**Abstract (Word count 287)**

**Objectives** To describe dye distribution and spinal nerve involvement after a simulated erector spinae plane (ESP) block performed on fresh equine cadavers.

**Study design**Experimental cadaver study.

**Animals**A group of 11 adult equine cadavers.

**Methods** The spinal region surrounding the 16th thoracic vertebra (Th16) of one cadaver was removed and underwent magnetic resonance imaging. In 10 adult equine cadavers (549 ± 58 kg, mean ± standard deviation), 0.2 mL kg-1 of a 50:1 2% lidocaine:dye solution was injected bilaterally (*n* = 20 injections) into the fascial plane between the transverse process of Th16 and the erector spinae muscles. An in-plane ultrasound-guided technique with a convex transducer was used to guide injection.

Dissection was performed immediately following injection. The craniocaudal and lateral extent of dye distribution was measured (cm) and the number of vertebral bodies involved were counted (*n* = 20). Abdominal and thoracic cavities as well as the epidural space were also examined for presence of dye (yes/no) (*n* = 20). Further dissection was performed to evaluate if staining of the dorsal rami (DR) and ventral rami (VR) of the spinal nerves and sympathetic chain occurred (*n* = 14).

**Results** The thoracolumbar fascia was stained in 17/20 (85%) injections and three injections terminated intramuscularly. Multisegmental staining of the DR and VR was observed in the 14 injections where staining of DR and VR was evaluated. Epidural migration was observed in 4/20 (20%) of the injections. No evidence of dye was found in the thoracic and abdominal cavities or on the sympathetic chain.

**Conclusions and clinical relevance** Theerector spinae plane block may prove beneficial to desensitise structures innervated by the DR of the thoracic spinal nerves. Further investigation is needed to evaluate complications due to epidural contamination.

***Keywords*** back pain, dorsal root, equine, nerve block, regional anaesthesia, spinal nerves

**Introduction (Word count 3121)**

Ultrasound (US) guided erector spinae plane (ESP) block consists of infiltration of local anaesthetic within the inter-fascial plane between the erector spinae muscular complex and the transverse process of the thoracic vertebrae (Forero et al. 2018).

In horses, the erector spinae is a muscular complex formed by the iliocostalis, longissimus and spinal muscles. It is covered by the thoracolumbar fascia and located on the dorsal surface of the transverse process and ribs (Payne et al. 2004). The erector spinae occupies the space between the spinous and transverse processes of the lumbar, thoracic and cervical vertebrae (Zaneb et al. 2013). Cranial and caudal spread of local anaesthetic from the ESP injection site desensitises the dorsal rami (DR) of the relevant spinal nerves (El-Boghdadly & Pawa, 2017). Erector spinae block at the level of thefifththoracic transverse process is a recently described technique in human medicine. Distribution in the ESP from this injection site has been shown to extend caudally to the eighththoracic transverse process in fresh cadavers. (Chin et al. 2017). This correlates with the observed pattern of analgesia and sensory loss in human clinical patients [Forero et al. 2016; Forero et al. 2018; Hernandez et al. 2018; Ferreira et al. 2019]. In humans, the ESP block is indicated for the treatment of acute or chronic pain following thoracic, abdominal, hip and spinal surgeries (Forero et al. 2016; Restrepo-Garces et al. 2017; Hernandez et al. 2018; Bugada et al. 2019).

A porcine cadaver study and two canine cadaver studies reported ESP injection to be a fairly simple to perform with no evidence of intrathoracic, mediastinal or epidural spread of dye (Ferreira et al. 2019; Portela et al. 2019; Otero et al. 2020). Considering the anatomical distribution of dorsal spinous process (DSP) impingement in horses (Walmsley et al. 2010), the authors hypothesised that the ESP injection performed more caudally could desensitise the relevant nerves and provide effective local analgesia for DSP ostectomy and desmotomy (Walmsley et al. 2010).

We investigated the magnetic resonance imaging (MRI) and sonographic anatomy required to develop an equine ESP injection technique. Authors used post-ESP injection dye distribution in equine cadavers to evaluate the potential benefits, by assessing nerve staining, and identify possible complications associated with procedure.

**Material and Methods**

Study design

Institutional ethics committee approval(RETH000689) was granted and owner´s consent was obtained for horses included in this study.

A total of 11 skeletally mature horses presented to the Leahurst Equine Hospital (University of Liverpool) and were euthanised for reasons unrelated to conditions affecting the vertebral column spine < 24 hours prior to use were included in the study. Variables such as body weight (kg), age (years), breed, sex, occipito-coccygeal distance (cm) and withers to coccygeal distance (cm) were recorded.

*Anatomical characterisation*

The caudal thoracic spine (13th thoracic to 2nd lumbar vertebra) of one cadaver was removed from the body. MRI was performed using a 1.5 Tesla unit equipped with a body coil, dStream anterior torso coil with 16 channels and a posterior coil in the table (Ingenia 1.5T CX, Philips Healthcare, UK). T2-weighted (T2W), T1-weighted (T1W) and thin-slice T1W (3D-T1W) images were acquired in the transverse plane. Multiplanar reconstruction of the 3D-T1W images was performed as necessary. The intention was to precisely delineate the gross musculoskeletal and neuroanatomy of the 16th thoracic vertebra (Th16), the site for the ESP injection. The MRI focused on anatomical factors critical to ESP injections, the epaxial muscles, continuity of the associated fascia, and relevant nerves (dorsal rami, ventral rami (VR) and grey rami communicans) as they exit the intervertebral foramen.

ESP injections

Ultrasound-guided ESP injections of a lidocaine-dye mixture targeting the transverse process of Th16 were performed bilaterally in 10 cadavers with a total of 20 injections (10 right-sided and 10 left-sided). The solution, a 50:1 ratio of 2% lidocaine hydrochloride (Hameln pharmaceuticals, UK) and a yellow permanent tissue marking dye (The Davidson Marking System, Bradley Products, MN, USA) was prepared immediately prior to use. To position the cadavers in sternal recumbency bilateral transaxillary and transinguinal incisions were performed, allowing the thoracic limbs to be extended laterally and the pelvic limbs backwards. Hair was clipped from the dorsal midline (approximately 20 cm on each side) from the tenth thoracic to the fourth lumbar vertebrae.

An US scanner (Vivid I, GEMS Ultrasound, Israel) with a convex transducer (5 MHz) was used to visualise the 18th rib as an anatomical landmark for identifying the TP of Th16. The transducer was positioned parasagittally and orientated longitudinally just lateral to the dorsal midline once the transverse process of Th16 was identified.

With the target site in the middle of the screen, an 18 gauge, 20 cm spinal needle (MILA International, Inc, USA) was advanced in-plane craniocaudally towards the middle TP of Th16 (Fig. 1). Needle advancement stopped in a distinctive way when the bevel of the needle, orientated ventrally, touched the bony surface of the transverse process. In the event of unsatisfactory positioning, the needle was withdrawn (partially or completely) and reoriented. When proper needle position was achieved the stylet was removed and an extension tube (ConnectaPE Line M/F 150 cm internal volume 1.4 mL m-1 Merit Medical Ireland Ltd, Ireland) with a 20 mL syringe(BD Plastipak, Becton, Dickinson and Company Limited, Ireland) were attached. A small volume (5 – 20 mL) of saline solution(Aqupharm NaCl 0.9%, Animalcare Ltd, UK) was then injected to try and separate the plane between the erector spinae muscles and the transverse process (hydrodissection). When the operators were satisfied with needle placement a lidocaine-dye mixture (0.2 mL kg-1)was injected over 2-3 minutes. To prevent leakage the stylet was replaced prior to withdrawing the needle. The procedure was repeated on the contralateral side.

The distances from the point of needle insertion to the dorsal midline and from the skin surface to the dorsal aspect of transverse process, on the US image, were recorded. All procedures were performed by one of two operators.

Dissection

Dissection of the injection sites to confirm dye distribution was performed immediately after bilateral ESP injections. All dissections were performed by two pathologists (GR and RV). The primary anatomisation was carried out in a dorsoventral approach previously described (Ferreira et al. 2019). The craniocaudal and lateral extent of dye distribution was measured, and the number of vertebral bodies involved were counted in 10 cadavers, hence 20 injections (*n* = 20).

The spine was then removed from the cadaver, incorporating a minimum 10 cm margin of tissue beyond any stained areas as determined by preliminary dissection and immediately frozen. The pleural and abdominal cavities were opened and examined for dye contamination (yes/no). The frozen spine was sawn in a sagittal plane to evaluate dye distribution in the epidural space.

Additional dissection was performed in seven specimens (*n* = 14) to evaluate nerve staining. The DR, VR, and sympathetic chain were considered successfully stained if the dye was found surrounding the nerve for a length ≥ 1cm.

**Statistics**

Data were analysed using IBM SPSS Statistics for Windows, Version 20.0. (IBM Corp, NY, USA), normality was tested using the Shapiro-Wilk test. Data are presented as mean ± standard deviation (SD) if normally distributed and as median plus interquartile range, (IQR) if non normally distributed.

**Results**

The horses used in this study weighed 549 ± 58 kg and were 9.9 ± 3.9 years of age. The study population consisted of four Thoroughbred horses, two Warmbloods, two Sports Horses, one Irish Draught, one Cob, and one Arabian horse. There were seven mares and four geldings. Occipito-coccygeal distance was 210.1 ± 38.2 cm and distance from occipital region to withers was 121.9 ± 17.3 cm.

Imaging Results

In all sequences, the *longissimus dorsi* muscle was observed lateral to the *fusiform multifidus* muscle, separated by discrete linear T2 and T1W hyperintensity (fat) within the *fascia thoracolumbalis*. The fascia of the *longissimus dorsi* muscle was indistinguishable from the T2 and T1W hyperintensity (fat) surrounding the spinal nerve roots following their exit from the intervertebral foramina into the paravertebral space. The intermuscular fascial septa and lateral segmentations of the *longissimus dorsi* muscle were also noted (Fig. 2). The other muscles of the equine erector spinae complex (the *spinalis* and *iliocostalis* muscles) were outside the field of view of the images acquired for this study (Mark Schultz & Sødring Elbrønd 2018).

On 3D T1W sequences the spinal nerve roots were identified as they exited the intervertebral foramen before branching dorsally and ventrally. The 16th *ramus communicans* separated from the ventral portion of the 16th spinal nerve prior passing ventromedially towards the ventral part of the vertebra. Post-mortem gas tracking along the fascial planes of the epaxial muscles precluded further visualisation of the path of the DR. The MRI findings frame the anatomical landmarks for the ESPblock in horses.

ESP injections

The relevant sonographic landmarks (transverse process and *longissimus dorsi* muscle) were identifiable in all animals. The needle tip was visualised before and during the injection in all cases.

Although not recorded, in some cases US identification of the *thoracolumbaris fascia* or visualisation of hydrodissection was not possible. The insertion point of the needle to the dorsal midline was 4.2 (3.58-7.75) cm. US measured distance from skin to TP was 9 (5.88 -10) cm.

Dissection and dye spread

The *thoracolumbar fascia* was stained in 17/20 injections (85%). Staining of the *longissimus dorsi* muscle was noted in 3/20 injections (15%).

Dye spread was observed cranially, caudally, and medially towards the spine and laterally towards the *multifidus* muscles from the injection site. Staining was also observed lateral to the *fusiform multifidus* muscles within the *thoracolumbar fascia* (Figs 3a, b).

Staining was observed on 4.8 ± 1.3 vertebral bodies within the *thoracolumbaris fascia.* Cranio-caudal dye distribution was 25 (18-31.5) cm. Staining of at least one dorsal rami was observed in each injection evaluated (*n* = 14) (Fig. 3c). Multi-segmental staining was observed in some injections (Fig. 4). Ventral rami (intercostal nerve) staining was observed in three injections (Fig. 3d). No evidence of dye was found in the thoracic and abdominal cavities or on the sympathetic trunk.

Epidural staining was observed in 4/20 injections (20%). The staining involved one spinal nerve corresponding to the injection site in 3 cases and two intervertebral spaces (16th and 17th thoracic) in the remaining instance (Figure 5). Epidural involvement coincided with VR staining in two instances.

**Discussion**

This study describes the first investigation of a novel US-guided ESP injection technique in horses that may prove beneficial in the diagnosis and treatment of back problems. As this block is US-guided, it offers the advantage of being more accurate and efficacious than traditional blind injections. In the authors experience, US-guided injections allow the clinician to identify the desired location, visualise the needle and the surrounding structures in real time. Ultimately this ensures that the solution can be accurately injected at the intended site.

MRI confirmed the gross anatomy of the thoracic spine and salient sonographic landmarks necessary to facilitate the ESP injection technique (Fig. 2). The anatomic landmarks used are similar to those reported in previous equine studies (Vandeweerd et al. 2007; Ehrle et al. 2017).

In the present study dye distribution was predominantly medial (between the *fusiform multifidus* muscle and the *thoracolumbar fascia*) and lateral to the transverse process of Th16 (Figure 3 a, b). This is in agreement with previously reported findings in human and dog cadavers (Ivanusic et al. 2018; Ferreira et al. 2019; Portela et al. 2019).

The primary landmark used to identify the ESP injection site was the transverse process of Th16, similar to previous reports in other species (Forero et al. 2018; Portela et al. 2019) (Figure 1). Identifying the transverse process of Th16 using the ribs as sonographic landmarks was feasible, but not easy in all instances. This was primarily due to the moderate formation of post-mortem emphysema, which reduces US image quality.

The needle tip was always visible prior to and during injection. In some instances visualisation of the needle tip was enhanced by rapid ‘in-and-out’ motion as previously reported (Ferreira et al. 2019). This technique may need other refinements (e.g. skin block or “needle track” block) to improve tolerance in standing horses. The use of echogenic needles is another option to improve US needle visualisation. Hydrodissection confirms correct needle position within the *thoracolumbar fascia* (Ferreira et al. 2019). Unfortunately, hydrodissection was not visualised in all instances in the present study and, sadly, this was not noted. The weight of the *longissimus dorsi* alone or in conjunction with post-mortem changes (partial rigor) may have impaired separation of the muscle from the transverse process.

Visualising the needle tip against the dorsal face of the transverse process was used to verify proper position for the ESP injection technique. The success rate was slightly lower than previously reported studies (Ferreira et al. 2019; Portela et al. 2019; Vidal et al. 2018). Inexperience due to the novel nature of the ESP injection technique in horses could have contributed to the lower success rate. Nevertheless 17/20 injections were successful.

In two recent canine cadaver studies reported staining of multiple dorsal rami (mean 2 and 4, respectively) following ESP injection of dye solution (0.5 vs 1 and 0.3 vs 0.6 ml kg-1, respectively) (Ferreira et al. 2019; Portela et al. 2019). Multi-segmental spread (based on DR staining) was also observed in this study (mean 2) (Figure 4). The staining patterns indicate ESP injections could provide analgesia to structures innervated by the DR. Despite isolating all DR associated with the thoracolumbar staining, tissue disruption during dissection made accurately assessing nerve staining challenging. The definition of successful staining in the present study was based on previously studies (Portela et al. 2019). Sensory or motor nerve blockade cannot be excluded in nerves with staining < 1cm.

Effective analgesia for thoracic procedures (VR blockade) following ESP injection in humans has been reported (Jones et al. 2019; Kuş et al. 2019; Nair and Seelam. 2019). The spread of solution to the paravertebral space may occur via perforations in the intertransverse connective tissue through which the dorsal rami of spinal nerves emerge, or through the superior costotransverse ligament described in humans, though the exact mechanism has not yet been elucidated (Forero et al. 2016; Forero et al. 2018; Altinpulluk et al. 2019; Portela et al. 2019). In the present study VR staining occurred in 3 injections, suggesting there may be similarities in the anatomy between humans, dogs and horses (Vidal et al. 2018; Ferreira et al. 2019). The fact that dissection was performed in a dorsoventral approach may have influenced dye distribution through the costotransverse ligament. Further research is needed to clarify the mechanism by which the VR could be blocked in horses.

Of the 20 injections, 3 were intramuscular (*longissimus dorsi*) and produced no staining of the DR or VR. A marked reduction in resistances was noted during these injections. This underscores the importance of both experience and continued technique development (e.g. hydrodissection visible on US) to ensure correct needle position.

Unilateral epidural staining was observed in 4 injections (Fig. 5). Epidural staining consisted of 1 spinal nerve (16th thoracic) in three instances and 2 spinal nerves (16th and 17th thoracic) in the other. Epidural involvement may result from the same mechanism proposed for VR staining following ESP injections (Forero et al. 2018).

The epidural involvement following ESP injection is a potential concern in horses. Motor blockade to either the thoracic or pelvic limbs can result in instability, unintending recumbency and possible fatal outcome. Fortunately, the 16th and 17th thoracic spinal nerves do not supply the limbs so blockade limited to this area would desensitise areas of the thoracic wall and dorsal aspects of the back, but should not impair ambulation (Levine J.M 2007). Further investigation is needed to define the risk associated with ESP blockade in equine patients, as well as dose and volume administered and rate of delivery impact, taking into account the potential to lead to unintended recumbency.

Innervation of each vertebra in horses is supplied by the DR. In the present study the DR was observed to divide at the level of the transverse process into medial and lateral branches. The medial branch further divides into two branches before exiting the intertransverse space. These branches continue dorsocaudal towards the surface of the vertebral lamina (Vandeweerd et al. 2007). Anatomically, ESP blockade at Th16 should desensitise DR expected to be involved in the pain associated with DSP pathology. In the present study the DR were consistently stained. A longer zone of blockade could make pain localization more challenging, but might prove more valuable in treatment applications. Further investigation is needed to determine the role of ESP blockade in the diagnosis and treatment of DSP pathology.

This study has several limitations, including the small sample size. The main limitation is related to the model used. The use of cadavers to evaluate dye spread may not represent the situation in vivo (Mowbray & Wong, 1988). Studies with live animals are imperative to evaluate the mechanism of action and efficacy of this block. The results reported in this study are specific to injections performed at the 16th thoracic level. The performance of this block at different spinal levels may change the distribution of the solution within the tissue. The amount of thoracic ribs was not recorded in any of the specimens. Anatomical differences consistent with lower number of ribs have been reported in previous study (Stecher 1962). The amount of thoracic ribs was not recorded in any of the cadavers which may have influenced the observed cephalo-caudal spread. The operators performing the injections and the pathologist doing the dissections were not blinded. This could have biased the interpretation of the results.

**Conclusion**

Ultrasound-guided ESP injections are reasonably simple to perform because the relevant sonographic landmarks are readily identifiable in equine. In the present study ESP injections consistently stained DR of the spinal nerves. Erector spinae plane injections may prove useful for desensitising structures innervated by DR of the thoracic and lumbar spinal nerves, most notably the diagnosis and treatment of DSP disorders. The results in this study showed no evidence of dye contamination of the intrathoracic or abdominal cavities. However, further investigation is warranted to refine the technique, determine clinical usefulness and to evaluate risks due to epidural contamination before attempting in live horses.

Further cadaver and clinical studies are needed to compare and validate the efficacy and mechanism of action of the block presented in this study, as well as to evaluate different methods to confirm the correct position of the needle before injection. Dorsal spinous process disorders are a recognized problem in equine patients. The results of this study suggest the ESP block as a promising technique to treat acute and chronic pain originating from structures innervated by the DR of the spinal nerve**.**

**References**

Altinpulluk E.Y, Ozdilek A, Colakoglu N et al. (2019) Bilateral postoperative ultrasound-guided erector spinae plane block in open abdominal hysterectomy: A case series and cadaveric investigation. Rom. J. Anaesth. Intensive Care 26, 83–88.

Bugada D, Zarcone A.G, Manini M et al. (2019) Continuous Erector Spinae Block at lumbar level (L4) for prolonged postoperative analgesia after hip surgery. J. Clin. Anesth 52, 24–25.

Chin K.J, Malhas L, Perlas A (2017) The erector spinae plane block provides visceral abdominal analgesia in bariatric surgery a report of 3 cases. Reg. Anesth. Pain Med. 42, 372–376.

Ehrle A, Ressel L, Ricci E et al. (2017) Structure and Innervation of the Equine Supraspinous and Interspinous Ligaments. J. Vet. Med. Ser. C Anat. Histol. Embryol. 46, 223–231.

El-Boghdadly K, Pawa A (2017) The erector spinae plane block: plane and simple. Anaesthesia 72, 434–438.

Ferreira T.H, St James M, Schroeder C.A et al. (2019) Description of an ultrasound-guided erector spinae plane block and the spread of dye in dog cadavers. Vet. Anaesth. Analg 46, 516–522.

Forero M, Adhikary S.D, Lopez H et al. (2016) The erector spinae plane block a novel analgesic technique in thoracic neuropathic pain. Reg. Anesth. Pain Med 41, 621–627.

Forero M, Rajarathinam M, Adhikary S et al. (2018) Erector spinae plane block for the management of chronic shoulder pain: a case report. Can. J. Anesth 65, 288–293.

Hernandez M.A, Palazzi L, Lapalma J et al. (2018) Erector Spinae Plane Block for Surgery of the Posterior Thoracic Wall in a Pediatric Patient. Reg. Anesth. Pain Med 43, 217–219.

Ivanusic J, Konishi Y, Barrington M.J (2018) A Cadaveric Study Investigating the Mechanism of Action of Erector Spinae Blockade. Reg. Anesth. Pain Med 43, 567–571.

Jones M.R, Urits I, Shnider M.R et al. (2019) Confirmation of Erector Spinae Plane Block Analgesia for 3 Distinct Scenarios. A A Pract 12, 141–144.

Kuş A, Üniversitesi K, Fakültesi T et al. (2019) The Effect of Erector Spinae Plane Block on Postoperative Pain Following Laparoscopic Cholecystectomy: A Randomized Controlled Study. JARSS 27, 9–14.

Levine J.M, Levine G.J, Hoffman A.G et al. (2007) Comparative Anatomy of the Horse, Ox, and Dog: The Vertebral Column and Peripheral Nerves. Compendium Equine.<https://vetfoliovetstreet.s3.amazonaws.com/mmah/c6/5837c24b2042b3ab205b5fdd405101/filePVE_02_09_279_0.pdf>

Mark Schultz R, Sødring Elbrønd V (2018) Novel dissection approach of equine back muscles: new advances in anatomy and topography-and comparison to present literature. SPG BioMed 1, 1–13.

Mowbray A, Wong K.K.S (1988) Low volume intercostal injection. A comparative study in patients and cadavers. Anaesthesia 43, 633–634.

Nair A.S, Seelam S (2019) The risks associated with erector spinae plane block in patients with abnormalities of coagulation. Korean J. Anesthesiol 72, 275–276.

Otero P.E, Fuensalida S.E, Russo P.C et al (2020) Mechanism of action of the erector spinae place block: distribution of dye in a porcine model. Reg Anesth Pain Med 45, 198–203.

Payne R.C, Veenman P, Wilson A.M (2004) The role of the extrinsic thoracic limb muscles in equine locomotion. J. Anat 205, 479–490.

Portela D.A, Castro D, Romano M et al. (2019) Ultrasound-guided erector spinae plane block in canine cadavers: relevant anatomy and injectate distribution. Vet. Anaesth. Analg 47, 229–237.

Restrepo-Garces C.E, Chin, K.J, Suarez P et al. (2017) Bilateral Continuous Erector Spinae Plane Block Contributes to Effective Postoperative Analgesia After Major Open Abdominal Surgery: A Case Report. A A case reports 9, 319–321.

Stecher R.M (1962). Anatomical Variations of the Spine in the Horse. J. Mammal 43, 205–219.

Vandeweerd J.M, Desbrosse, F, Clegg P et al. (2007) Innervation and nerve injections of the lumbar spine of the horse: A cadaveric study. Equine Vet. J 39, 59–63.

Vidal E, Giménez H, Forero M et al. (2018) Erector spinae plane block: A cadaver study to determine its mechanism of action. Rev. Esp. Anestesiol. Reanim 65, 514–519.

Walmsley J.P, Petterson H, Winberg F et al. (2010) Impingement of the dorsal spinous processes in two hundred and fifteen horses: case selection, surgical technique and results. Equine Vet. J 34, 23–28.

Zaneb H, Peham C, Stanek C (2013) Functional anatomy and biomechanics of the equine thoracolumbar spine: A review. Turkish J. Vet. Anim. Sci 37, 380–389.