**Objective methods of nerve localisation to facilitate performing locoregional anaesthetic techniques for horses undergoing surgical procedures**

**Abstract (up to 300 words)**

Perineural anaesthesia is a useful technique in equine surgery, providing pre-emptive targeted anaesthesia of the surgical site, reducing volatile anaesthetic requirements, improving recovery quality and providing post-operative pain relief. Surgery under standing sedation in horses has increased in popularity, mandating the need for effective locoregional anaesthesia and analgesic techniques. Nerve location techniques offer greater accuracy than blind techniques when placing injectate. These methods can help to avoid structures such as blood vessels and minimise direct nerve damage during needle placement, reducing the risk of procedure-related complications. This review will discuss the most pertinent research in the veterinary literature where objective methods of nerve location have been used to perform peripheral nerve blocks in horses. The efficacy of using objective methods to perform nerve blocks in equine anaesthesia is discussed by the authors, providing useful information to equine anaesthetists, and potentially improve the quality of anaesthesia and analgesia in horses.

**Introduction**

Neuraxial and peripheral regional anaesthetic and analgesic techniques have been widely utilised in equine anaesthesia. These techniques provide pre-emptive analgesia, reduce adjunctive anaesthetic requirements, improve recovery quality and provide post-operative analgesia in horses subjected to surgical procedures under general anaesthesia (Goodrich *et al.* 2002, Morris *et al.* 2020, Louro *et al.* 2020, Hector *et al.* 2020). The growing popularity of standing surgery and the increasing focus on pain management in horses have created a demand for effective regional anaesthesia and analgesia techniques. Although direct palpation of nerves can be highly effective, its application is limited to superficially located nerves, restricting its use to target nerves that are located closer to the skin.

In recent years, hundreds of studies in human and small animal anaesthesia have been conducted as a result of the widespread use of new methods and technologies, including peripheral nerve stimulation and ultrasound-guided injections. The application of these methods for targeting peripheral nerves in small animal anaesthesia have been previously reviewed and have been demonstrated to improve accuracy and precision, as well as reduce complications associated with regional anaesthesia and analgesia (Portela *et al.* 2018a, Portela *et al.* 2018b).

The purpose of this review is to discuss the most pertinent research in the veterinary literature where objective methods of nerve location have been used to perform peripheral nerve blocks in horses (Table 1.). This review will exclude objective methods used to perform peripheral nerve blocks of the head, as these have been recently reviewed elsewhere (Hermans *et al.* 2019, Johnson *et al.* 2021).

1. **Peripheral nerve stimulation**

Anatomical landmarks and response to needle placement are typically employed to ensure that the needle tip is placed near the target nerve when performing peripheral nerve blocks in conscious horses. A peripheral nerve stimulator, on the other hand, uses motor reactions to electrical stimulation in order to determine the proximity of a peripheral nerve (Dalrymple and Chelliah 2006). Although anatomical landmarks are still used to guide needle approach, direct contact between the needle and the target nerve is not required, avoiding potential trauma to the nerve associated with blind techniques (Dalrymple and Chelliah 2006).

In 1780, Luigi Galvani, discovered that the muscles of dead frogs' legs contracted when an electrical stimulus was applied (Galvani 1791), but it was not until 1912 that Georg Perthes described the safe and atraumatic localisation of peripheral nerves using electrostimulation for the purpose of performing regional anaesthesia (Perthes 1912).

1. **Clinical use of peripheral nerve stimulators**

The nerve stimulator applies a small amount of direct current (DC) to the tip of the needle. Depolarisation of the neurons will occur if the needle is close enough to a motor neural structure, if the stimulus is sufficiently strong and applied for an appropriate duration (Dalrymple and Chelliah 2006). This will produce an action potential which will propagate and generate a response from the effector organ (e.g. skeletal muscle). Even when the stimulation is sustained or prolonged, a low intensity stimulus will not result in an action potential. Likewise, if a high intensity stimulus is administered for a brief period of time, an action potential will not be generated (Dalrymple and Chelliah 2006).

A peripheral nerve stimulator (Figure 1.) is composed of a case featuring an LCD display, an on-off switch, and a dial for regulating the target stimulation current. Additionally, the stimulator is equipped with several buttons to adjust the current range, stimulus duration, and frequency, providing precise control over the stimulation parameters. To establish the circuit, two leads are utilised, with one lead connected to the skin electrode through a skin clamp or an ECG skin pad, while the other lead is attached to the nerve stimulation needle. In both the United Kingdom and the United States of America, the cathode (negative terminal) and anode (positive terminal) are commonly color-coded as black and red, respectively. However, it is essential to note that colour coding may vary among different manufacturers worldwide (Krone 2012). The cathode cable is attached to the nerve stimulation needle, whereas the anode is attached remotely to the patient in order to complete an electrical circuit (Krone 2012). A constant current is produced between the anode and cathode which is maintained irrespective of the impedance of the tissue. The shaft of the nerve stimulation needle is insulated (e.g., Teflon) so that the electrical current carried through the needle is applied to the tissues only from the tip of the needle (Krone 2012).

Peripheral nerve stimulators used in veterinary medicine allow an adjustable constant current output from 0.1 to 2 mA which can be controlled by a dial (Otero *et al.* 2019). Initially, a high current (>1.0 mA) is employed, which will stimulate neural structures through tissue and fascial planes distant from the tip of the needle (Dalrymple and Chelliah 2006). When a correct motor response is first elicited, the current is gradually decreased, in decrements of 0.1 mA, to 0.5-0.3 mA; the ‘threshold current’. If a motor response is still present at the threshold current but not if further reduced to 0.2 mA, it indicates the tip of the needle is likely to be close to the neural structure, but not penetrating the nerve (Otero *et al.* 2019). If a response is still elicited at 0.2 mA, it is likely the needle tip is directly impinging on, or embedded in, the nerve and it should be withdrawn slightly to avoid intrafasicular injection and iatrogenic nerve damage. When correct needle placement has been achieved it is appropriate to inject local anaesthetic, preceded by initial aspiration to confirm inadvertent vascular needle placement has not occurred (Otero *et al.* 2019). Initially, a small volume of local anesthetic or saline solution (1mL) is administered to verify the absence of excessive resistance. This injection typically leads to an immediate halt in the motor response, commonly known as the Raj test. Initially, it was believed that this cessation resulted from the displacement of neural structures away from the needle tip, causing an increase in the needle-to-nerve distance and subsequently requiring more current to elicit a response. However, we know understand that the loss of response is actually attributed to decreased current density surrounding the needle, brought about by the dispersion of local anaesthetic (Tsui et al. 2004, Ercole 2008). Therefore, beware that this phenomenon may be noticed even if the nerve is located further away from the nerve stimulator needle (Otero *et al.* 2019). Aspiration of blood prior to injection, excessive resistance to injection, or failure to abolish muscle twitch after the test dose has been administered should alert the operator to intravascular or intraneural needle placement and the needle should be withdrawn and repositioned.

The frequency of feedback can be adjusted by the operator, determining the rate at which a motor response is produced. Usually, the frequency is set between 1 to 2 Hz. Drifting from these parameters may result in patient discomfort, block failure or complications due to challenges in precisely locating the nerve (Otero *et al.* 2019).

1. **Physics of peripheral nerve stimulation**

The relationship between the nerve stimulation needle and the nerve can be described by Coulomb’s law, $E=k\frac{Q}{r^{2}}$, where E is the required stimulating charge, k is a constant, Q is the minimal required current to evoke stimulation of a neural structure and r is the radius reflecting the electrode-to-nerve distance. Consequently, the closer from the nerve the tip of the needle is, the less current is needed to elicit an action potential.

Different peripheral nerve fibres have distinct electrophysiological characteristics, depending on fibre diameter, function, conduction velocity and degree of myelination (Dalrymple and Chelliah 2006). The threshold stimulation of a neural structure will also depend on the constant current applied and the duration of the stimulus. Rheobase is the minimal current necessary to activate an action potential in the nerve (Figure 2.). Even if the current is applied for an extended period, it cannot initiate an action potential below this threshold. Chronaxie is the length of time that the current must be delivered to initiate an action potential, when the constant current applied is twice the rheobase (Dalrymple and Chelliah 2006) (Figure 2.).

Aα motor nerve fibres, which are fast conducting nerves, have a short chronaxie due to a shorter refractory period when compared to sensory nerve fibres, such as Aδ or unmyelinated C fibres, which are slower conducting nerves. Because of these characteristics, at low constant current intensities it is possible to stimulate a motor nerve (Aα fibres) but not the sensory nerve (Aδ or unmyelinated C fibres). Therefore, a motor response can be elicited without producing pain (Dalrymple and Chelliah 2006). As previously mentioned, a muscular motor response serves as feedback to locate the nerve accurately. Hence, having motor fibers in close proximity to the stimulation point is crucial for the effectiveness of this technique. Peripheral nerve stimulators used in veterinary medicine will allow an adjustable duration of stimuli from 0.1 msec to 0.3 msec which can be controlled in the peripheral nerve stimulator (Otero *et al.* 2019).

1. **Use of peripheral nerve stimulation guided techniques in horses**

One of the first descriptions of the use of a peripheral nerve stimulator to aid loco-regional anaesthetic techniques in horses was reported in 2009, by Cheetham and colleagues who performed bilateral hypoglossal nerve blocks at the level of the ceratohyoid bone, in two separate occasions, in ten horses (total of 20 blocks) to study the role of this nerve in upper airway stability (Cheetham *et al.* 2009). These authors used a constant current of 2 mA at a frequency of 2 Hz and duration of 0.15 msec, with synchronous caudal retraction of the tongue seen as effector organ response. Following a positive response, the current was reduced in 0.2 mA increments until a threshold current of 0.2–0.4 mA was reached. When this was achieved, 0.5 mL of mepivacaine (concentration not stated by the authors) was injected following aspiration and if a positive Raj test was observed and no resistance to injection was noted, the remainder (1.0 –2.5 mL) of the mepivacaine was injected slowly (Cheetham *et al.* 2009). Tongue tone was tested 30 minutes later to assess success of the technique, and was found to be absent in only two out of 19 cases. Nevertheless, using this technique, nasopharyngeal instability, manifested as dorsal displacement of the soft palate, was reported in ten of 19 cases (Cheetham *et al.* 2009).

Peripheral nerve stimulation has also been described for performing pudendal nerve block in horses (Gallacher *et al.* 2016). These authors performed an initial cadaveric study to evaluate dye distribution after blocking the pudendal nerve using electrolocation, followed by clinical evaluation of the block in horses undergoing reproductive surgical procedures under standing sedation or general anaesthesia.

In the cadaveric study the authors determined that a combination of anal and perineal twitch, rather than only anal or perineal twitch alone, provided better results in terms of dye distribution over the pudendal and caudal rectal nerves (Gallacher *et al.* 2016). The authors also noted that there was no difference in dye distribution or length of nerve staining if a high volume (20 mL) was injected compared to a low volume (10 mL). During the cadaveric study, complications were reported in two out of seven horses, including one case of rectal puncture and one case of vaginal puncture as a result of the block (Gallacher *et al.* 2016).

In the clinical part of the study, a bilateral pudendal nerve block was performed in 27 horses (both mares and geldings) undergoing various reproductive surgical procedures (Gallacher *et al.* 2016). In all cases, the block was performed under standing sedation, using the ischial tuberosity, semimembranosus muscle, the external anal sphincter and either the dorsal vulvar lips or the retractor penis muscle as visual or palpable landmarks. The ventral aspect of the external anal sphincter served as the upper limit and the lateral limit is indicated by the semimembranosus muscle. Following skin desensitisation, an insulated 21G, 100mm needle was inserted at an angle of 45° to the sagittal plane adjacent to the ventrolateral aspect of the external anal sphincter. Initial stimulation variables were reported as a current of 1 mA, frequency 1-2 Hz and stimulus duration of 0.1 msec. When contractions of both the anal sphincter and perineal muscles or vulvar lips were observed at a threshold current of 0.2–0.4 mA, an appropriate anaesthetic solution was injected. For male patients, a 10 mL injection of either lidocaine 2% or mepivacaine 2% solution was injected at each site. For female patients, a 20 mL injection of lidocaine 2%, mepivacaine 2%, or bupivacaine 0.5% solution was injected, depending on the required duration of anaesthesia for the specific surgical procedure. The authors reported a needle insertion depth of 5-10 cm depending on patient size and perineal conformation, with variable onset of action between five to fifteen minutes. In males, time from injection to penile extrusion was reported to range from one to ten minutes and lasted for less than five hours (Gallacher *et al.* 2016). The block was successful at first attempt in 25 cases, with two cases requiring an additional unilateral injection of 10 mL of local anaesthetic, after which the block was deemed effective.

A single case report describes successful nerve-stimulator guided thoracolumbar paravertebral nerve block in a thoroughbred mare undergoing a flank laparoscopy for unilateral ovariectomy under standing sedation (Santos and Gallacher, 2017). Using the approach described by Moon & Suter (1993), these authors initially performed local anaesthetic infiltration of the skin, subcutaneous tissue and superficial musculature (5 mL of lidocaine 2% per site). A 20 G, 150 mm insulated needle was inserted perpendicular to the skin, towards the relevant lumbar transverse processes, which in the adult horse are located at a depth of approximately 9 cm. If bone was contacted, the needle was walked off the cranial edge until penetration of the intertransverse ligament occurs. Application of a current of 1 mA was used to evoke contraction of the external and internal oblique and transverse abdominal muscles. A volume of 5 mL of mepivacaine 2% was infiltrated at maximum positive stimulation and another 5 mL was infiltrated when retracting the needle 2 cm above this point. This procedure was repeated at three sites to desensitise nerves T18, L1 and L2 at the transverse process of L1, L2 and L3 respectively. The adequacy of the block was checked 15 minutes later by sensory loss of the surgical field to pinprick sensation (Santos and Gallacher 2017).

1. **Ultrasound guided nerve blocks**

Ultrasound is a non-invasive imaging technique which allows for the precise localisation of anatomical structures. Ultrasound provides a real-time, dynamic image of the scanned anatomical structures, which has revolutionised the field of locoregional anaesthesia (Krone 2012). The introduction of ultrasound-guided technique for peripheral nerve blocks has enabled more accurate needle placement in relation to the target nerve, and the spread of the local anaesthetic can be observed in real-time (Krone 2012). In recent years, the importance of this tool has been recognised in small animal anaesthesia and has recently gained some attention in equine anaesthesia, leading to the development of new equine locoregional anaesthesia techniques. In humans, using ultrasound to locate nerves for peripheral blockade has been shown to be more successful than the use of peripheral nerve stimulation guided techniques, and reduced the rate of vascular puncture (Munirama and McLeod 2015). While studies have shown that this technique results in faster onset times, longer block durations, better predictability of block success and reduced requirement for supplemental analgesia, it is yet unclear whether ultrasound truly provides better success and safety, especially in terms of reducing nerve injury, than other techniques in both humans and small animal patients (Marhofer and Fritsch 2017).

**a. Erector spinae plane block**

Erector spinae plane (ESP) block is a technique involving the infiltration of local anaesthetic within the inter-fascial plane between the transverse process of the thoracic vertebrae and the erector spinae muscular complex (formed by iliocostalis, longissimus dorsi, and spinal muscles), resulting in desensitisation of the structures innervated by the dorsal rami of the thoracic spinal nerves. Delgado et al. (2021), conducted an experimental study to assess the viability of utilising an ultrasound guided approach to performing ESP block by assessing nerve staining and yellow permanent tissue marking dye distribution in equine cadavers. The procedure was conducted using a convex transducer (5 MHz) at the level of the 16th thoracic vertebra (T16). The transducer was positioned parasagittally and orientated longitudinally just lateral to the dorsal midline, allowing for the identification of the transverse process of T16 (Figure 3.). An 18 gauge, 200 mm spinal needle was then advanced in-plane craniocaudally towards the transverse process of the T16. With the bevel orientated ventrally, the needle was advanced until the bevel touched the bony surface of the transverse process. A small volume of saline solution was injected in order to confirm needle placement by observing hydrodissection between the erector spinae muscles and the transverse process prior to injection of a lidocaine-dye mixture (0.2 mL/kg). Dye distribution was subsequently assessed during dissection of the cadavers.

Staining of the thoracolumbar fascia was observed in 85% of the 20 cases studied, with an average dye distribution length of 4.8 ± 1.3 vertebral bodies. Although no dye was found in the thoracic and abdominal cavities or on the sympathetic trunk, staining of the epidural space was observed in 20% of the 20 injections performed (involving one or two intervertebral spaces). In only 14 cases further dissection to evaluate nerve staining was performed. At least one dorsal rami of the thoracic nerves were stained, whereas the ventral rami (intercostal nerves) were stained in only three injections.

This loco-regional technique may be useful for desensitising the structures supplied by the dorsal rami of the thoracic and lumbar spinal nerves, specifically to identify and treat conditions affecting the spinous processes of the vertebrae. Recently, three case-reports have been published demonstrating the use of ultrasound-guided ESP (UG-ESP) block in horses undergoing ostectomy and interspinous ligament desmotomy (Chiavaccini et al., 2022; Perez et al., 2023; Rodriguez et al., 2022). Perez et al (2023) performed the procedure bilaterally under standing sedation, targeting the transverse process of T13 and T18. Utilising 20 mL 2% lidocaine hydrochloride at each of the four sites, the authors reported an effective block for at least 140 minutes, after which a top-up dosage of 5 μg/kg of detomidine and a splash block with 40 mL of 2% lidocaine in the surgical area were required due to an increase of 27% in the pulse rate and slight movement of the horse during surgical manipulation (Perez et al., 2023). Rodriguez and co-workers performed the UG-ESP block under general anaesthesia, bilaterally at the level of T15 utilising a total volume of 53.5 mL (0.1 mL/kg) 0.5% bupivacaine per injection site (Rodriguez et al., 2022). Additionally, Chiavaccini et al. (2022) utilized this loco-regional technique in a case of surgical wound exploration and debridement in a horse with multiple lumbar spinous process fractures (Chiavaccini et al., 2022). These authors performed bilateral UG-ESP block using a combination of 0.25% bupivacaine (55.6 mL per site; 0.1 mL/kg) and dexmedetomidine (1 μg/mL) (Chiavaccini *et al.* 2022). These clinical case reports suggest that UG-ESP block is effective in providing analgesia to horses undergoing surgery of the dorsal spinal processes. Despite the potential epidural spread of local anaesthetics reported in the cadaveric study conducted by Delgado et al., 2021, no motor deficits have been reported in clinical cases, utilising either lidocaine or bupivacaine at different concentrations. A prospective randomised controlled trial is needed to compare the effects of UG-ESP block to the blind loco-regional techniques commonly used for these surgical procedures. Additionally, it is crucial to evaluate the influence of epidural spread on motor blockade in both the front and hind limbs. This assessment is necessary to understand the potential consequences, such as weakness or ataxia, that may impact either the front or hind limbs and could potentially lead to recumbency.

**b. Transversus abdominus plane block**

The transversus abdominus plane (TAP) block desensitises the sensory branches of the thoracic and lumbar spinal nerves, which run within the interfascial plane between the transversus abdominis and internal oblique muscles. Local anaesthetic injection into this fascial plane should anaesthetise the relevant areas of skin, abdominal wall muscles and parietal peritoneum (Cevik *et al.* 2022). Ultrasound is commonly used to accurately locate the targeted fascial plane prior to injection in humans, dogs, cats and horses (Cevik et al., 2022). When performed successfully, the TAP block can be beneficial in desensitising the ventral abdominal wall, providing analgesia and muscle relaxation during abdominal surgical procedures. It is important to mention that the TAP block is not expected to provide analgesia for visceral pain associated with intra-abdominal pathology in horses.

So far, three different techniques have been described for administering the TAP block in cadaver studies in ponies and horses: subcostal, ventral intercostal, and flank approaches (Baldo *et al.* 2018, Küls *et al.* 2020, Freitag *et al.* 2021) (Figure 4.). The first report of the TAP block in pony cadavers was performed using a flank approach in dorsal recumbency (Baldo *et al.* 2018). A 6-13 MHz linear US transducer was positioned midway between the caudal aspect of the last rib and the cranial aspect of the iliac crest (Figure 4. blue dot) at a point perpendicular to the caudal aspect of the umbilicus (Figure 4. dashed with line). The ultrasound transducer was positioned in a transverse plane and shifted ventro-dorsally and caudo-cranially until the external oblique, internal oblique and transversus abdominis muscles were identified as three separate layers. Once the ultrasonography landmarks were identified, a 21G, 100 mm needle was inserted in-plane in a ventral-dorsal direction (Figure 4. red dot). To confirm correct needle placement between the internal oblique and transversus abdominis muscles, a small amount of dye and local anaesthetic was injected to induce hydrodissection. If separation of the two muscles did not occur, the needle was repositioned. Upon confirmation of proper needle position, a total volume of 0.5 mL/kg of bupivacaine 0.5% and dye (methylene blue 1%) was injected. The authors reported the technique was simple to perform and resulted in >75% success rate in staining the ventral branches of nerves T16-L2 (Baldo *et al.* 2018). The authors speculated that a TAP block performed using a flank approach will only desensitise the middle to caudal and ventral abdominal wall and parietal peritoneum, not the cranial abdominal wall (Baldo *et al.* 2018), due to the innervation of the equine abdominal wall originating from spinal nerves ranging from T5 to L2 (Cevik et al., 2022). Like any other cadaveric study, the efficacy and safety profile of these loco-regional techniques must be validated through in vivo studies. Specifically, for this technique, it is crucial to evaluate the feasibility, practicality, and safety of injecting such a large volume (0.5 mL/kg of local anaesthetic) within the interfascial plane between the transversus abdominis and internal oblique muscles of a horse.

A ventral intercostal approach was developed by Küls, et al. (2020) to address the limited cranial spread of local anaesthetic observed with the flank approach. In this cadaveric study each hemiabdomen received a TAP block at three different points: caudal to the 9th, 14th and 18th rib. Cadavers were positioned in lateral recumbency and the rib corresponding to T18, T14 and T8 identified. A 5-10 MHz linear transducer was positioned perpendicular to the rib, 10 cm ventrally to the costochondral junction of the corresponding intercostal space (Figure 4. red dots). Once the ultrasonography landmarks were identified, a 21G, 100 mm needle was inserted in-plane in a caudo-cranial direction. A test injection to confirm hydrodissection of the plane between the internal oblique and transversus abdominis muscles was performed, prior to injection of 0.3 mL/kg bupivacaine 0.5% and 1% methylene blue dye, equally divided between the three injection sites. With this technique all spinal nerves originating from T8 to T18 were stained. These authors subsequently investigated the efficacy of this block in a prospective, blinded, controlled trial in six Shetland ponies under standing sedation, using either 0.1 mL/kg bupivacaine 0.125% or a saline solution at each injection site (Küls *et al.* 2020). Efficacy of the block was assessed using cutaneous pinprick at the level of T8 to L2. The authors reported a success rate of four out of six ponies, although it failed in the remaining two cases. The results presented, however, are pooled for all cases, making it challenging to precisely determine the overall success rate. A noteworthy concern is that a truly successful block should result in the absence of a pinprick response. However, the authors reported a statistically significant reduction in response for nerves T8–T18, suggesting some level of efficacy but not complete success. An onset of effect of 30 minutes was reported and a duration up to 120 minutes for nerves T12–T17 and up to 180 minutes for nerves T8–T11 (Küls *et al.* 2020).

The subcostal approach to performing a bilateral TAP block is a two-point technique described in horses in either lateral or dorsal recumbency (Freitag *et al.* 2021). For this procedure a line is drawn between the xiphoid cartilage and the umbilical scar (Figure 4. blue dots) and the ventral border of the cutaneous trunci muscle is identified. The first point of injection is located midway between these two landmarks ventral to the cutaneous trunci muscle limits (Figure 4. dashed red line leading to point of injection: red dot). The second point of injection is located at the first third of the line drawn from the umbilical scar and the fist injection point, ventral to the cutaneous trunci muscle limits (Figure 4. dashed white line leading to point of injection: red dot). A 7.5-12 MHz linear transducer is positioned perpendicular to the central axis at one of these points. The cutaneous trunci, rectus abdominis and transversus abdominis muscle are the ultrasonographic landmarks to be identified. An 18G, 100 mm needle was inserted in-plane in a dorso-ventral direction. A test injection confirmed hydrodissection of the plane between the rectus abdominis and transversus abdominis muscles, followed by a total volume of 30 mL methylene blue 0.5% injected at the two sites, corresponding to a total volume of 60 mL (0.12-0.16 mL/kg). The authors reported correct needle placement at first attempt in 86.7% of hemiabdomens injected, with all failures occurring when the horse was positioned in dorsal recumbency (Freitag *et al.* 2021). Cadavers placed in lateral recumbency had a higher percentage of nerve staining (>57.1%) between T9-T17 than those in dorsal recumbency (>50%) between T13-T18, and this difference was statistically significant (p = 0.0249). This technique is believed to be a feasible solution for desensitising the ventral cranial abdomen (Freitag *et al.* 2021).

1. **Rectus abdominis sheath block**

In an anatomical description and prospective, crossover, placebo-controlled, blinded study, Ishikawa et al., (2023) described a technique to perform an internal rectus abdominis sheath (RAS) block for providing antinociception to the abdominal midline in horses (Figure 4.). The abdominal wall is composed by four muscles: the external abdominal oblique muscle, the internal abdominal oblique muscle and the transversus abdominis muscle, which are located anterolaterally compared to the rectus abdominis muscle which is located immediately lateral to the linea alba in a more midline position than the transversus abdominis muscle (Budras *et al.* 2012). The first part of the study conducted by Ishikawa et al., (2023) utilised two cadavers and compared a one-point injection technique, performed on the right hemiabdomen with a two-point injection technique performed on the left hemiabdomen. The xiphoid and the umbilicus were used as main landmarks (Figure 4. blue dots) and a line between this two structures was used as a reference for the injections (Figure 4. full white line). A linear ultrasound transducer (6-13 MHz) was placed over the abdominal midline in a transverse orientation to the reference line in order to locate the linea alba at the midpoint of the reference line. The probe was moved 5 to 10 cm laterally toward the right side of the abdomen to view the interior RAS between the lateral aspect of the right rectus abdominis muscle and the medial aponeurosis of the right transverse abdominal muscle (Figure 4. dashed white line leading to point of injection: red dot). At this site, an 18G, 90 mm spinal needle was inserted in-plane at a 30-45° angle in a dorsomedial direction. Correct placement was confirmed when a test dose (2.0 mL) of the prepared 1% methylene blue and 0.5% bupivacaine solution was administered and hydrodissection of the rectus abdominis muscle from the transverse abdominal muscle was identified. The two-point technique involved a similar technique, with the first and second points located close to the abdominal midline between the cranial and middle thirds and the middle and caudal thirds of the reference line, respectively. A total volume of 0.25 mL/kg of 1% methylene blue and 0.5% bupivacaine solution was injected at each point. The authors reported successful staining of the ventral branches of the ninth thoracic (T9) to second lumbar (L2) nerves when using the two-point injection technique. Incomplete spread was observed with the one-point injection technique that did not consistently stain T9 and T10 (Ishikawa *et al.* 2023). For the second part of the study, the authors conducted a prospective, crossover, placebo-controlled, blinded study using a two-point injection technique with 0.2% bupivacaine (0.25 mL/kg per injection site) in standing horses (Ishikawa *et al.* 2023). Antinociception, evaluated using mechanical nociceptive threshold with a 1 mm probe tip, demonstrated the technique provided antinociception for at least five hours at the abdominal midline without evidence of pelvic limb weakness, leading the authors to hypothesise that this technique may be useful for reducing incisional pain associated with exploratory laparotomy (Ishikawa *et al.* 2023).

**d. Cervical plexus block**

Laryngoplasty is commonly performed under standing sedation. In order to allow this procedure, surgeons perform extensive infiltration of the skin and peri-laryngeal structures with local anaesthetic (Rossignol *et al.* 2015). An ultrasound-guided cervical plexus block (targeting C2 and C3 nerve roots) has been described in horses, providing an alternative to the more traditional local infiltration approach (Campoy *et al.* 2018). The wing of the atlas, the vertebral body of C2, the linguo-facial vein, and the jugular vein are used as anatomical landmarks. A linear transducer is placed at the midlevel of the second cervical vertebral body, caudal to the parotid gland and ventral to the omotransversarius muscle. The transducer is then rotated to obtain a short axial view of the ventral spinal branch of C2 located in the interfascial plane between the longus capitis and the cleidomastoideus muscles. A 20G, 90mm Tuohy needle was then advanced in-plane through this fascial plane, and a small amount of saline is injected to confirm needle placement by observation of hydrodissection of the two muscle planes and approximately 40mL 2% mepivacaine injected. Additionally, the authors recommended an injection of 10 mL 2% mepivacaine subcutaneously in a vertical plane, 10 cm caudal to the most caudal aspect of the incision site in order to anaesthetise the cutaneous branches of C3 (Campoy *et al.* 2018). Performing a cervical plexus block improved surgical conditions in comparison to conventional tissue infiltration. The local anaesthetic used during conventional tissue infiltration distorts tissues sufficiently to obscure essential surgical landmarks (e.g. linguofacial vein) which are utilised to guide the first skin incision. Moreover, the majority of local anaesthetics typically used induce local vasodilation, thereby increasing the probability of surgical haemorrhage and tissue oedema. This can further complicate recognition of important intraoperative landmarks. Despite these encouraging results, it is important to note that sensory innervation of the larynx is not provided by the cervical nerves. In addition, no corresponding reduction in surgical time, duration of procedural sedation, or amount of sedative or local anaesthetic was observed by the authors (Campoy *et al.* 2018). The efficacy of this block in horses undergoing left-sided prosthetic laryngoplasty under general anaesthesia was compared to horses undergoing the same procedure without a locoregional block in a retrospective study (Morris *et al.* 2020). The authors noted that the group receiving a cervical plexus block needed less additional anaesthesia compared to the non-blocked group (Morris *et al.* 2020). Due to the neuroanatomy of the area it is possible that transient Horner’s syndrome and/or transient laryngeal hemiplegia of the right arytenoid may occur in some horses. The latter is suspected to occur due to diffusion local anaesthetic resulting in blockade of the right caudal laryngeal nerve, resulting in altered laryngeal dysfunction (Morris *et al.* 2020). However, no intra- or post-operative complications were reported following a cervical plexus block in either study (Campoy *et al.* 2018, Morris *et al.* 2020).

In a prospective experimental cadaveric study, researchers aimed to assess the feasibility of ultrasonography-guided perineural injection of the C7 and C8 ramus ventralis in four equine cadavers (Touzot-Jourde *et al.* 2020). Using the cervical vertebrae C6 and C7 as anatomical landmarks, a micro-convex 5-8 MHz ultrasound probe was positioned to identify the C7-T1 articular process joint and a longitudinal section of the C8 ramus ventralis was identified. A 20G, 88mm spinal needle was inserted 2 cm caudo-ventrally to the transducer and advanced in-plane to within 5mm of the nerve surface for injection of either 7 or 14 mL of methylene blue (Touzot-Jourde *et al.* 2020). The study demonstrated successful staining of a portion of the nerve root in all injections, with eight rami showing uniform transversal staining extending over 2 cm. However, incomplete staining was observed in one C7 and one C8 nerve root. Epidural contamination was reported by the authors, with injections closer to the articular processes resulting in more epidural diffusion. Despite the variations in injection points and volume, all injections were selective for the targeted nerve. The study suggests that ultrasonography-guided perineural injection of C7 and C8 ramus ventralis is a feasible technique with potential applications in multimodal analgesia in horses (Touzot-Jourde *et al.* 2020).

A similar technique was described for injecting the caudal cervical spinal nerve roots (C5 to C7) in 14 equine cadavers (Cruz-Sanabria *et al.* 2021). However, in this study, the distribution of the injectate was assessed using magnetic resonance and computed tomography imaging before cadaver dissection. The perineural injection consistently delivered contrast agent to the targeted caudal cervical spinal nerve root region in all cases. The authors suggested that this technique holds potential for diagnosing and treating cervical pain in horses, especially when intra-articular cervical articular process joint injections have not yielded desired results (Cruz-Sanabria *et al.* 2021).

**e. Pudendal nerve block**

An ultrasound-guided technique for performing a bilateral pudendal nerve block has been described in male donkeys (El-Khamary *et al.* 2017). In contrast to the blind technique and nerve-stimulator guided technique discussed earlier in this review, this approach offers the advantage of real-time visualisation of crucial anatomical structures, including the rectum, blood vessels, and nerves, during needle placement. This capability has the potential to lower the risk of inadvertent damage to these structures, hypothetically making the technique safer and more precise and accurate. After desensitising the skin at the dorsal aspect of the ischiorectal fossa, a 5-10 MHz transrectal probe was positioned ventrolaterally to locate the ischium, and dorsally to that, the internal pudendal blood vessels were identified using colour Doppler. The pudendal nerve, which is not always easily visible with ultrasound, was located approximately 1 cm dorsal to the internal pudendal vessels. To target this nerve, an 18G, 200 mm needle was inserted dorsally in the ischiorectal fossa and directed cranioventrally. The needle was then visualised on ultrasound and advanced dorsally, reaching a depth of about 8 cm dorsally to the internal pudendal artery and vein. A mixture of 10 mL lidocaine 2% and 1 mL of methylene blue 1% (0.05 mL/kg at each site) was injected to the targeted area. The same procedure was repeated on the opposite side (El-Khamary *et al.* 2017). Anaesthesia of the penis and perineal region was achieved within five minutes and lasted for an average of 47 minutes. Penile protrusion was obtained in eight minutes and maintained for 90 minutes. No persistent priapism or paraphimosis were observed in the animals studied (El-Khamary *et al.* 2017).

**f. Head nerve blocks**

A number of advanced techniques for the equine head have recently been developed, such as ultrasound-guided, advanced imaging-guided and nerve stimulator-guided techniques. This topic has been recently reviewed in two excellent narrative reviews, where the authors discussed the use of perineural nerve blocks for the equine head, exploring the advantages and disadvantages of blind approaches as well as approaches guided by ultrasound, nerve stimulation, or advanced imaging techniques (Hermans *et al.* 2019, Johnson *et al.* 2021). Furthermore, the authors highlighted the wide range of clinical applications for ultrasound-guided procedures of the equine head and compared human and equine literature, examining the potential of perineural techniques for both diagnostic and therapeutic purposes. As techniques in the field are constantly evolving, ultrasound guidance is becoming an increasingly popular and utilised modality worldwide for locoregional anaesthesia of the head in equine patients (Hermans *et al.* 2019, Johnson *et al.* 2021). Consequently, the authors of the current review have not included locoregional techniques involving the head for dental or ocular procedures.

1. **Further research applications**

There is an obvious growing interest in objective methods of nerve location in equine anaesthesia, which follows the prolific research performed in small animal and human anaesthesia. Nevertheless, much of the published literature consists of cadaveric studies and case reports, with only a few studies combining cadaveric anatomical description with clinical efficacy studies using a limited sample size. The need for further research on the impact of locoregional techniques in equine anaesthesia is apparent, specifically in terms of evaluating the effects on anaesthetic requirements, post-operative analgesia and pain scores, the ability to performing standing surgery using such techniques, and the incidence of complications associated with these objective methods of nerve location. Moreover, larger sample sizes should be used to better understand and assess the impact of these methods of nerve location in comparison to the traditional blind locoregional anaesthetic techniques which are already popular in equine anaesthesia and analgesia.

1. **Conclusion**

The use of objective methods for locoregional anaesthesia in equine patients has led to the development of various new techniques for targeting different nerves and desensitising various anatomical areas, allowing for standing surgery as well as reduced intraoperative pain during surgery performed under general anaesthesia. To assess the advantages and disadvantages of these objective methods in comparison to traditional blind locoregional anaesthetic techniques, and to determine their impact on equine anaesthesia, analgesia, and surgery, further research is necessary. So far, objective methods such as nerve stimulation and ultrasound-guided injections have been found to improve accuracy and precision, as well as reduce complications associated with locoregional anaesthesia and analgesia in small animal and human anaesthesia. Thus, gaining a comprehensive understanding of the impact of these objective methods could significantly enhance the integration of such techniques into the routine practice of equine veterinary surgeons. This, in turn, will enable clinicians to make well-informed decisions when choosing the most suitable locoregional techniques for their equine patients, leading to improved patient care and surgical outcomes.

Table legends:

Table 1. Summary of locoregional anaesthesia techniques using objective methods of nerve localisation for use in horses undergoing surgical procedures

Figure Legends:

Figure 1. Peripheral nerve stimulator (Plexygon, Vygon (UK) Ltd, Swindon, UK). The peripheral nerve stimulator is a small device that enables the localisation of nerves through the emission of a low-intensity electrical current.

Figure 2. A graphical representation illustrating the concepts of chronaxie and rheobase in nerve fibers. Chronaxie is the minimum duration of an electrical impulse required to elicit a response at twice the rheobase current intensity. Rheobase is the minimum current intensity required to elicit a response with a stimulus of infinite duration. These parameters are crucial in understanding the excitability and responsiveness of nerve fibers to electrical stimulation.

Figure 3: (a) Parasagittal ultrasounography image of the region of thoracolumbar region at the level of the fifteenth and sixteenth transverse processes (TP). (b) Schematic representation indicating key ultrasonography landmarks for conducting the erector spinae plane block: longissimus dorsi (LD) muscle, thoracolumbar fascia (TLF), and parietal pleura (PP).

Figure 4: Techniques for transverse abdominis plane (TAP) and rectus abdominis sheath (RAS) blocks. This schematic figure illustrates various approaches to the transverse abdominis plane (TAP) block and the technique for performing an internal rectus abdominis sheath (RAS) block. Three distinct methods have been identified in cadaver studies for administering the TAP block in ponies and horses: subcostal, ventral intercostal, and flank approaches (Baldo et al. 2018, Küls et al. 2020, Freitag et al. 2021). Additionally, the technique for performing an internal RAS block to provide antinociception to the abdominal midline in horses is shown (Ishikawa et al. 2023). Anatomical landmarks are indicated by blue marker and white lines, while points of injection are represented by red marker.

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