nerve was first identified by palpation (firm cordlike 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 a 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.

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 were by a hand-held digital algometer (Prod, TopCat Metrology) attached to a long custom-made handle (Figure 3). 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 3) 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.



FIGURE 3 Images show 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.

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 and 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 3), 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).

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 2mL 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

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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.

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.

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 \geq 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.

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 \pm SD, 10 \pm 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 (two left hindlimb and one right hindlimb), one horse underwent one US-guided injection and one blind injection (both right hindlimb) and one horse underwent two blind injections (one left hindlimb and one 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 USguided injections. The boarded surgeons performed the remainder: JW one blind injection and six US-guided injections, MM two blind injections and 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).

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 4a. 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 4b.

The mechanical nociceptive threshold values recorded for both groups are shown in Figure 5.

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 6.

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.

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



FIGURE 4 (a) Histogram shows 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).



Skin sensation measurements after blocking

FIGURE 5 Histogram shows mechanical nociceptive threshold (MNT) measurements in Newton (N) for the medial and lateral heel bulb recorded after performing tibial perineural analgesia grouped in ranges.

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



FIGURE 6 Box plot shows 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.

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 intraobserver 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 5). 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 an US-guided technique using a 25 gauge, 5/8-inch needle and a 6-10MHz 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, 4-10mL less local anaesthetic agent were infiltrated around the nerve. Van der Laan et al. (2021) compared the accuracy of a conventional 'blind' technique and an US-guided technique for perineural injection of the tibial nerve, using cadaveric limbs and a low volume of dye (1mL 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 8% 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 20mL 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 Laan et al. (2021). The length facilitated repositioning of the needle for injection of local anaesthetic agent around the nerve without 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

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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 USguided 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 ($275.7 \pm 20.6 \text{ s}$ vs $115.7 \pm 24.9 \text{ s}$; p < 0.001). The US-guided injection however was completed in less than 5 min 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 8% 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-guided 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.

AUTHOR CONTRIBUTIONS

N. Bellitto is the primary author with substantial contribution to study design, data collection, interpretation and analysis of the data and manuscript preparation. L. Voute contributed to study design, interpretation of the data and critical revision of the manuscript. R. Reardon contributed to the interpretation of the data, data analysis and critical revision of the manuscript. J. Withers contributed to study design, data collection and critical revision of the manuscript. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared.

FUNDING INFORMATION

None.

ETHICS STATEMENT

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

INFORMED CONSENT

Owners gave consent for the horses to participate in this study.

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