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ADVANCEMENTS IN ULTRASOUND-GUIDED, NEUROSTIMULATED AND BLIND APPROACHES TO LOCOREGIONAL ANAESTHESIA IN COMPANION ANIMALS

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ABSTRACT

Peripheral nerve blocks (PNB) play nowadays an important role in perioperative pain management, both in human and in veterinary medicine. Several approaches have been described and validated in companion animals undergoing especially orthopaedic surgeries. Thus, considering the state of art in veterinary medicine, studies presented in this dissertation focus on pain management relate to soft tissue surgeries and medical conditions. An ultrasound-guided approach was used to assess cervical plexus block (US-CPB) in dog cadavers and to preliminary assess spermatic cord block (US-SCB) in dogs undergoing orchiectomy. A Neurostimulated approach to mandibular and facial nerves was assessed in dogs and rabbits undergoing total ear canal ablation and lateral bulla osteotomy (TECA-LBO). Furthermore, a different technique was described as a case report in a rabbit undergoing partial ear canal ablation and lateral bulla osteotomy (PECA-LBO), considering the different surgical technique used and the individual and specie-specific characteristics. Blind celiac plexus block is reported in a dog to manage severe pain related to acute pancreatitis irresponsive to conventional systemic analgesia. All these new techniques resulted to be feasible and those assessed in a clinical setting showed enhancement in anaesthetic stability and pain management, appearing to have promising application in clinical practice.

SOMMARIO

L'anestesia locoregionale riveste un ruolo fondamentale nella gestione del dolore intra e postoperatorio in medicina umana e veterinaria. Le tecniche riportate in letteratura ed utilizzate nella pratica clinica sono molte ed applicate principalmente alla chirurgia ortopedica. Considerando lo stato dell'arte in medicina veterinaria, gli studi presentati in questa tesi di dottorato si concentrano sulla gestione del dolore in corso di chirurgia dei tessuti molli e correlato a patologie mediche. È stato quindi descritto un approccio ecoguidato al blocco del plesso cervicale nel cane (studio cadaverico) ed è stata eseguita una valutazione preliminare di fattibilità ed efficacia del blocco ecoguidato del funicolo spermatico in cani sottoposti ad orchiectomia. Il blocco neurostimolato dei nervi facciale e mandibolare è stato descritto in ambito clinico nel cane e nel coniglio sottoposti a rimozione totale del condotto auricolare esterno e osteotomia laterale della bolla timpanica. Inoltre, considerando la differente tecnica chirurgica e le esigenze specifiche del soggetto, una variante di tale blocco è stata eseguita su un coniglio sottoposto a rimozione parziale del condotto auricolare esterno e osteotomia laterale della bolla timpanica. È stato infine descritto l'utilizzo clinico del blocco del plesso celiaco "alla cieca" in un cane affetto da pancreatite acuta per il controllo del dolore refrattario ai trattamenti analgesici classici. Tutte le tecniche descritte per la prima volta nella presente raccolta sono risultate di relativa facile esecuzione; in particolare, quelle valutate in ambito clinico hanno dimostrato di fornire ai pazienti elevata stabilità emodinamica durante l'anestesia generale e migliorato la gestione del dolore. Le future applicazioni nella pratica clinica sembrano, pertanto, promettenti.

INTRODUCTION

In 1906, Sr. Charles Sherrington (1857-1952) set a new approach to the understanding of the nervous system, foreseeing the pattern of spinal reflexes and their interaction. In addition to a mere physiological description, an attempt was made to go beyond the motor system to the psychical events: "*we may agree that if such sensations and feelings (…) do accompany the reactions we have studied, the neural machinery to whose working they are adjunct lies not confined in the nervous arcs we have so far traced but in fields of nervous apparatus that, though connected with those arcs, lie beyond them, in the cerebral hemispheres*" (Sherrington, 1906). An early concept of "nociception" and "pain" was thus introduced to mark a distinction between the detection of a noxious stimulus and the psychological and pathophysiological response to it. While nociception is now defined as the detection of a noxious stimulus that might cause tissue damage by specialized receptors, pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Loeser & Treede, 2008).

Despite the relatively recent definition and disclosure of its neurological pathways, an effective effort of modern medicine to treat pain dates back to the first half of the ninth century, when ether and chloroform potential to induce general anaesthesia was discovered. It was then in 1885 that Dr. JL Corning performed the first spinal anaesthesia using cocaine, while in 1905 Dr. Heinrich Braun published "Local anaesthesia; its scientific basis and practical use", after perfecting what he called *the block of nervous conduction* during the previous two decades (Rose, 1993; Wulf, 1998).

Locoregional anaesthesia plays nowadays an essential role in perioperative pain management in human as well as and in veterinary medicine.

Neuraxial and perineural deposition of local anaesthetics (LA) determines a blockade in impulse conduction, mainly through sodium ion channel inhibition, leading to a localized temporary loss of sensory and motor function (Weidmann, 1955). Thus, an effective central or peripheral nerve block allows a reduction in general anaesthetic requirement and perioperative opioids supplementation, providing better pain control, faster post-operative recovery and reduced hospital stay both in human and in veterinary patients (Xu et al., 2014; Atchabahian et al., 2015; Romano et al., 2016).

Three kind of approaches are currently described in literature to perform peripheral blocks: a blind, a nerve-stimulated and an ultrasonographic approach, which have been developed through the years aiming to improve the efficacy and to minimize procedure-related risks. Blind blocks are performed according to anatomical landmarks only and entail the higher failure rate and risk. Despite still finding a limited application, this approach was discontinued in human medicine especially in anatomically complex regions. The brachial plexus block in men is an example, as the blind supraclavicular route was scarcely used and caused *a certain amount of fear and trepidation* (Accardo & Adriani, 1949) in the anaesthetist due to the high incidence of complications (i.e. pneumothorax, block of the stellate ganglion, vagus and phrenic nerves, persistence of hyperesthesia or paraesthesia) that overcame the well-known advantages (De Jong, 1961). Furthermore, a high failure rate was reported due to anatomical variations and the impossibility to accurately locate the nerves. In 1962, Greenblatt and Denson (1962) introduced the use of the nerve stimulator in clinical practice, determining the fundamentals on which our current use of this technique is still based upon. In particular, they stated that only nerves with a motor component can be identified, as the current delivered with a first rudimental insulated needle elicited a muscular twitch. The intensity of current delivered, the distance between the tip of the needle and the target nerve, and the quality of muscular twitch were investigated as well, as factors that could influence the success rate of the block. A substantial reduction in the volume of local anaesthetic was reported, from half to one third of the usual volume administered, together with an increase of block effectiveness and accuracy especially for those blocks defined as "difficult". In addition, this new method seemed compatible with a busy operating schedule (Greenblatt & Denson, 1962). The peripheral nerve stimulation (PNS) technique has been considered the gold standard for three decades, as it improved perioperative analgesia management, reducing also the use of general anaesthesia in adult human patients (Sbarigia et al., 1999; De Andrés & Sala-Blanch, 2001). With PNS techniques, nerve location is related to current intensity, as needle-to-nerve relationship is considered to be inferior to 2 mm when muscular twitch persists under 0.4 but not 0.2 mA. Positive stimulation at 0.2 mA might in fact indicate an intraneural location of the needle (Hadzic & Vloka, 2004). The introduction of ultrasound (US) in clinical practice dates back to 1978, when a Doppler ultrasound blood flow detector was used to facilitate supraclavicular brachial plexus block (La Grange et al., 1978). At this time, detailed knowledge of the ultrasonographic appearance of neural structures was poor, and the ultrasound technology was not suitable for visualization of nerves. The first direct use of US for locoregional anaesthesia was in 1994, again for supraclavicular brachial plexus block. In this case, the direct visualisation of the needle tip and surrounding anatomical structures such as the cervical pleura, subclavian and axillary vessels and the target nerve, allowed to perform an effective and safe block (Kapral et al., 1994). Since then, a great effort was made within the scientific community to describe new ultrasound-guided approaches to peripheral nerve blocks (PNB) and the idea of a new gold standard method to be applied in everyday clinical practice started to take shape (Marhofer et al., 2005). As regional anaesthesia consists in putting *the right dose of the right drug in the right place* (Denny & Harrop-Griffiths, 2005), the argument for the widespread use of US is that direct visualization of the needle, the anatomy, the neural structures, and the spread of LA can enhance the anaesthetist's ability to satisfy this demand. After an initial enthusiasm that led to the description of several new US-guided blocks, the debate about their real additional benefits over the PNS technique is still open due to a lack of large multicentre study (Aguirre et al., 2009). Nonetheless, some clinically relevant points have been stated. First of all, the direct visualization of the target nerve, the adjacent structures and the needle as well as the spread of local anaesthetics during the injection. This can prevent maldistribution and intravascular injections, especially when anatomical variations are present (Weintraud et al., 2008). On the other hand, the direct visualization of the injection allowed to reassess the statement that intraneural injections result in nerve damage. Whilst the debate is still open, it has been proved that eliciting paraesthesia to verify needle positioning when performing a blind block, generally ends in subepineural injection. Bigeleisen (2006) reported that the administration of LA under the epineurium in axillary block does not necessary cause short- and long-term damage *per se*, moving the focus on the volume and the pressure of the injection (Bigeleisen, 2006; Borgeat, 2006). Cappelleri and colleagues (2016) found that intraneural popliteal sciatic nerve block provided a faster onset and better success rate compared with subparaneural one, without complications.

Most comparative studies have shown faster onset time and longer duration of blocks when using US in comparison to other techniques (Marhofer et al., 2004; Kapral et al., 2008), leading to minor perioperative systemic analgesic supplementation with a faster discharge. These appear also significant factors for cost management. A substantial reduction in the volume of local anaesthetic administered has been described, even though its clinical relevance is yet to be evaluated, apart from the possibility to limit the risk of toxicity. A novel method based on the measurement of nerve cross-sectional area allowed to determine a volume of 0.7 ml to perform ulnar nerve block (Eichenberg et al., 2009), and 1.7 ml for the sciatic block in average adult patients (Ladzke et al., 2010). Overcoming the first concept of "doughnut sign", i.e. the circumferential spread of hypo-echoic LA around the nerve structure (Marhofer et al., 2005), the definition of the ED_{99} equates to a 99% success rate for peripheral nerve blocks. This could contribute to future considerations of US-guided techniques as "gold standard", even if these studies were performed by experts in ultrasound-guided regional anaesthesia, aiming to describe the minimum volumes that can be used. In clinical practice, the current minimum volume advised is two to three times the aforementioned.

The need for continuous education and training is mandatory to achieve high success rate, as US-guided block outcome is strongly skill- and operator-dependent. The minor discomfort caused in patients compared to the use of PNS techniques especially on injured limb should be considered as well, despite few comparative studies have been performed yet (Marhofer et al., 2010; Dhir et al., 2016).

Ultrasound technology also allowed the development of intrafascial and compartmental blocks, such as TAP (transverse abdominal plain) and PECS (pectoralis nerve and serratus plane) blocks (Kanazi et al., 2010; Baeriswly et al., 2015; Asmaa Elsayed et al., 2017; Doo-Hwan et al., 2018).

Using a combined technique, i.e. using nerve stimulation along with US guidance for additional confirmation of nerve identification, might allow to associate the benefits of both techniques minimizing risks. However, several studies in children demonstrate that visible needle tip placement under US guidance may not result in an end motor response (Tyler et al., 1993; Geffen & Gielen, 2006). These findings are consistent with Portela and colleagues' (2013a) findings in dogs. Ponde and colleagues (2010) stated that no significant difference in block characteristics between patients who exhibited a motor response with electrical stimulation and those who did not occurred in children undergoing sciatic nerve block (Ponde et al., 2010). This may be explained due to the nonhomogeneous distribution of motor, sensitive and connective component of the nerve (Urmey & Stanton, 2002; Hogan, 2003; Perlas et al., 2006). In addition, Sinha and colleagues (2007) showed that in US-guided interscalene block performed eliciting a motor response with intensity below or above 0.5 mA had no impact on success or duration of upper trunk block. Since no increase in success rate of trained anaesthetist is reported in clinical setting (Gürkan et al., 2010; Hara et al., 2014), the combined technique is thus advised whenever the operator is not familiar enough with a particular US-guided approach. As the needle tip appears to be close to the nerve (<2 mm), the nerve stimulator is turned on at low voltage to confirm needle-to-nerve proximity (Kumar et al., 2007).

Regardless to the technique used, locoregional anaesthesia performed together with procedural sedation instead of general anaesthesia increased safety during several procedures (e.g. orthopaedic and vascular surgeries), improving both short- and long-term outcome in terms of postoperative cognitive deficit, health-related quality of life, and cancer recurrence (Lewis et al., 2008; Bodenham & Howell, 2009).

From a different perspective, in paediatric anaesthesia locoregional blocks are advised to be performed after heavy sedation or general anaesthesia. Infants and children are not able to provide any information regard paraesthesia or early symptoms of LA toxicity, and can be defined as "uncooperative" patients according to their age, ability to speak and to accept block performance (e.g. needle puncture, nerve stimulator-related contractions, cold ultrasound transducer). General anaesthesia removes the risk of sudden movement and increases safety. The rate of postoperative neurologic symptoms is reported to be 7 times higher in awake and sedated children receiving locoregional anaesthesia than in anaesthetized ones (Krane et al., 1998; Bernards et al., 2008; Taenzer et al., 2014).

STATE OF ART OF PHERIPHERAL NERVE BLOCK IN COMPANION ANIMALS

Despite several locoregional blocks have been described in companion animals, further studies should be performed focusing on surgeries where a lack in locoregional approaches is present. Most cadaveric studies find an application in everyday clinical practice as part of multimodal and balanced anaesthesia (Campoy et al., 2010). Possible side effects of PNB are haematoma, intravascular or intraneural injection, intrathecal spread of LA or staining of untargeted nerves according to the region of injection (Campoy, 2006). To reduce these risks, syringe aspiration prior to local anaesthetic administration is advised regardless to the approach used. In PNS blocks the desired muscular twitch is found at 1-2 mA with an insulated needle, while LA administration should be performed at 0.4 to 0.2 mA (Campoy, 2006). With US-guide approaches, the needle tip should be always visualised during injection. Despite the difficulties to reach the sample size of human multicentre studies (Nordin et al., 2003), many efforts have been made to validate PNB techniques in companion animals' anaesthesia. Locoregional blocks currently reported in literature in companion animals are briefly described thereafter.

Pheripheral nerve blocks of the head

Peripheral nerve blocks are widely used to provide perioperative analgesia in patients undergoing maxillofacial and ophthalmologic surgeries. The trigeminal nerve (V cranial nerve) is responsible for most of the sensory innervation of the head. Further its emergence, it divides in three main branches, whose sensory neurons have their soma in the Gasser's ganglion. Ophthalmic and maxillary nerves have sensory fibres only, while the mandibular nerve also provides motor function to the masticatory muscles (masseter, digastric, internal and external pterigodeus muscles) (Esteves et al., 2009). According to this characteristic, the branches deriving from the ophthalmic and maxillary nerves can only be blocked with a blind approach at their emergence from the skull, or with a US-guided one. Maxillary blind block is described at the level of the infraorbital foramen located above the caudal root of the maxillary third premolar (infraorbital nerve block). Either a proper needle or an intravenous catheter can be inserted in the foramen in a cranio-caudal direction. Depending on the depth of deposition and volume of LA, anaesthesia of the nasal planum and most of bridge nose as well as teeth, alveolar bone, and buccal soft tissue from either the canine tooth forward to the midline (Pascoe, 2016), from the third premolar forward to the midline, or the entire maxillary arcade is achieved (Woodward, 2008). A more caudal technique can be performed when anaesthesia of the entire maxillary quadrant is advised (Cremer et al., 2013). The needle is placed at the level of the root tips of the last molar with either and intra- o extra-oral approach. Recently, a trans-orbital approach was also reported in cadavers. The block consists in retropulsing the eye pressing on the upper eyelid and inserting the needle through the conjunctiva close to the medial cantus, in a dorso-ventral direction, until the tip reaches the underlying bony structure (pterygopalatine fossa). In the short communication by Langton and Walker (2017), a good success rate (88.2%, 15 out of 17 blocks) was obtained injecting 1 ml of methylene blue. Nonetheless, evaluation of clinical efficacy and possible side effects (e.g. oculocardiac reflex triggering, intravascular injection, etc) has yet to be performed (Langton & Walker, 2017). The major palatine nerves supply innervation of hard and soft palatal structures, thus the deposition of LA near the foramen (i.e. midway between the palate midline and the dental arcade, at the level of the maxillary fourth premolar tooth) provides analgesia during oronasal and palatal surgery. Deep infraorbital canal catheterisation, until the tip of the catheter reaches the latera cantus of the eye, is reported to produce a caudal spread of dye able to stain also the palatine nerve in cadavers (Viscasillas et al., 2013).

The mandibular nerve can be blocked blindly as well, at the level of the middle mental and the inferior alveolar foramina. The mental nerve emerges from the middle mental foramen in close proximity to the labial frenulum. Its blockade should provide analgesia to the cranial portion of the mandibula (up to the canine tooth) and the associate soft tissues, despite contradictory evidence has recently been provided (Krug & Losey, 2011). Inferior alveolar block is generally preferred due to the possibility to extend the block caudally. The foramen is located approximately halfway between the angular process and the cranial aspect of the coronoid process of the mandible, caudal to the last mandibular molar. Either a proper needle or an intravenous catheter can be inserted in the foramen in a cranio-caudal direction. All dental and bony structures of the ipsilateral mandible are blocked. A reported side effect is the retrograde block of the lingual nerve, whose branches take off caudally to the mandibular foramen to innervate the tongue. Tongue paraesthesia and loss of sensation are reported leading to possible self-mutilation. The advised volume of LA currently reported to be administered in the blind techniques described rage between 0.1 and 0.2 ml per site in cats and up to 0.4 ml per site in dogs (Woodward, 2008). Effective local analgesia is provided in patients undergoing oncologic and orthopaedic surgery with excision of hard and soft tissues, reconstructive maxillofacial surgery (i.e. oronasal fistula and palatal defects), dental scaling, extractions (Beckman & Legendre, 2002; Lantz, 2003; Gauthier & Gilbert, 2004; Gracis, 2013) and rhinoscopies (Cremer et al., 2013).

A combination of blind mandibular and great auricular nerve blocks was investigated in clinical setting in dogs undergoing TECA-LBO surgery. No significant improvement in perioperative pain management and outcome was reported (Buback et al., 1996). This approach has been recently tested in foxes and beagle cadavers. The injection of 0.04 ml kg^{-1} of methylene blue-bupivacaine solution injected near the caudo-lateral margin of the zygomatic arch and the masseteric muscle provided adequate stain of the auriculotemporal nerve (0.6 mm). The injection of 0.2 ml kg^{-1} at the level of transvers process of the atlas provided adequate staining of the great auricular nerve (ventral rami of C2) as well (Stathopoulou et al., 2018). Neurostimulated and US-guided blocks of the mandibular nerve have also been reported. In the first case, the block was bilaterally performed in a geriatric dog undergoing rostral mandibulectomy to treat a malignant melanoma. The ventral portion of the zigomatic arch was palpated in a cranio-caudo direction until reaching the temporomandibular joint. The needle was inserted caudally to it with a caudo-cranial latero-medial orientation, obtaining a twitch of the digastricus, pterigodeus medialis and lateralis and masseter muscles with movement of the jaw and the auricular pinna. The bilateral injection of 2 mg kg^{-1} of ropivacaine 0.75% provided effective perioperative analgesia up to 8 hours, as no analgesic drugs were administered neither in premedication, nor intra- or post-operatively (Carotenuto et al., 2011). In the second case, US-guidance was used to perform trigeminal block in a dog undergoing radical oncologic surgery consisting in exenteration of the left orbit and excision of the zygomatic arch with partial caudal maxillectomy. A microconvex probe was placed caudal to the orbital ligament angled in a cranio-caudal direction to visualise the lateral aspect of the frontal bone and the sphenoid complex, where the ophthalmic, maxillary and mandibular nerves leave the cranial cavity through the orbital fissure, rostral alar foramen and oval foramen, respectively. Due to impossibility to directly visualize the nerves, the adjacent maxillary artery was considered the target structure. After its identification with colour Doppler, a perivascular injection of 2.5 ml of ropivacaine with an *in-plane* approach was performed. The block provided effective intraoperative and perioperative analgesia up to 3 hours (Viscasillas & Ter Haar, 2017).

Ocular regional anaesthetic techniques provide analgesia, akinesia and possible oculocardiac reflex protection during ophthalmologic surgery (e.g. keratotomy, vitreoretinal surgery, cataract extraction, enucleation). The ophthalmic branch of the trigeminal nerve emerges from the orbital fissure, adjacent to the optic foramen, from which the optic nerve emerges. Ocular locoregional blocks also involve the optic, oculomotor, trochlear and abducens nerves (II, III, IV, VI cranial nerve respectively), which pass inside the muscular cone (except the trochlear nerve) providing innervation to the globe and peribulbar structures (Murphy et al., 2013). Retrobulbar anaesthesia (RBA) consists in the intraconal administration of a relatively small volume of LA, while peribulbar anaesthesia (PBA) consists in the extraconal administration of a larger volume of LA that diffuses in the corpus adiposum of the orbit, including the intraconal space. RBA is performed in dogs and cats with a 22G spinal needle, bent to form an angle of approximately 20°. The needle is inserted through the inferior eyelid at the junction of its middle and temporal thirds and advanced until a slight popping sensation is detected. The tip of the needle is then redirected slightly dorsally and nasally and advanced for further 3-4 mm. This technique has been suggested as a potential alternative to the administration of neuromuscular-blocking agents for ophthalmic surgery avoiding the need for mechanical ventilation (Accola et al., 2006; Hazra et al., 2008). PBA is described either as a single- or a double-injection technique. In the first case, the needle is inserted in the conjunctiva of the eyelid at the medial cantus and advanced in close proximity to the orbital wall. In the second case, the needle is inserted through the inferior eyelid conjunctiva at the ventrolateral region and half of the total volume of LA is injected. The other half is injected in the dorsomedial region of the upper eyelid conjunctiva (Shilo-Benjamini et al., 2017a). Several papers assessed and compared RBA and PBA in order to evaluate their efficacy. When effective, a variable lasting analgesia from 1 to 11 hours is provided, while both appear to cause a transitory increase in intraocular pressure, lasting up to 10 minutes, together with exophthalmos and mydriasis. More serious complications are conjunctival oedema and haemorrhage, more frequent after PBA, as well as globe perforation, optic nerve damage, intravascular injection and systemic toxicity, which are more likely to occur after RBA performance. The incidence of such complications has not been reported in veterinary medicine yet (Hazra et al., 2008; Myrna et al., 2010; Shilo-Benjamini et al., 2017a). The latest experimental study in dogs suggested that a total volume of 5 ml divided in two administration for the peribulbar block provided effective corneal and periocular anaesthesia. A volume of 2 ml for the retrobulbar approach resulted adequate in spread but provided a less reliable corneal analgesia (Shilo-Benjamini et al., 2018). A trans-eyelid ultrasound technique to perform PBA has also been described. A microconvex transducer is sagittally placed on the upper eyelid, angled 45° to 60° so to visualize the globe, the ocular muscles and the optical nerve. The needle is then inserted with an *in-plane* approach in the lateral third of the lower orbital margin. Once the tip is correctly placed, LA is injected. In particular, Wagatsuma and colleagues (2014) assessed the US-guided administration of 0.3 ml kg^{-1} of ropivacaine 1% compared with blind administration of the same volume in healthy dogs. Effectiveness and duration of motor and sensory block appeared similar, while a reduced increase in intraocular pressure was detected in dogs receiving the US-guided block. A cadaveric (Shilo-Benjamini et al., 2013) and a subsequent experimental study in cats found consistent results. Peribulbar anaesthesia seemed to provide a more reliable corneal and peribulbar analgesia compared to RBA, lasting up to 3 hours. Shilo-Benjamini and colleagues (2014) stated also that the use of US-guidance to perform RBA might lead to wrong needle positioning due to the curvature of the needle, reducing the success rate of intraconal injection. The volume suggested to be effective for RBA and PBA in cats was 1 and 3 ml respectively, despite the particular viscosity of the mixture administered in the study (bupivacaine 0.5% and iopamidol with or without saline solution) might have influenced the spread (Shilo-Benjamini et al., 2014). Local anaesthetic volume and concentration seem to be particularly important in this species due to anatomical features and to the particular sensitivity to bupivacaine toxicity. Doses up 2 mg kg^{-1} administered with the ventral retrobulbar approach were proved to be safe in healthy cats (Shilo-Benjamini et al., 2017b). On the other hand, a case report by Olivier & Bradbook (2012) highlights that a change in the administration technique and a higher volume might have caused brainsteam anaesthesia in a cat undergoing enucleation. Sub-Tenon blocks has been suggested as an alternative to systemic neuromuscular blockade during phacoemulsification. The mediodorsal portion of the bulbar conjunctiva (3-5 mm from the limbus) is incised with tenotomy scissor. The conjunctiva and sub-Tenon capsule are bluntly dissected from the underlying sclera and LA from 1 to 5 ml (according to body weight) is injected with a dedicated atraumatic cannula. In both an experimental and clinical study, no increase in intraocular pressure was detected, despite higher level of vitreal expansion, a longer time to perform block and no analgesic improvement with the control group were shown. In addition, the presence of engorged episcleral blood vessels might be a contraindication to block performance due to the high risk of haemorrhage (Ahn et al., 2013; Bayley & Read, 2018).

Combined blind approaches to the major occipital nerve (dorsal ramus of the second cervical nerve), frontal nerve (deriving from the ophthalmic branch of the trigeminus) and zygomaticotemporal nerve (deriving from the maxillary branch of the trigeminus) was recently described in a cadaveric study, aiming to develop an alternative to systemic opioid administration for perioperative pain management in craniotomies (Kushnir et al., 2018).

Pheripheral nerve blocks of the thoracic limb

The innervation of the thoracic limb is provided by the brachial plexus, which is composed by the ventral branches of the cervical spinal nerves from C6 to C8 and of the first thoracic nerve (T1). Minor contribution from the fifth cervical nerve and the second thoracic nerve is variably reported (Allam et al., 1952). The roots exit the intervertebral foramina and intertransversus musculature, emerging as cords through and cross the ventral border of the scalenus muscle where they extend to the thoracic limb by traversing the axillary space, in close proximity to the axillary artery and vein and the subscapular vein. Cranial pectoralis, suprascapular, subscapular, axillary, musculocutaneous, radial, median and ulnar nerves exit the plexus in a cranio-caudal direction (Allam et al., 1952; Kitchell, 2013) and provide motor and sensory innervation of the skin, muscles and joints of the thoracic limb. Paravertebral brachial plexus block is reported to provide perioperative analgesia for procedures involving the forelimb up to the shoulder. The nerves of the brachial plexus are blocked as they exit intervertebral foramina. A blind, neurostimolated and US-guided techniques have been described in dogs and cats. The blind block is performed relying only on the anatomical landmarks, i.e. the transverse process of C6 and the head of the first rib. With the patient in lateral recumbency, the scapula is shifted caudally. After palpating the transverse process of C6, 1 to 3 ml of local anaesthetic are injected above the dorsolateral surface of the transverse process at each site, in order to block the ventral branches of C6 and C7. The ventral branches of C8 and T1 are located dorsal to the cranial and caudal margin of the head of the first rib, which should be isolated by holding it ventrally with a finger. Injection of 1-3 ml of LA is performed dorsal to the head of the rib at each site (Lemke & Dawson, 2000; Lemke & Creighton, 2008). A modified approach consists in the location of the axillary artery and the costochondral junction. A single injection is then performed 1-2 cm above these landmarks so to block C8 and T1 at the point of their convergence (Lemke & Creighton, 2008). Hofmeister and colleagues (2007) suggested a parasagittal dorso-ventral insertion of the needle, the first one with an inclination of 45 degrees at the level of the 6th cervical vertebra and the second one with an inclination of 90 degrees at the level of the 2nd thoracic vertebra. A total volume of 3 to 5 methylene blue solution was administered, nonetheless success rate in cadavers was only of 33% (Hofmeister et al., 2007). The neurostimulated technique relies on the same anatomical landmarks of the blind block. In addition, a reorientation of the insulated needle towards the transverse process of C7 is also performed, so three point of LA injection are identified (Lemke & Creighton, 2008). Stimulation of the cranial branch of C6 causes contraction of the brachiocephalicus, supraspinatus, and infraspinatus muscles in addition to outward rotation of the shoulder, and stimulation of the caudal branch causes inward rotation of the shoulder. Stimulation of the cranial branch of C7 causes contraction of the biceps and outward rotation of the arm, stimulation of the middle branch causes contraction of the deltoideus and inward rotation of the limb, and stimulation of the caudal branch causes contraction of the triceps and extension of the carpus. Stimulation of C8 causes contraction of the triceps and extension of the elbow, carpus, and digits, and stimulation of T1 causes flexion of the carpus and digits (Allam et al., 1952). A US-guided approach has been reported on cadavers based on the ultrasonographic anatomy of the innervation of the thoracic limb described by Guilherme & Benigni (2008). Monticelli and colleaugues (2018) introduced further anatomical and ultrasonographic landmarks with a preliminary MRI phase of their study, modifying thus the approach of Rioja and colleagues (2012) which did not appear to be effective. With the cadaver in lateral recumbency, a linear transducer was placed in a transverse plane on the neck to identify the ventral border of C6 vertebral body and moved laterally to visualize the ventral and dorsal lamina of its transverse process. The probe was then moved 2-3 mm cranially, until the concave surface ridge of the lamina ventralis disappeared. A spinal needle was inserted *in-plain* oriented in a dorso-lateral ventro-medial direction and 0.05 ml kg^{-1} of methylene blue was injected at this level to block C6 spinal nerve. A second injection of the same volume was performed caudally with the same ultrasound landmarks to block C7 spinal nerve. The transducer was then moved caudally so as to visualise the first rib. An injection of 0.1 ml kg^{-1} of methylene blue was performed at the cranial border of the first rib in a triangle-shaped fascial plane medial to the insertion of the serratus ventralis muscle. Cadaveric dissection, CT and MRI examinations revealed a high success rate of this technique (100% for C6-C8 and 92% for T1) on a total of 24 blocks. Nonetheless, epidural contamination due to dye spread (66%), intravascular injection and phrenic nerve staining (17%) are reported (Monticelli et al., 2018). Despite the use of cadavers could have influenced the outcome, further studies are suggested to assess the safety of this technique before clinical application. Transient Horner's syndrome has been reported in an experimental study in dogs receiving 0.2 ml per site of lidocaine with or without adrenaline (Choquette et al., 2017). In a case report by Viscasillas and colleagues (2012), transient Horner's syndrome was shown in a dog after neurostimulated paravertebral brachial plexus block. As no signs of intrathecal spread were shown, a blockade of cervicothoracic ganglion related to volume administration (2 ml per site of ropivacaine 0.75%) was suspected.

In veterinary literature, only one experimental work on 34 dogs by compares blind, PNS and US-guided approaches to paravertebral brachial block. Both the modified blind technique and the neurostimulated technique by Lemke & Creighton (2008) were used, while an US-guided approach was described *ex novo* based on Guilherme & Benigni's (2008) ultrasonographic anatomical study. A volume of 0.1 ml kg^{-1} mixture of methylene blue and lidocaine was administered at each injection point, regardless to the approach used. The deposition of local anaesthetic with US-guidance was performed adjacent but not in contact with the nerves. After receiving the block in general anaesthesia, the dogs were euthanized and dissected to evaluate nerve staining. Low success rate and high incidence of possible side effects is reported with all three techniques. The low volume used in the blind and neurostimulated techniques could partially explain the low success rate (Rioja et al., 2012).

Axillary brachial plexus block provides analgesia for procedures involving the elbow and the distal thoracic limb. Skelding and colleagues (2018) recently described and assessed two new blind approaches in addition to the traditional one, aiming to improve performance and success rate of a technique that would not need particular equipment or skill. The traditional blind block was performed with the animal in lateral recumbency and the limb to be blocked uppermost. A spinal needle was inserted was inserted at the level of the acromion, medial to the scapula, in a cranio-caudal direction held parallel to the body axis. The perpendicular approach was performed positioning the cadaver as for the traditional technique. The spinal needle was inserted perpendicular to the scapula, caudal to the scapulohumeral joint, aiming cranial to the first rib at a level that corresponded to the midpoint between the transverse process of C6 and the point of the shoulder. A third approach was performed with the dog in dorsal recumbency, with the limb to be blocked at a standing angle. A spinal needle was introduced in a ventro-dorsal direction, lateral and cranial to the first rib and medial to the scapula. As the needle was advanced enough to locate at the midpoint between the transverse process of C6 and the point of the shoulder, injection was performed. In all three techniques, a single injection of 0.2 ml kg^{-1} yellow tissue marking dye was administered. A success rate of 33%, 50% and 58% was reported based on the anatomical dissection and stain evaluation for the three aforementioned approaches respectively (Skelding et al., 2018).

The use of peripheral nerve stimulator was widely described and the suitable volume to perform brachial plexus block has been defined in experimental and clinical studies. The patient is held in lateral recumbency, with the arm to be blocked uppermost. Landmarks include the scapulohumeral joint, the first rib, the jugular vein and the axillary artery. A proper insulated needle is inserted in a cranio-caudal direction medial to the scapula at the level of the acromion, held parallel to the body axis. Neuro-location can be performed in different ways. Neuro-location according to the specific muscular twitch of each nerve allows selective injection of local anaesthetic. In particular, stimulation of the musculocutaneous nerve (C7) causes flexion of the shoulder, elbow, and carpus. Stimulation of the axillary nerve (C7 and C8) causes flexion of the shoulder, abduction of the elbow, and inward rotation of the carpus. Stimulation of the radial nerve (C7, C8, and T1) causes extension of the elbow, carpus, and digits in addition to splaying of the toes. Stimulation of the combined median-ulnar nerve trunk (C8 and T1) causes flexion of the elbow, carpus, and digits. Contractions of the biceps brachii muscle and flexion of the elbow (i.e. musculocutaneous nerve stimulation) could be considered the endpoint for injection as described by Campoy (2006). A less specific flexion or extension of the carpus or elbow can be elicited as well. Either the whole volume or part of the local anaesthetic is then injected. In the second case, the remaining is administered as the needle is retracted. Volumes of 0.2 to 0.3 ml kg^{-1} are reported to be sufficient for effective block performance (Futema et al., 2002; Wenger, 2004; Wenger et al., 2005; Campy et al., 2008; Riccò et al., 2013). According to Riccò and colleagues (2013), the neurostimulated approach using 0.2 ml kg^{-1} has comparable results with the injection of 1 ml kg^{-1} with the blind technique, thus allowing a considerable reduction in LA administration. A volume of 0.6 ml kg^{-1} of a mixture of lidocaine 1% and bupivacaine 0.5% appeared effective in perioperative pain management in cats undergoing distal thoracic limb surgery, reducing intraoperative isoflurane requirements and postoperative pain in the early postoperative period (Mosig et al., 2010).

An US-guided approach was described both in dogs and cats. The dog is placed in dorsal recumbency, with the thoracic limbs flexed and oriented caudally. A linear array transducer is placed in a parasagittal plane, caudal to the thoracic inlet, cranial to the pectoral muscles, medial to the brachiocephalicus muscle and lateral to the sternum. A short axis scan of the axillary vessels (artery and vein) is obtained and C7, C8 and T1 roots of the brachial plexus are visualized dorsal and in close proximity to the vessels. A proper needle is inserted *in-plane* in a cranio-caudal direction. In the study by Campoy and colleagues (2010), a combined technique was used in order to confirm the correct identification of the nervous structures. A volume of 0.15 ml kg^{-1} of methylene blue and lidocaine was administered and circumferential spread of the solution was observed in real time. Post mortem anatomical dissection confirmed nerve roots staining and correct block placement. The same approach can be performed in cats, despite Ansòn and colleagues (2014) showed a higher success rate in cat cadavers when the limb to be blocked is abducted of 90°. A total volume of 1 ml of either blue dye solution or iodinated contrast medium appeared adequate to stain the brachial plexus as confirmed either by anatomical dissection or CT examination.

Successful PNS and US-guided perineural catheter placement are reported to provide postoperative repeated brachial plexus blocks in dogs (Mahler & Reece, 2007; Vettorato & Taeymans, 2017).

To date, the comparison between the neurostimulated and US-guided approach to the brachial plexus has been assessed only in one prospective experimental trial in dogs. Success rate did not differ significantly between the two techniques with the administration of 0.4 ml kg^{-1} of 0.25% bupivacaine. On the other hand, a faster onset and a longer duration of the block was reported for the US-guided approach (Akasaka & Shimizu, 2017).

Possible specific complications related to brachial plexus block are stellate ganglion block and consequent Horner's syndrome, unilateral phrenic nerve block and pneumothorax (Lemke & Dawson, 2000; Campoy, 2006). The latter can develop several hours after block performance. Careful monitoring of respiratory function is thus advised during both early and late postoperative period (Bhalla & Leece, 2015). A case of severe ventricular arrhythmias has also been reported in a dog, related to inadvertent insertion of the needle in the thorax during PNS block performance. Lidocaine CRI (1.8 mg kg^{-1} h^{-1}) led to resolution of ventricular tachycardia within 6 hours (Adami & Studer, 2014). Slight abduction of the forelimb ad the scapula is suggested during neurostimulated brachial plexus block to create a virtual space between thoracic wall and axillary region, making inadvertent intra-thoracic needle placement less likely.

Individual blockade of the radial, ulnar, musculocutaneous, and median nerves (RUMM) has been described as an alternative to the axillary brachial plexus block in dogs to provide analgesia distal to the elbow. Described reference points are the lateral epicondyle of the humerus and the brachial artery. In particular, the radial nerve is palpated above the lateral epicondyle of the humerus between the brachialis and lateral head of the triceps. The needle is inserted proximal to the lateral epicondyle, and local anaesthetic is injected along the nerve. Since the musculocutaneous nerve lays cranial to the brachial artery, and the median and ulnar nerves are located caudally, the needle is inserted proximal

to the medial epicondyle over the brachial artery, and local anaesthetic is injected along its cranial and caudal margins. A volume of 1-2 ml of LA is advised for each injection (Lemke & Creighton, 2008). A different blind approach consists in a more proximal blockade of nerves. To perform radial nerve block, the dog is placed in lateral recumbency with the arm to be blocked placed upwards and the elbow flexed at 90°. The radial nerve is located between the middle and the distal thirds of the humerus, between the brachialis muscle and the triceps. The brachialis muscle is displaced cranially applying digital pressure until the humerus is felt under the operator's thumb. A spinal needle is then inserted in a cranio-caudal direction, caudal to the thumb at a 45° angle, perpendicular to the long axis of the humerus and LA is injected. The ulnar, musculocutaneous and median nerve block is performed with the animal in lateral recumbency with the arm to be block placed downwards and the elbow flexed at 90°. Nerves are located halfway along the humerus. digital pressure is applied to the medial aspect of the limb until the humerus is palpated and a spinal needle inserted in a cranio-caudal direction, caudal to the thumb at a 45° angle, perpendicular to the long axis of the humerus. A volume of 0.25 ml kg^{-1} (with 0.1 ml kg^{-1} administered laterally and 0.15 ml kg^{-1} administered medially) is reported to provide incomplete block, in particular for the ulnar nerve (Trumpatori et al., 2010).
The use of a neuro-locator is described as well in order to increase precision of the block (Campoy, 2006). Bortolami & Love (2012) reported the additional use of peripheral nerve locator to the technique described by Trumpatori and colleagues (2010) to perform RUMM block in a conscious dog affected by generalized neuro-muscular disease. The injection of 0.1 ml kg^{-1} per site of 0.5% bupivacaine together with systemic analgesia (methadone IV 0.3 mg kg^{-1}) allowed muscle biopsy and electromyography to be performed without general anaesthesia. In a case report by Portela and colleagues (2013b), the use of US-guidance with a linear array transducer allowed a short axis visualisation of the radial nerve on the lateral aspect of the midhumerus. The musculocutaneous nerve was identified in a short axis scan cranial to the brachial artery, while the ulnar and median nerves were found between the brachial artery and vein. An *in-plain* approach together with neurostimulation of the target nerves were used to administer 0.09, 0.1 and 0.05 ml kg^{-1} for the radial, median and ulnar, and musculocutaneous nerves respectively. A modified US technique reported by Castiñeiras and colleagues (2015) consists in visualizing and blocking all four nerves from the medial aspect of the brachium. In particular, the median, ulnar and musculocutaneous nerves were identified as described previously described, while the radial nerve was identified from the medial aspect reducing the frequency of the probe. In particular, the probe was moved slightly distally and caudally until a hyperechoic structure could be visualized caudal to the humerus. All nerves were blocked with and *out-of-plain* approach using a total volume of 0.18 ml kg^{-1} of 0.75% ropivacaine and was repeated postoperatively.
Despite the successful use of neurostimulator and US-guidance was reported in the aforementioned papers, no clinical prospective or retrospective studies are currently present in veterinary literature assessing the consistency of these findings.

Pheripheral nerve blocks of the thorax and compartmental blocks

Blind selective intercostal nerve blocks have been widely described and performed in order to provide analgesia in dogs and cats undergoing intercostal thoracotomy or presenting rib fractures. The block is performed as dorsally as possible, near the insertion of the epaxial musculature and the intervertebral foramen. Since intercostal nerves and vessels lie adjacent to the caudal border of each rib, the tip of an adequate needle is positioned over the rib and walked caudally until the caudal border of the rib is overcame. Two or three intercostal spaces on either side of the thoracotomy access should be blocked, due to overlapping nerve supply in this region (Duke, 2000; Lemke & Dawson, 2000). In human beings, the injection of an adequate volume of local anaesthetic in the thoracic paravertebral space results in a longitudinal spread that provides multisegmental analgesia (Saito et al., 2001; Marhofer et al., 2013; Krediet et al., 2015). In dogs, this spread to contiguous spinal nerves is not present as assessed in two cadaveric and experimental studies. Thus, thoracic paravertebral block is advised to be performed with multiple injections of 0.05 ml kg^{-1} of LA. The use of a neurostimulator could increase the accuracy as currents of 0.5 mA elicit the contraction of intercostal muscle. On the other hand, US-guided reduced the incidence of intrapleural migration compared to the neurostimulated technique (Portela et al., 2012; Portela et al., 2017).

The use of ultrasonography in regional anaesthesia allowed the development of intrafascial and compartmental blocks. The spread of local anaesthetic within musculofascial planes allows to anesthetize multiple small nerves or plexuses, rather than targeting specific nerve structures. Serratus, pectoralis and erector spinae plane blocks have been described in humans in order to provide widespread analgesia during thoracic and spinal surgeries, while quadratum lumborum and transverse abdominis plane blocks are reported for abdominal surgeries (Chin et al., 2017; Gürkan & Kus, 2017). The serratus plane block has been preliminary assessed in dog cadavers. A 10-14 MHz linear transducer was placed perpendicular to the ribs at the level of the serratus muscle. A mixture of methylene blue and contrast medium (1 ml kg^{-1}) was administered with an *in-plain* technique between the serratus and intercostal muscles. Subsequent CT examination and cadaveric dissection showed spread of the solution from the 1st up to the 6th rib (Drozdzynska et al., 2017). Different approaches to the transversus abdominis plane (TAP) block have been investigated in dogs and cats. Innervation to the canine abdominal wall and peritoneum is provided by the branches of T11, T12, and T13 cranially and branches of L1, L2, and L3 caudally (Evans, 1993; Castañeda-Herrera et al., 2017). Cadaveric studies focused on different sites and volume of injection of dye either alone or with contrast CT medium in order to evaluate the spread and the stain of the branches. The transversus abdominis muscles lies over the peritoneum and the targeted fascia can be seen as a hyperechoic rim between the transversus abdominis and the internal abdominis oblique muscles with a linear array transducer. An *in-plane* approach is used to reach the fascia with a proper needle and the correct spread of the solution is visible as hydrodissection, i.e. the separation of the muscles due to fluid interposition within the fascia. Schroeder and colleagues (2011) performed a single-injection TAP block assessment in dog cadavers. The probe was positioned in a transverse orientation midway between the iliac crest and the caudal aspect of the rib cage and approximately 5 cm lateral to midline on the left side of the abdomen. A volume of 1 ml kg^{-1} of methylene blue and bupivacaine per hemi-abdominal wall was injected. Anatomical dissection showed an adequate spread of the solution from T12 to L2. Staining of T11 and L3 occurred only in 20-30% of cadavers respectively. As different volumes of injected solution seem to significantly affect cranial to caudal spread within the TAP, a volume of 1 ml kg^{-1} with the single-injection technique has been assessed to cover the most dermatomes within the hemi-abdomen (Bruggink et al., 2012). A two-point and a three-point injection technique were assessed as well. In the two-point technique by Johnson and colleagues (2018), a cranial injection is performed caudal to the thirteenth rib at the level of the costochondral junction, while a caudal injection is performed cranial to the tuber coxa. A total volume of 0.6 ml kg^{-1} per hemiabdomen (0.3 ml kg^{-1} per injection point) of methylene blue was used, resulting in poor stain of T12 and adequate stain of T13 to L3. In the three-point technique, the first injection point is located caudal to the costal arch with the probe held parallel to it and oblique to the midline. After the injection, the hydrodissected fascia is followed caudo-laterally and a second injection is performed at the end of the spread. The procedure is repeated and a third injection is performed. In the study of Drozdzynka and colleagues (2017), 3.3 ml were injected at each point in beagle cadavers weighting 13 ± 2 kg. Adequate stain of T9 to T13 was reported, while caudal spread to L1 and L2 was reported only in 33 and 11% of cases respectively. A preliminary retrospective clinical evaluation of TAP block in dogs undergoing both radical and regional mastectomy was carried out. Despite the lack of a standardised anaesthetic and analgesic protocol, the outcome suggests the TAP block might be a useful technique to provide multimodal analgesia in dogs undergoing mastectomy. Nonetheless, prospective controlled studies in dogs are advised to evaluate intraoperative isoflurane and perioperative opioid sparing effect (Portela et al., 2014). A clinical prospective control study has been performed in cats undergoing ovariectomy. Transverse abdominis plane block was performed according to the single-injection technique described in dogs (Schroeder et al., 2011). A volume of 1.5 ml of either lidocaine and bupivacaine solution or saline solution was injected for each hemi-abdomen. The group receiving the treatment showed a significant reduction in perioperative analgesic supplementation compared to control group, suggesting that TAP block could improve perioperative pain management in cats undergoing ovariectomy in addition to systemic analgesia (Skouropoulou et al., 2018). Bilateral continuous transversus abdominis plane block via epidural catheter has been described in three dogs to manage acute pancreatitis related pain (two dogs) and perioperative pain related to splenectomy and partial pancreatectomy (one dog). The approach of Schroeder and colleagues (2011) was performed, using an 18G, 9 cm Tuohy needle to reach the fascia. After hydrodissection was performed with 0.3 ml kg^{-1} of 0.5% bupivacaine, a 18G epidural catheter was inserted through the needle and advanced 4-5 cm inside the facial plane. Bupivacaine 0.5%, 0.3 ml kg^{-1} was administered every 6 hours to provide continuous analgesia. The good outcome reported in this case report suggest that TAP block might be useful to manage pain related not only to surgical but also to medical conditions (Freitag et al., 2018).

Pheripheral nerve blocks of the pelvic limb

The innervation of the pelvic limb in dogs is mainly due to the lumbosacral plexus, composed by the ventral branches of the spinal nerves from L3 to S2. The lumbar plexus provides the roots for the genitofemoral (L3-L4) lateral cutaneous femoral (L4), femoral (L4-L6), and the obturator nerves (L4-L6). The sciatic nerve arises from the last two lumbar nerves and the first two sacral nerves (L6-S2). The sacral plexus provides the roots for the pudendal nerve, the caudal cutaneous femoral nerve and the gluteal nerves (Evans & de Lahunta, 2013).

Several locoregional techniques are reported both in experimental and clinical studies in order to provide perioperative analgesia in dogs and cats undergoing hindlimb surgery.

A neurostimulated paravertebral approach to the lumbar and sacral plexus has been described. Three points of injection are performed, with the insulated needle introduced paravertebrally between the intervertebral spaces, 1-2 cm lateral to the midline until specific muscular contraction is evoked. In particular, contractions of the sartorius or quadriceps muscle are elicited when the needle is introduced at the L4-L5 intervertebral space, contraction of the quadriceps with clear extension of the knee joint at L5-L6 and contraction of the gluteal or biceps femoris muscles with extension of the hip at L6-L7. The injection site to perform the parasacral plexus block is identified at the junction between the cranial and mid thirds of a line drawn between the cranial dorsal iliac crest and the ischiatic tuberosity. The stimulating needle is inserted at this level until the stimulation of the sciatic nerve roots is detected observing the contractions of the gastrocnemius muscle or tarsus flexion or extension. The injection of a combination of three different volumes and concentrations of bupivacaine was assessed by Portela and colleagues (2010) while describing the technique. A volume of 0.05 ml kg^{-1} per injection point of 0.5% bupivacaine was sufficient to produce a high degree sensory and motor block assessed with pressure from Halsted clamp in the femoral innervation zone, while it resulted in partial block in 25% of the dogs for the tibial and peroneal nerves. Furthermore, concentration more than volume appeared a critical parameter in terms of percentage of success, degree and duration of blocks, consistently with experimental studies in rats (Nakamura et al., 2003). A possible advantage of this approach is the inclusion of the femorocutaneous and proximal gluteal sciatic branch in the block.

Considering the several approaches reported in literature, a description for the femoral and sciatic nerves followed by the clinical relevance will be carried out.

Femoral nerve blocks: The femoral nerve lies within the iliopsoas muscle. It then passes into the proximal pelvic limb where it enters the quadriceps femoris between the vastus medialis and the rectus femoris, splitting into branches to innervate quadriceps femoris. Before leaving the iliopsoas muscle, the saphenous nerve arises from the cranial side of the femoral nerve and a muscular branch takes off to innervate the cranial and caudal bellies of sartorius muscle. The femoral artery and vein run craniomedial to the femoral nerve. The saphenous nerve supplies skin on the medial side of the thigh, stifle, tarsus and paw as well as fibres to the medial articular nerve (O'Connor & Woodbury, 1982).

Psoas compartment block both with PNS and US-guided has been described. A parasagittal approach described by Campoy (2006) is performed with the stimulating needle advanced at the level of the transverse process of L5 in a sagittal orientation, aiming for a contraction of the femoral quadriceps muscle. A volume of 0.4 ml kg^{-1} of lidocaine and methylene blue solution appeared sufficient to adequately stain the femoral nerve within the psoas compartment in a subsequent experimental study, despite epidural spread was reported in 8.7% of cases (Campy et al., 2008). Portela and colleagues (2013c) described a lateral pre-iliac approach. An imaginary line is drawn from the spinal process of L6 perpendicular to the spine in a dorso-ventral direction. The needle insertion is performed at the intersection with a second line drawn from the most cranial aspect of the iliac crest parallel to the spine. The needle tip is directed in a caudo-medial direction with 30-45° of inclination. The injection is then performed after a twitch of the quadriceps with extension of the stifle is elicited. A volume of 0.1 ml kg^{-1} solution of lidocaine and methylene blue was assessed for nerve staining in the experimental phase of the study. An adequate stain of the femoral nerve was reported and was probably due to the anatomic characteristic of the caudal portion of the psoas compartment which forms a defined sheath around the nerve. The obturator nerve was also stained in three out of four cases. The same volume of ropivacaine was evaluated in the clinical phase. The femoral block was combined with a lateral sciatic nerve block (Campy et al., 2008) without systemic analgesia in dogs undergoing orthopaedic pelvic limb surgery. Success rate in preventing intraoperative nociception raged between 86.6% and 93.3%. The use of US-guidance to identify the femoral and obturator nerves within the iliopsoas muscles was described both with a ventral and a lateral approach. To perform the ventral suprainguinal approach, the dog is placed in dorsal recumbency and the linear array transducer is positioned cranial to the inguinal nipple, so as to obtain a short axis scan of the iliopsoas muscle (Mahler, 2012; Echeverry et al., 2012a; Echeverry et al., 2012b). A further anatomical landmark is the external iliac artery, which is described to lay at a mean distance of 5.8 mm in cats and 8.2 mm in beagle dogs from the targeted nerve (Mogicato et al., 2015). Once the femoral nerve is identified, a proper needle is inserted *in-plane* in a latero-medial direction. Volumes of 0.2 to 0.4 ml kg^{-1} were assessed to be sufficient to stain both the femoral and obturator nerve in dogs (Echeverry et al., 2012b). According to further investigations, injection of 0.4 ml kg^{-1} bupivacaine results in a longer motor nerve blockade than sensory blockade. Sensory nerve blockade and complete nerve blockade rate are comparable for both volumes, suggesting that 0.2 ml kg^{-1} might be a more appropriate choice (Shimada et al., 2017). In cats (3.99 ± 0.41 kg mean weight), 1 ml of lidocaine 2% is reported to provide effective nerve block with this technique (Haro et al., 2016). For the lateral approach, the dog is placed in lateral recumbency with the limb to be blocked uppermost. A linear array transducer is placed perpendicular to the spine under the cranial margin of the iliac wing in the lumbar area and tangent to the iliopsoas muscle. As the short-axis scan of the femoral and obturator nerves is visualised within the psoas muscle, an *in-plane* approach is used to perform the block. Tayari and colleagues (2017) reported that the sciatic nerve is also visible in the same ultrasonographic window with this approach. A volume of 0.1 ml $kg^{-1}$of methylene blue was reported to be sufficient for adequate nerve staining in the cadaveric part of the study. The same volume of ropivacaine at two different concentrations (0.3 and 0.5%) was administered in the clinical prospective control trial of the study. A 90% success rate in terms of intra- and post-operative sparing analgesic effect was reported. Ropivacaine concentration seemed to influence the duration of the block rather than its effectiveness. With a similar technique, a US-guided lumbar plexus catheter placement was described in dog cadavers. A Tuohy needle was inserted with an *out-of-plain* approach, in a ventro-caudal direction with a 30 degrees inclination. A volume of 0.2 ml kg^{-1} was injected prior catheter placement. The catheter was advanced blindly and further 0.2 ml kg^{-1} of methylene blue was injected. Anatomical dissection showed obturator and femoral stain in 12 out of 14 cases (Monticelli et al., 2016).

The femoral nerve can be blocked with an inguinal approach to provide analgesia to the distal part of the hindlimb. Anatomical landmarks include the femoral triangle and the femoral artery. With the animal in lateral recumbency, the limb to be blocked is placed upwards and abducted of 90 degrees. The puncture site is located between the femoral artery and the medial belly of the sartorius muscle both for blind and PNS approach (Campoy, 2006; Mahler & Adogwa, 2008). The use of US allows the visualization of the femoral artery which remains the main landmark as the femoral nerve is not always visible (Echeverry et al., 2010). A linear array transducer is placed at the level of the femoral triangle so to obtain a short axis scan of the artery. A proper needle is inserted with an *in-plain* approach in a cranio-caudal direction through the quadriceps. A volume of 0.1 ml kg^{-1} of LA injected deep and cranial to the femoral artery has been considered sufficient to provide effective blockade (Campoy et al., 2010; Echeverry et al., 2010). The superficial circumflex iliac artery was recently identified as a further landmark to perform femoral nerve block in canine isolated pelvic limbs (Garcia-Pereira et al., 2018). The saphenous nerve is a sensory branch of the femoral nerve. Seen the lack of motor fibres, only the use of ultrasound allows its block. The combined sciatic and saphenous nerve block produces selective anaesthesia of the hind limb distal to the stifle in dogs (Echeverry et al., 2010; Shilo et al., 2010; Costa-Farré et al., 2011).

Sciatic nerve blocks: A neurostimulated transgluteal approach to the sciatic nerve was described by Mahler & Adogwa (2008), despite no evaluation of LA volume was carried out. The dorsal iliac spine and the ischiatic tuberosity are connected by a line. A second line is drawn perpendicular to the distal third of the first line. A third line is drawn from the dorsal iliac spine parallel to the midline, crossing the perpendicular line. The needle-insertion site is on the perpendicular line, midway between the two intersections. Contractions of the biceps femoris, semitendinosus, semimembranosus (extension of the hip joint and abduction of the limb), gastrocnemius (extension of the tarsus), digital flexors or extensor muscles (flexion and extension of the digits) are observed. A similar US-guided approach was assessed by Shilo and colleagues (2010), based on the ultrasonographic anatomy of the sciatic nerve described by Benigni and colleagues (2007). The nerve is visualised with a linear array transducer caudal to the sacroiliac joint in a long axis scan. A proper needle is used to perform the injection with an *in-plane* approach. Three different volumes of 0.5% bupivacaine were assessed (0.05, 0.1 and 0.2 ml kg^{-1}) in

the experimental trial, 2/3 of which were administered with the described approach and 1/3 to block the saphenous nerve. None of the volume resulted in complete block conduction and a high variability in sensory and motor block onset and duration was reported. In the lateral approach, the sciatic nerve is blocked one-third the distance along a line that joins the femoral greater trochanter and the ischiatic tuberosity. The muscular contraction elicited with the neurostimulator causes either dorsiflexion or plantar extension of the foot (Campoy, 2006). A volume of 0.05 ml kg^{-1} of methylene blue and lidocaine is reported to provide adequate staining of the nerve in an experimental study (Campy et al., 2008). A linear array transducer placed immediately distal to the femoral greater trochanter and the ischiatic tuberosity allows the visualization of the sciatic nerve in short axis. The deposition of local anaesthetic (0.05 to 0.1 ml kg^{-1}) is performed with a caudo-cranial *in-plain* orientation of the needle (Campoy et al., 2010; Echeverry et al., 2010) In experimental cats, 1 ml of diluted lidocaine is reported to provide effective nerve block with this technique (Haro et al., 2012). In dogs, an *out-of-plain* approach was described as well, despite a low success rate is reported (Costa-Farré et al., 2011).
A blind technique has been evaluated to provide analgesia below the thigh (Rasmussen et al., 2006a). Despite its feasibility, the blind administration of bupivacaine to block the saphenous (0.13 ml kg^{-1}), tibial and common peroneal nerve (0.26 ml kg^{-1}) in dogs undergoing extracapsular cranial cruciate ligament surgical stabilization did not improve perioperative analgesia (Rasmussen et al., 2006b). In a more recent experimental trial, US guidance was used to performed the aforementioned block. Methylene blue (0.3 ml kg^{-1}) was injected in the adductor canal. Stain of the saphenous, tibial and common peroneal nerves was reported to be 55%, 30% and 20% respectively (Castro et al., 2018).

The use of peripheral nerve blocks described so far is supported by clinical evidence in veterinary medicine. In a retrospective clinical assessment, PNS combined lumbar plexus and sciatic nerve blocks in dogs undergoing pelvic limb surgery showed a mean success rate of 78.5%. In particular, the dorsal paravertebral approach to the lumbar plexus associate with lateral sciatic nerve block (DPV-SN) showed a mean 74.7% success rate, while the lateral pre-iliac approach to the lumbar plexus associated to lumbar paravertebral sciatic nerve block (LPI–LPVSN) showed a mean 82.3% success rate. According to the authors, the higher success rate of LPI–LPVSN could be due to the increased probability of blocking the obturator nerve, providing a more complete analgesia of the hindlimb. An intraoperative isoflurane and fentanyl sparing effects was reported, and 66.3% and 72.9% of dogs receiving DPV-SN and LPI-LPVSN respectively did not require postoperative methadone administration (Vettorato et al., 2013). Similar results were shown in cats undergoing pelvic limb surgery receiving four different combinations of lumbar plexus and sciatic nerve blocks. Peripheral nerve block was considered successful in 81.2% of cats, with a mean end-expiratory fraction of inhalational anaesthetic agent of 1.12% MAC, reflecting a significant sparing effect. In 68.1% of patients no additional intraoperative analgesia was needed (Vettorato & Corletto, 2016). Furthermore, as effective peripheral nerve blocks reduce the need for postoperative analgesic drugs, opioids administration should be consistent with pain assessment. In fact, the administration of opioids at fixed time points regardless to pain assessment seems to increase the incidence of side effects without any improvement in pain management (Bini et al., 2018). Prospective studies comparing the use of PNB and epidural anaesthesia suggest that similar intraoperative nociception and postoperative analgesia are provided (Campoy et al., 2012a; Caniglia et al., 2012; McCally et al., 2015; Bartel et al., 2016). Both neuraxial anaesthesia and peripheral nerve blocks seem to prevent endocrine and metabolic stress response to surgery in dogs compared with fentanyl infusion (Romano et al., 2016). In humans, an advantage of PNB over neuraxial anaesthesia is the reduction in possible complications (Capdevila et al., 1999; Davies et al., 2004; Singelyn et al., 2005). In veterinary anaesthesia, the incidence of hypotension and urinary retention after epidural administration of LA and morphine compared to PNB is still controversial, due to the number of confounding factors and the small sample size of studies (Campoy et al., 2012a; Bartel et al., 2016). Neurostimulated psoas compartment and sacral plexus block was reported to be effective in providing short-term perioperative analgesia in dogs undergoing pelvic limb amputation. As the plexus was blocked at the cranial parasacral level, all sensory and motor nerve branches detaching from it were included, providing analgesia up to the coxofemoral junction and to the surrounding musculature (Congdon et al., 2017). Procedural sedation, i.e. a semi-conscious state that allows human patients to be comfortable during certain surgical or diagnostic procedures, has been reported in dogs in experimental trials (Bortolami & Love, 2012; Riccò et al., 2013). In a case series by Campoy and colleagues (2012b), procedural sedation was assessed in clinical setting in dogs ASA I and II undergoing surgery of the stifle or of the distal part of the hindlimb. A relatively light sedation plane (thoracic limb stretching, yawning, lingual movements and swallowing, occasional spontaneous blinking) was maintained with propofol and dexmedetomidine intravenous infusion, while analgesia was provided by sciatic and femoral block. Cardiovascular and ventilatory stability were reported, as well as adequate perioperative analgesia lasting up to 24 hours after extubation (Campoy et al., 2012b). Continuous sciatic nerve block in two dogs has been recently reported by Viscasillas and colleagues (2018). The placement of an epidural catheter under the paraneural sheath of the sciatic nerve allowed surgical debridement without GA and opioid-free pain management for seven days.

GENERAL AIM

As widely described in the introduction of this dissertation, locoregional anaesthesia plays an essential role in companion animals. True pre-emptive analgesia is provided, preventing central sensitization and wind up, which grants a faster postoperative recovery and improves pain management compared to systemic modulation of pain (Corletto, 2007). Peripheral nerve blocks allow a reduction in both inhalant and opioid anaesthetics administration, preventing their cardiovascular and respiratory depressant effect and other side effects such as excitement, nausea and vomiting (Bini et al., 2018). Furthermore, as a reduction in systemic opioid and inhalants anaesthetics provided by PNB has been firmly stated, opioid-free anaesthesia and its implication appears still an unexplored field (White et al., 2017; Forget, 2018).

In the last decade, several locoregional blocks have been described and the great dedication to this branch of anaesthesia is witnessed not only by peer-reviewed studies, but also by books that have been published especially in the last two years. (Lerche et al., 2016; Otero & Portela, 2017)
Nonetheless, the major field of study and application of PNB is orthopaedic surgery, while the interest for PNB applied to soft tissue surgery is at its early stage, powered by the increasing use of ultrasound guidance. The possibility to directly visualize the needle, the surrounding anatomical structures and the injection of local anaesthetic, increases the success rate and duration of blocks, leading also to a reduced onset and volume LA. Furthermore, veins and arteries are now considered reference point and ultrasonographic landmarks rather than only structures to be avoided as in most neurostimulated and blind blocks. In addition, as the use of ultrasound is traditionally reserved to other specialists, such as sonographers, obstetrics and cardiologists, the anaesthetist is required to develop new skills and abilities in order to handle the technique properly and to make it fit in the busy operating schedule of the clinical practice. Among others, the adequate knowledge of anatomy and ultrasonographic anatomy of every region that could possibly undergo surgery, the development of an adequate eye-to-hand coordination and interpretation of the resulting ultrasonographic image during block performance are required. As surgery evolves and new approaches are assessed and applied to companion animals, so should the anaesthetic techniques do.

During these three years of PhD course, after an accurate analysis of literature, which highlighted a poor application of locoregional anaesthesia to soft tissue surgeries, the decision to focus on this field was taken. This was due also according to the increasing need to provide effective pain management in patients undergoing aggressive or demolitive surgeries in our clinical setting. Regions involved were the head in dogs and rabbits, and neck, the inguinal region and the splanchnic compartment in dogs. The need to implement multimodal analgesia for surgeries and medical conditions related to the region was the common thread of the studies, while the choice of the technique to develop (i.e. US-guided, neurostimulated or blind block) and the type of study (cadaveric or clinical) was taken according to the particularity of the region or surgery itself. Based on these considerations, the aim of this research project is thus to explore, assess and describe new locoregional blocks for those surgeries that lack a PNB approach and would benefit from it.

ULTRASOUND-GUIDED CERVICAL PLEXUS BLOCK IN DOG: A CADAVERIC PRELIMINARY STUDY

INTRODUCTION

The cervical plexus block (CPB) is widely used in human medicine in order to provide perioperative analgesia in patients undergoing thyroid and parathyroid surgery, carotid endarterectomy and cervical lymph nodes excision (Andrieu et al., 2007; Egan et al., 2013). Its efficacy during elective procedures has been proved not only in association to general anaesthesia, but also alone (Tobias, 1999; Mukhopadhyay et al., 2012) and the incidence of intraoperative conversion from loco-regional to general anaesthesia is reported to be between 0.28% and 2% (Pandit et al., 2007; Pasin et al., 2015). Furthermore, CPB seems to play a role in both maxillofacial surgeries (Shteif et al., 2008; Kanthan, 2016) and non-surgical pain management, such as neuralgia related to herpes zoster (Herring et al., 2012; Shin et al., 2014). Superficial and deep CPB are both described in humans, according to the anatomy of the cervical plexus which consists of the first four cervical anterior nerve branches (C2 – C4) and their anastomosis (ansa cervicalis) with the sympathetic chain and the hypoglossal, facial and vagus nerves. In particular, the superficial portion of the cervical plexus is composed of the anterior sensitive branches of C2 – C4, i.e. the lesser occipital, greater auricular, transverse cervical and supraclavicular nerves, emerging in a fascial compartment (superficial cervical fascia) at the level of the caudal margin of the sternocleidomastoid muscle (SCM). Innervation to the skin, neck structures and partly of the head and shoulder is provided. The deeper components of the plexus are located near the transverse processes of the cervical vertebrae, in the layer superficial to the scalenus medius and levator scapulae (Gray, 2009; Arbona et al., 2011). As for other perineural blocks, ultrasound-guidance has been shown to have a better success rate, a reduction of the volume of local anaesthetic used, a faster onset time and a decrease in complications such as inadvertent vascular puncture and intravascular injection (Marhofer et al., 1997; Marhofer et al., 1998) compared to blind and neurostimulated approaches in humans. The ultrasound guided superficial CPB is performed at the level of the superficial cervical fascia, which is visible as a hyperechoic rim. *In-plane* and *out-of-plane* approaches have been described depending on the type of needle insertion. In both cases, the needle tip is placed to inject local anaesthetic under the SCM and superficial to the prevertebral fascia (Herring et al., 2012). In dogs, the cervical plexus is also composed by the ventral branches from the second to the fourth cervical nerves, providing innervation to the region of the neck and the auricular pinna. In particular, the ventral branch of the second cervical nerve divides in two branches, i.e. the transverse cervical and great auricular nerves. The transverse cervical nerve provides innervation to the parathyroid, intermandibular and mandibular region. At this level, its cutaneous area overlaps with rami of the mandibular nerve. The great auricular nerve innervates the convex, lateral and concave surface of the pinna. Its arborisations overlap with the auricular branches of the facial nerve cranially and the greater occipital nerve caudally (Whalen & Kitchell, 1983a). The ventral branches of the third and fourth cervical nerves origin the supraclavicular nerves, that merge with the accessory nerve and frequently have a connection with the second cervical nerve. They supply the skin and muscles of the ventrolateral part of the neck, i.e. the longus capitis, longus colli, intertransversarius cervicis, omotransversarius, and brachiocephalicus muscles (Whalen & Kitchell, 1983b). The cervical plexus in dogs is not classified in a deep and a superficial portion in a comparable way with that of the human beings. To the authors knowledge, cervical plexus block has not been described in veterinary medicine. The aim of this study is thus to preliminary assess the feasibility of the ultrasound guided CPB in dog cadavers.

MATERIALS & METHODS

Six fresh dog cadavers were used within 24 hours from euthanasia in this preliminary study. All dogs were euthanized for reasons not related to the study. They were all mesaticephalic dogs (brachycephalic and dolichocephalic dogs were excluded) and did not have any muscular or neurological disease, nor showed any anatomical alteration of the neck. Owner informed written consent and agreement to the disposal of the body was obtained. Each dog was assigned to three different size categories, namely small (up to 10 kg of body weight), medium (from 11 to 24 kg of body weight) and large size (over 25 kg of body weight). The two small-size dogs were mixed-breed females weighing 8 kg and 10 kg, the two medium-size dogs were both mixed-breed males of 15 and 19 kg of body weight, and the two large-size were a mixed-breed male of 37 kg and a female of 26 kg. A predetermined volume of methylene blue 15% was assigned to each size category, i.e. a total of 5, 10, 20 ml per block for the small-, medium- and large-size cadavers respectively (Table 1).

Size Category	***Gender***	***Weight***	***Breed***	***Volume***
Small size	Female	8 kg	Mixed-breed	5 ml
	Female	10 kg	Mixed-breed	
Medium size	Male	15 kg	Mixed-breed	10 ml
	Male	19 kg	Mixed-breed	
Large size	Female	26 kg	Mixed-breed	20 ml
	Male	37 kg	Mixed-breed	

Table 1 Description of dog cadavers assigned to size category and total volume of methylene blue injected to perform the block for each side of the neck

Ultrasonographic study

The cadavers were placed in lateral recumbency. The hair of the neck was widely clipped and the skin cleaned with alcohol solution from the mastoid process to the cranial edge of the scapula. After coupling gel application, a 7.5 -12 MHz linear array transducer (Esaote MyLab 70, Genova, Italy) set at 12 MHz was positioned with the marker oriented dorsally to obtain a short-axis scan of the transverse process of the atlas (Figure 1a). The probe was then moved in a cranio-caudal direction until reaching the transverse process of C4, which was visible as a marked hyperechoic structure determining an anechoic acoustic shadowing (Figure 1b). Considering the transverse process of C4 as a landmark, the probe was held in a transverse position with the marker oriented dorsally. The deep layer of the superficial cervical fascia was identified as a double hyperechoic rim, and a 22G, 70 mm spinal needle (Quinke spinal needle, Terumo srl, Milan, Italy) was inserted with an *in-plane* approach in a caudo-cranial direction (Figure 2a & b). When the tip of the needle reached the cervical fascia, the predetermined volume of methylene blue 15 % was injected in two times: half of the volume over the cervical fascia and half beneath it. The procedure was carried out bilaterally in all cadavers: probe positioning was performed by an expert sonographer while needle insertion and injection was performed by an anaesthetist.

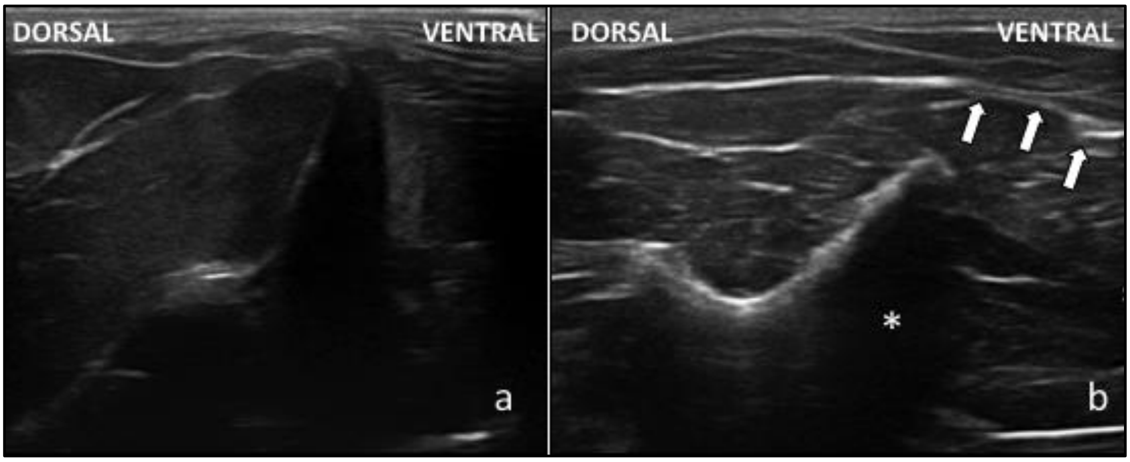

Figure 1a) short axis scan of the transvers process of the atlas, visible as an anechoic structure underlying a hyperechoic edge. **b)** Short axis scan of the transverse process of C4 (white asterisk: acoustic shadowing determined by the surface of the transverse process of C4) and identification of the cervical fascia, visible as a double hyperechoic rim (white arrows).

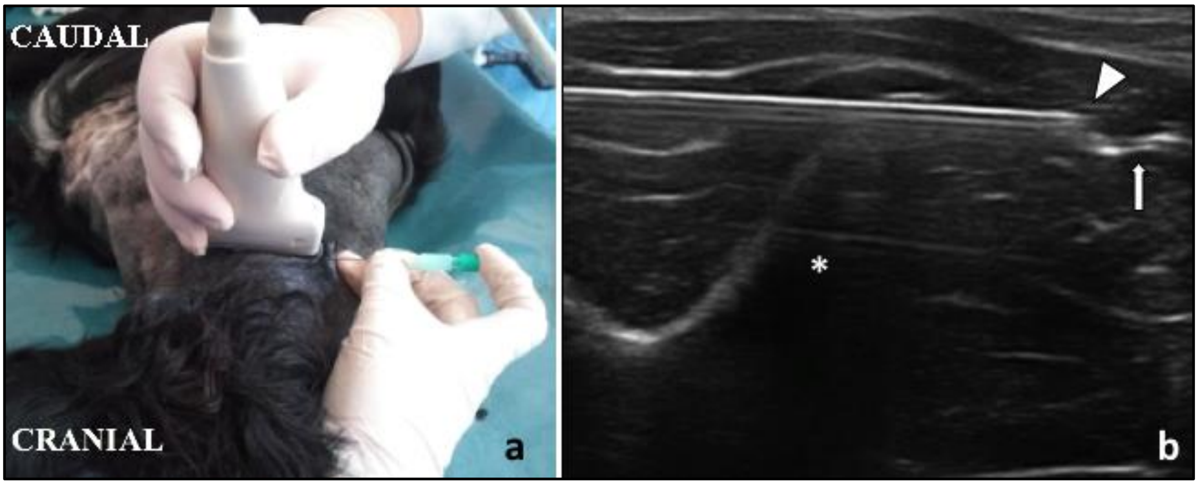

Figure 2a) Dog placed in lateral recumbency and widely clipped on the neck region. *In-plain* needle insertion in a cranio-caudal direction is shown. **b)** The needle is advanced until the tip (white arrow tip) reaches the cervical fascia (white arrow). White asterisk: acoustic shadowing determined by the surface of the transverse process of C4.

Anatomical dissection

Anatomical dissection was performed in order to evaluate the dye spread and nerve staining. The skin and the platysma muscle of the ventro-lateral portion of the neck were removed, followed by the omotrasversarius and the cleidocephalic muscles in order to expose the cervical plexus within the cervical fascia. Assessment of methylene blue spread was evaluated in a cranio-caudal direction, considering the ventral rami of C2 and C4 as the cranial and caudal reference point respectively. In particular, whenever the dye exceeded the reference point of more than 1 cm, anatomical dissection was continued up to the atlas cranially, or to the transverse process of C5 caudally. The depth of the spread towards the transverse processes of the cervical vertebrae and, ventrally, towards the jugular vein was evaluated as well. A block was defined successful according to the number of ventral branches involved (from C2 to C4) and the length of the stained portion which should be over 2 cm (Campy et al., 2008)

RESULTS

Both anatomical and ultrasonographic landmarks were easily identified in all cadavers, regardless to size and weight. All blocks were successful and homogeneous staining of the ventral branches of the second, third and fourth cervical nerves was confirmed (figure 3). Overall, the volume of methylene blue administered during the block was between 0.5 ml kg^{-1} (small size dog, 10 kg of body weight) and 0.77 ml kg^{-1} (large size dog, 26 kg of body weight). In particular, the mean volume administered in was 0.56, 0.59 and 0.65 ml kg^{-1} in small, medium and large size group respectively. In the smallest size dog (mixed-breed female weighing 8 kg), anatomical dissection was bilaterally expanded cranially as dye deposition exceeded of more than 1 cm over the ventral rami of C2. Spread of methylene blue was observed up to the atlas and to the transverse processes of the cervical vertebrae, as well as to the jugular vein. In seven out of twelve blocks, the volume of the deepest injection was found between the ventral intertrasversarius muscle and the fascia, while in the remaining blocks it was found within the muscle itself.

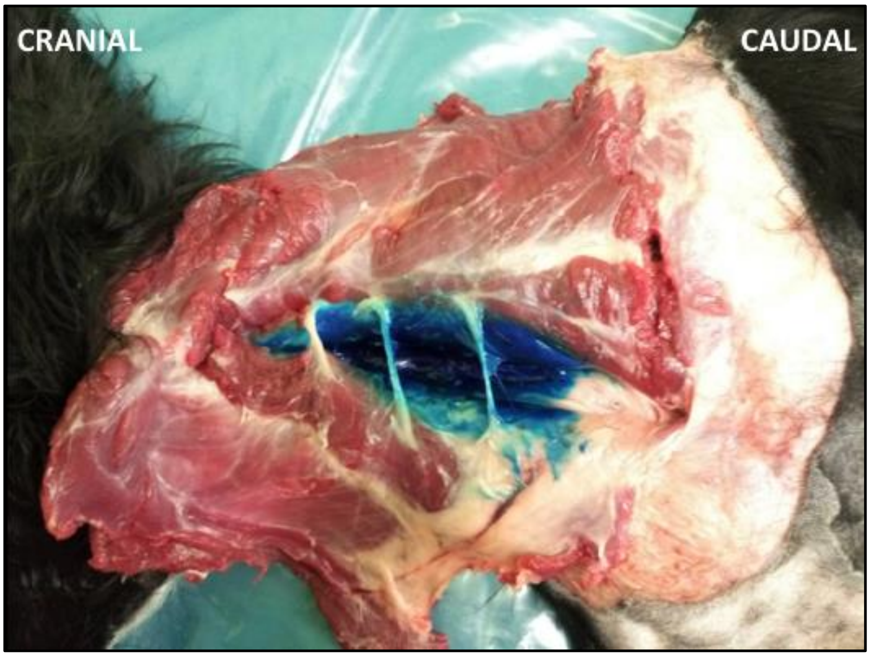

Figure 3 Anatomical dissection of the neck after block performance. Ventral roots from C2 (lesser occipital) to C4 are exposed after removal of the overlying muscles and dye spread is well visible as well.

DISCUSSION

In the authors' knowledge, the CPB has not been described in dogs. Ultrasonographic approach was chosen to take advantage of the direct visualisation of reference points, targets and of the injection itself. The anatomical and ultrasonographic landmarks, i.e. the transverse process of the atlas, the transverse process of the fourth cervical vertebra and the cervical fascia under the cleidocephalic and omostrasversarius muscles, were chosen accordingly to the dog anatomy. In fact, the ventral branches of the second to the fifth cervical nerves pass between the muscle bundles of the intertrasversarius cervicis, reaching the medial surface of the omostrasversarius (Evans & de Lahunta, 2014). Injections were performed at the level of the fourth cervical vertebra, as the cervical fascia was better visualized and could prevent excessive cranial spread of the LA as described in human patients (Pandit et al., 2007). Ultrasound landmarks were easily identified in all cadavers, regardless to size and body weight. The cervical fascia appeared as a well-defined double hyperechoic rim which did not seem to be negatively affected by the amount of adipose infiltration of the surrounding muscles that is known to generate a less sharp US image (Sites et al., 2007). Despite these considerations, the influence of body condition score on CPB performance should be investigated in a future prospective study, as well as other factors such as breed and morphotype. According to literature, a fascial block requires higher volume of local anaesthetic in order to grant an adequate spread compared with single perineural deposition (Marhofer et al., 2010). In human patients, the total volume administered rises up to 30 ml (Stoneham et al., 2015) for the superficial and deep cervical plexus block combined (despite the anatomical features of the cervical tract). In our study most of the spread appeared to be due to the fascial injection. The deepest injection did not appear to contribute to nerve staining as dye was found either within the ventral intertrasversarius muscle or between it and the overlying fascia. Effective volume might thus be considered half of the initial one. Interestingly, only in the smallest size dog the spread was noted up to the atlas and at the level of the transverse processes of the cervical vertebra, despite receiving a total volume of 0.62 ml kg^{-1}, with a probable effective volume of 0.31 ml kg^{-1}. In small size mesaticephalic dogs a lower pro kg dosage might be more suitable to reduce the risk of excessive distribution. Horner syndrome is a rare complication described in humans after CBP whenever excessive spread of local anaesthetic occurs. Despite this side effect is reported to be self-limiting in men, further studies should be performed in dogs (Flores et al., 2015). Ventral dye distribution was found at the level of the jugular vein in the smallest size dog. In humans, no side effects related to migration of LA in the visceral neck space is reported. Furthermore, intraoperative topical application of lidocaine directly in the visceral neck space by the surgeon is reported in order to enhance cardiovascular stability and analgesia during carotid endarterectomy (Sbarigia et al., 1999). Thus, no major complications should occur even with accidental ventral spread of LA in dogs. Being a cadaveric study, root staining was considerate adequate according to Campoy and colleagues (2008) as 2 cm represent five times the minimal length exposure to block nerve transmission and might be adequate to provide clinical effectiveness of the block (Raymond et al., 1989; Campy et al., 2008). Nonetheless, in clinical practice a balance between volumes, doses and concentration of local anaesthetic needs to be achieved in order to provide block efficacy, minimizing possible side effects. Further clinical studies are granted to evaluate whether or not a volume comprised between 0.25 and 0.38 ml kg^{-1} of local anaesthetic at different concentrations might be suitable to achieve perioperative analgesia in surgery involving the region of the neck (e.g. tiroidectomy, laryngeal lateralization, ventral neck explorations, etc). Side effects should be investigated as well. Phrenic nerve paralysis is reported as a common complication in human patients. In dogs, the phrenic nerve arises from the fifth, sixth and seventh cervical nerves, with seldom contribution of the fourth cervical nerve (Evans & de Lahunta 2014). As no caudal spread of the dye was noted over 1 cm from C4, it is unlikely that dye of the phrenic nerve occurred. Nonetheless, as no specific research was carried out further assessment should be performed. In conclusion, the ultrasonographic approach to the cervical plexus described in this study seems to be feasible in dogs. A single injection at the level of the cervical fascia appeared to provide adequate nerve staining of the cervical plexus, despite further evaluation on injection volume is advised.

ULTRASOUND GUIDED SPERMATIC CORD BLOCK IN DOGS UNDERGOING ORCHIECTOMY: PRELIMINARY EVALUATION OF AN INTRAFUNICOLAR APPROACH

INTRODUCTION

Orchiectomy is a standardized surgical procedure commonly performed in veterinary medicine (Adin, 2011). Castration is likely cause of pain in animals (Hewson et al., 2001). In horses and farm animals, the use of locoregional anaesthesia during this surgery has been largely described. Intratesticular injection of lidocaine improved perioperative analgesia in horses (Haga et al., 2006; Portier et al., 2009), calves (Stafford et al., 2002), piglets (Ranheim & Haga, 2006), lambs (Molony et al., 1997) and other species (Vesal & Tabadei, 2007; Oujad & Kamel, 2009; Nickell et al., 2015; Kharbush et al., 2017). In donkeys, intratesticular block was proved ineffective due to the connective structure and innervation of the testicle (Wrobel & Moustafa, 2000; Suriano et al., 2014). Several studies were carried out in companion animals as well. In cats, intratesticular block is reported to alleviate intraoperative nociception compared to ketamine-midazolam based general anaesthesia (Moldar et al., 2013). Administration of 0.05 ml kg^{-1} of lidocaine resulted in comparable intraoperative analgesia of either sacrococcygeal epidural administration or intravenous (IV) methadone. Nonetheless, higher postoperative pain scores were shown with earlier administration of rescue analgesia (Fernandez-Parra et al., 2017). In dogs, controversial results are reported. Intratesticular administration of local anaesthetic (LA) decreases intraoperative anaesthetics requirements and serum cortisol concentration in dogs undergoing orchiectomy compared to placebo (McMillan et al., 2012; Perez et al., 2013; Kushnir et al., 2017). A mitigation in sympathetic response is described as well (Huuskonen et al., 2013). Nonetheless, no significant difference in perioperative rescue analgesia administration is reported. Confounding factors such as premedication with pure μ-agonists or NSAIDs are advocated (Stevens et al., 2013; Kushnir et al., 2017). Blind spermatic cord block (SCB) has been assessed in large animals. The deposition of LA directly within the tunica vaginalis is reported to have a shorter onset compared to intratesticular injection in horses and to improve perioperative pain after castration both "in standing" and under general anaesthesia (GA) (Haga et al., 2006). On the other hand, inadvertent venous puncture and subsequent hematoma is a reported contraindication of this technique (Schumacher, 1996). Blind SCB in dogs undergoing orchiectomy was assessed by the authors (unpublished data). Hematoma and venous puncture were complications observed during surgery despite no blood was detected prior injection. The main aim of this study is to describe an ultrasound (US)-guided spermatic cord block technique in dogs undergoing elective orchiectomy. In particular, we hypothesized that the use of US-guidance would allow correct deposition of local anaesthetic, avoiding venous puncture. A secondary aim was to preliminary assess its possible analgesic effect during surgery. We hypothesized that the block of testicular nerve with LA would prevent intraoperative nociception compared to saline solution control.

MATERIALS & METHODS

Animals

Eight client-owned male dogs referred to the Faculty of Veterinary Medicine (Univesità degli Studi di Milano) to undergo elective orchiectomy were enrolled in the study. Informed written consent to the procedure was obtained by the owner. Exclusion criteria were American Society of Anesthesiologists (ASA) physical status equal or superior to III, presence of inguinal or scrotal hernia and testicular or scrotal alteration (e.g. neoplasia), variation in surgical technique (oschiectomy). Patients presenting mitral valve disease or heart murmur were excluded as well. Food but not water was withdrawn 12 hours prior surgery.

Procedures

All dogs were premedicated with intramuscular (IM) dexmedetomidine (5 µg kg^{-1}; Dexdomitor, Orion pharma, Finland). An intravenous (IV) catheter was placed either in the left or right cephalic vein and general anaesthesia was induced with IV propofol (4 mg kg^{-1}; Proposure, Merial S.p.A., Milan, Italy). After endotracheal intubation was performed, GA was maintained with isoflurane in 100% oxygen and with a continuous rate infusion (CRI) of dexmedetomidine (3 µg kg^{-1} h^{-1}). Dogs were allowed to breathe spontaneously. Cefazoline IV (25 mg kg^{-1}; Cefazolina Teva, Teva, Milan, Italy) was administered once before surgery and IV ringer lactate solution (5 ml kg^{-1} h^{-1}) was administered throughout the procedure. In order to verify correct block performance, sterile methylene blue 1% (Metiltioninio, SALF SpA, Bergamo, Italy) was added to lidocaine 2% (Lidocaina, Eciphar, Spain) or saline solution 0.9% at a 1:1 ratio. Each dog received an intrafumicular spermatic cord block as described hereafter both on one side with lidocaine (treatment) and on the other side with saline solution (placebo). The two dye solutions were prepared by an operator not involved in block performance and assessment. Volumes were administered according body weight, i.e. 0.5 ml and 1 ml per block in dogs weighing up to 10 kg and more than 11 kg respectively. The operator performing blocks, which was an anaesthetist with intermediate skill in US-guided blocks, and the one performing intraoperative evaluation were unaware of the treatment. Time to perform blocks were recorded, starting from the moment the ultrasound probe was positioned on the testicle and ending as the second injection was completed. Dogs were connected to a multiparameter module (GE Datex-Ohmeda S/5, Soma Technology Inc., Bloomfield, CT, USA). Respiratory rate (f_R), PE'CO_2 and FE'Iso were monitored with a side stream sampling capnograph, while heart rate (HR) was recorded from electrocardiography. A pulse oximeter was applied to the tongue to monitor haemoglobin oxygen saturation. Invasive blood pressure (IBP) was recorded placing an arterial catheter in the posterior pedal artery. Blood pressure transducer was calibrated to atmospheric pressure and positioned at the level of the left cardiac projection. An open prescrotal surgical technique was performed (Hamilton et al., 2014) always by the same surgeon, which was asked to assess dye localization and possible complications (hematoma within the spermatic fascia, active bleeding, dye deposition in the subcutaneous layer or outside the spermatic fascia). Heart rate (HR), respiratory rate (f_R), IBP, PE'CO_2, FE'Iso and esophageal temperature (T) were recorded every five minutes during surgery. In particular, HR, MAP, DAP, SAP and f_R values considered for statistical analysis were those recorded before the beginning of the surgery (T0), at skin incision (T*i*), ligation and cut of the spermatic cord of the first and second testicle. At the end of the study, data concerning ligation and cut of the first and second testicle were divided in two groups according to the type of treatment received, i.e. group receiving **methylene blue-lidocaine block (ML)** and group **receiving methylene blue-saline block (MS)**. Thus, T_{ML} data for each patient correspond to the time point in which the spermatic cord infiltrated with methylene blue and lidocaine was ligated and cut. In the same way, T_{MS} data for each patient correspond to the moment the spermatic cord infiltrated with methylene blue and saline was ligated and cut.

Block performance

Animals were placed in dorsal recumbency. The surgical site was clipped and aseptically prepared with alcohol and chlorhexidine solution. A linear array transducer (6-13 MHz, Fujifilm SonoSite Inc., USA) connected to a portable ultrasonographic device (M-Turbo Ultrasound System, Fujifilm SonoSite Inc., USA) was set at a high frequency and placed on the first testicle in order to obtain a short axis scan of the parenchyma. Moving the probe in a cranial direction, the spermatic cord was identified. The testicular vein and artery were visualized with color Doppler and were used as the main reference point in order to follow the spermatic cord to the external inguinal ring. At this level, the spermatic cord appeared as an oval hypoechoic structure surrounded by the hyperechoic spermatic fascia (Figure 1a & b). The testicular vein and artery were still visible in a short axis scan within the spermatic fascia. A 21G, 50 mm spinal needle without stylet and flushed with saline solution to avoid air injection was inserted *in-plain* in a later-medial direction. As the tip of the needle entered the vaginal fascia, a syringe containing methylene blue solution ether with lidocaine or saline was connected and negative pressure was applied on the plunger. If no blood was detected, injection was slowly performed and intrafunicular dye spread was observed (Figure 2). The same procedure was repeated for the second testicle for a total of 16 blocks in eight dogs.

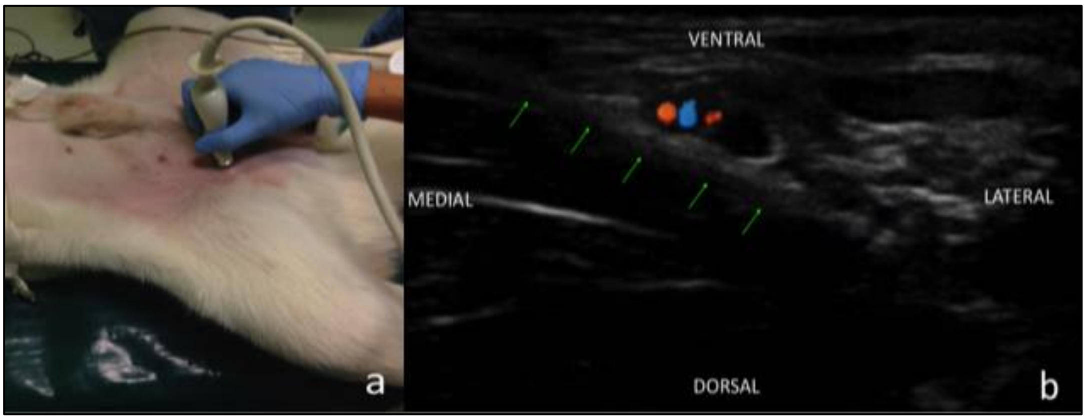

Figure 1: Probe positioning at the level of the external inguinal ring **(a)**. Short axis scan of the spermatic cord visualized at the level of the inguinal canal. It appears as an oval hypoechoic structure surrounded by a hyperechoic rim (spermatic fascia). The inguinal ligament is also visible (green arrows). The spermatic artery within is visualized in color Doppler (red) **(b)**.

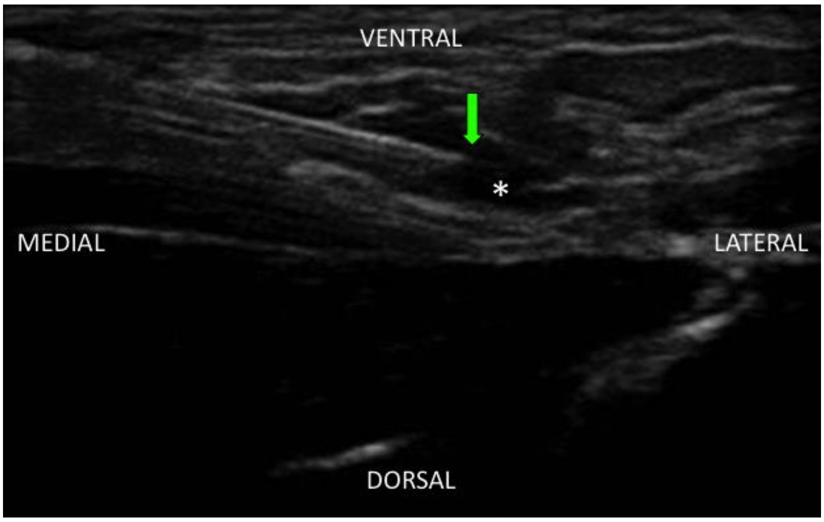

Figure 2: *In-plain* needle insertion and methylene blue solution injection. The tip of the needle (green arrow) is placed within the spermatic cord. Methylene blue is visible as an anechoic spread (white asterisk).

STATISTICAL ANALYSIS

This is a blind prospective matched-pairs randomized clinical trial. Data were statistically analysed using PASW 18.0 software (SPSS Inc, Chicago, USA). Normal data distribution was assessed with Shapiro-Wilk test. Repeated measures ANOVA, followed by Tukey Kramer's multiple comparison test, were used to compare the intraoperative physiological variables. In particular, values were compared between each other, i.e. T0 with T*i*, T_{ML}, and T_{MS}, T*i* with T_{ML} and T_{MS}, T_{ML} and T_{MS} between them. Significance was set for *p* values inferior to 0.05. Statistically highly significance was set for *p* inferior to 0.01.

RESULTS

Patients breed, age and body weight are resumed in table 1. Following data are presented as mean and standard deviation. Mean body weight was 22.9 ± 8.9 kg and mean age was 3.2 ± 2.3 years. Time of bilateral block performance was 4.8 ± 2.2 minutes. Time of general anaesthesia was 41.5 ± 3.4 minutes while time of surgery was 22.5 ± 1.6 minutes. Values are reported in table 2. Mean and SD values of HR, f_R, SAP, MAP, DAP for each time point are resumed in table 3. No statistically significative difference was found in HR between any time point. Values of SAP showed a significative difference between T0 and T*i* (p = 0.04, 108 ± 10.85 mmHg and 101.25 ± 10.44 mmHg respectively) and between T0 and T_{MS} (p = 0.02, 108 ± 10.85 mmHg and 112.62 ± 10.68 mmHg respectively). Values of MAP showed a significative difference (p = 0.02) between T0 and T_{MS} with values of 72.5 ± 6.52 mmHg and 82 ± 11.08 mmHg. Values of DAP showed a highly significative difference between T0 and T_{MS} (p = 0.01, 54.5 ± 11.53 mmHg and 61.87 ± 17.65 mmHg). Values of f_R showed a significative difference (p = 0.03) between T*i* (16.25 ± 5.73 breaths $minute^{-1}$) and T_{ML} (13.57±3.64 breaths $minute^{-1}$). No significative difference was found at any time point between T*i* and T_{MS} nor between T_{MS} and T_{ML}. Dye was found within the spermatic fascia in all 16 blocks performed, within the tunica vaginalis, distally from the testicle and close to the external inguinal ring in all subjects (Figure 3). Ligation and cut of the spermatic cord were performed between the testicle and the dye. No complication was detected.

	Breed	*Age*	*Weight*
Dog 1	Golden Retriever	7 years	35 kg
Dog 2	Mixed-breed	9 months	16 kg
Dog 3	Labrador Retriever	14 months	28 kg
Dog 4	Amstaff	2 years	20 kg
Dog 5	Pitbull	4 years	25 kg
Dog 6	Mixed-breed	3 years	25 kg
Dog 7	Mixed-breed	18 months	28 kg
Dog 8	Mixed-breed	6 years	6 kg

Table 1: Breed, age and weight of dogs enrolled in the study

	Bilateral block (minutes)	*General anaesthesia (minutes)*	*Surgery (minutes)*
Dog 1	6	47	21
Dog 2	8	41	20
Dog 3	8	45	25
Dog 4	5	38	23
Dog 5	3	40	23
Dog 6	3	37	22
Dog 7	3	41	22
Dog 8	3	43	24

Table 2: Table of bilateral block performance, time of general anaesthesia and time of surgery reported for each dog.

	HR (*beats minute*$^{-1}$)	f_R (*breaths minute*$^{-1}$)	*SAP* (*mmHg*)	*MAP* (*mmHg*)	*DAP* (*mmHg*)
T0	73.86 ± 18.94	13.62 ± 4.17	101.25 ± 10.44	72.5 ± 6.52	54.5 ± 11.53
Ti	79.25 ± 14.75	16.25 ± 5.73	108 ± 10.85	76.38 ± 10.44	57.13 ± 13.78
T_{ML}	76.62 ± 22.54	13.57±3.64	108.3 ± 15.45	77.62 ±13.88	60.12 ±12.63
T_{MS}	76.37 ± 22.44	15.62 ± 8.02	112.62 ± 10.68	82 ± 11.08	61.87 ± 17.65

Table 3: Values presented as Mean ± SD of HR, f_R, SAP, MAP and DAP at specific time points, i.e. at baseline (T0), skin incision (T*i*), ligation and cut of the testicle receiving Methylene blue-Lidocaine block (T_{ML}) and Methylene blue-Saline (T_{MS}).

Figure 3: Testicle extraction from the tunica vaginalis. Methylene blue deposition is visible distally to the testicle within the spermatic cord.

DISCUSSION

In dogs, the spermatic cord is a structure containing the vas deferens and blood vessels deriving from the testis. It is covered by an extension of the peritoneum (i.e. tunica vaginalis) and exits the abdomen through the inguinal canal. The testicular and cremasteric arteries, the pampiniform plexus of veins and lymphatic vessels are contained as well, together with the genital branch of the genitofemoral nerve and the ilioinguinal nerve. Innervation of the cremasteric muscle and testicle but not of the skin of the scrotum is provided (Evans & de Lahunta, 2014). In the authors' knowledge, this is the first paper describing the spermatic cord block in dogs. The choice to rely on an US-technique was based on previous results of blind SCB (unpublished data). The most common consequence was venous puncture leading to hematoma detected intraoperatively by the surgeon. As blood aspiration before injection always resulted negative and hematoma formation went undetected to anesthetists, we suspected that further vascular events such as intravascular injection (Campoy, 2006) might be hard to prevent as well. As small vessels are scarcely visible with ultrasound in cadavers, we decided to perform a preliminary clinical assessment of US-guided ISCB instead of a cadaveric study. In this case, the testicular artery and vein (derived from the pampiniform plexus) were also used as important landmarks and appeared easy to identify. Time to bilateral block performance decreased consistently from 8 to 3 minutes for the last four dogs, suggesting this could be an easy technique to handle for anesthetists with intermediate skill in US-guided block performance. Consistently, sixteen out of sixteen blocks resulted successful for what concerns methylene blue deposition and none of the side effects considered were shown. Dye was found within the tunica vaginalis, distally from the testicle and close to the external inguinal ring in all subjects. As spermatic cord ligation was performed distally to the site of injection, complete desensitization of the testis and cremaster muscle should occur whenever the block is effective. The volume administered has been standardized on body weight category rather calculated pro kg. This choice was made according to the poor correlation between body weight and testicular size in dogs (Eilts et al., 1993). Being a clinical study, skin incision was not extended to the external inguinal ring or further deep in the inguinal canal. Thus, actual length of the spread could not be measured, nor any accidental intraperitoneal spread could be retrieved. Intraoperative nociceptive assessment could have helped in evaluating that an adequate portion of the genitofemoral and ilioinguinal nerves was blocked. Intraoperative nociception is defined as an acute increase in blood pressure and heart rate over 20% of the baseline values (Vettorato et al., 2013). Despite a significative difference was found in blood pressure between T0 and T_{ML}, no increase over 20% of baseline values was shown. Furthermore, the comparison between the ligation and cut of spermatic cord infiltrated with lidocaine and with saline did not show significative difference. Heart rate appeared stable throughout the procedure. A possible explanation could be the anaesthetic protocol used, as dexmedetomidine CRI provides cardiovascular stability and analgesia (Kuusela et al., 2001; Uilenreef et al., 2008). The decision to perform dexmedetomidine CRI was based on the effort to avoid opioid administration to limit confounding factors. Stevens and colleagues (2013) stated that the use of morphine as a premedication might have influenced both intraoperative and postoperative pain assessment in dogs undergoing orchiectomy and receiving either lidocaine/bupivacaine or saline intratesticular block. Butorphanol as well could result as a confounding factor as stated by Kushnir and colleagues (2017). On the other hand, it was necessary to provide mild to moderate analgesia according to animal welfare. In particular, mild to moderate analgesia has been be ethically provided to reduce nociception related not only to skin incision and ligation of the spermatic cord receiving placebo, but also related to ligation of the spermatic cord receiving LA whenever the block failed. Overall, our data do not allow to draw any preliminary conclusion about the clinical efficacy of the ISCB in preventing intraoperative nociception in dogs undergoing orchiectomy. Nonetheless, as US-ISCB was performed successfully, further studies to evaluate its analgesic potency and clinical applications will be performed. Orchiectomy in dogs has been recently assessed to cause mild to moderate pain (Quarterone et al., 2017). Thus, the use of an effective locoregional block technique might be useful to perform opioid-free balanced anesthesia in patients undergoing orchiectomy for testicular neoplasia that cannot receive intratesticular block. Furthermore, seen the site of injection, analgesia for testicular biopsies and other surgeries could be provided as described in humans (Magoha, 1998; Wipfli et al., 2011). This study has some limitations. First of all, the small sample size that might have further influenced the preliminary analgesic assessment despite the matched-pairs design of the study. Secondly, the analgesic protocol used might have provided a strong cardiovascular stability that did not allow to assess nociceptive hemodynamic changes.

In conclusion, US-ISCB in dogs undergoing orchiectomy resulted to be feasible, easy to perform for anesthetists with intermediate experience in locoregional block performance and safe in the 16 blocks performed. Further assessment of clinical application will be performed on a larger sample, especially to evaluate the influence of SCB in perioperative pain management.

ASSESSMENT OF TRIGEMINAL AND FACIAL NERVES BLOCK IN DOGS UNDERGOING TOTAL EAR CANAL ABLATION AND LATERAL BULLA OSTEOTOMY (TECA-LBO) COMPARED TO SYSTEMIC MORPHINE ADMINISTRATION.

INTRODUCTION

Total Ear Canal Ablation and Lateral Bulla Osteotomy (TECA-LBO) is the most effective yet RADICAL surgical treatment of END STAGE external otitis described in dog and other species (White & Pomeroy, 1990; Bacon et al., 2003; Chow et al., 2011). Severe intra- and postoperative pain is frequently associate to the surgery, leading to rough recovery and to systemic opioids and sedative drugs supplementation (Buback et al., 1996). Multimodal analgesia consists in the combination of several drugs with a different mechanism of action affecting nociception and pain pathways (Corletto, 2007). Thus, the use of local anaesthetics in addition to systemic opioids has been investigated to manage TECA-LBO postoperative pain. (Radlinsky et al., 2005; Wolfe et al., 2006). In these studies, Continuous local infusions of both lidocaine and bupivacaine in combination with systemic morphine were assessed in dogs. Despite no significative improve in pain score and postoperative recovery, the clinical impression of a beneficial effect was reported in dogs treated with local anaesthetic infusion. Several perineural blocks have been described in veterinary medicine to manage perioperative pain in dogs and cats undergoing different type of surgeries, especially for highly painful procedures (Weger et al., 2005; Gurney & Leece, 2014; Vettorato & Corletto, 2016; Congdon et al., 2017). The blockade of impulse conduction prevents the sensory input to reach the spinal cord, providing effective pre-emptive analgesia and preventing neuroplasticity (Moiniche et al., 2002; Bolay & Moskowitz, 2002). A blind approach to great auricular nerve (ventral branch of the second cervical nerve) and the auriculotemporal branch of the trigeminus nerve aiming to manage perioperative pain in dogs undergoing TECA-LBO was reported by Buback and colleagues (1996). Bupivacaine 0.5% (0.5 ml per site regardless to body weight) was injected prior surgery in a line from the wing of the atlas to the caudal aspect of the vertical ear canal to block the great auricular nerve and between the caudodorsal aspect of the masseter muscle and rostral aspect of the vertical ear canal to block the auriculotemporal nerve. Despite the apparent correct location of nerves and subsequent block performance, no difference in pain score and other physiological parameters was found in dogs receiving the locoregional block with systemic opioids and those receiving either bupivacaine splash block with systemic opioids or systemic opioids alone. A clinical impression of a more stable anaesthetic plane and a better postoperative outcome was suggested (Buback et al., 1996). More recently, Stathopoulou and colleagues (2018) tried to perfection this block improving the anatomical landmarks in a cadaveric study in foxes and beagles. According to anatomical dissection, 0.04 ml kg^{-1} and 0.2 ml kg^{-1} appeared sufficient to stain over 0.6 cm of the auriculotemporal and great auricular nerve respectively. Neurostimulation increases the accuracy of nerve location, leading to a higher success rate of perineural blocks.

The aim of this study is thus to describe a new neurostimulated perineural block to provide perioperative analgesia in dogs undergoing TECA-LBO. In addition, we aim to assess the clinical efficacy of the block performed with two different local anaesthetics (LA) in comparison to systemic administration of morphine. We hypothesized that the locoregional block would have an isoflurane sparing effect during surgery and would reduce the need for perioperative rescue analgesia in dogs treated.

MATERIALS & METHODS

Animals

Eighteen client-owned dogs referred to the faculty of Veterinary medicine of Milan to undergo TECA-LBO surgery were enrolled in the study. Informed written consent was obtained by the owners. All dogs underwent physical, neurologic and otoscopic examination, followed by X-rays and CT examination of the head and bullas. Patients assessed to be American Society of Anesthesiologists (ASA) physical status I or II were considered to be eligible. Exclusion criteria included ASA III or more, heart disease, history of liver, renal or gastrointestinal disease or any contraindication for the use of the non-steroidal anti-inflammatory drugs or local anesthetic agents, skin infection at the sites of injection. Food but not water was withdrawn 12 hours prior surgery.

Procedure

All dogs received intramuscular (IM) acepromazine (0.03 mg kg^{-1}; Prequillan, Fatro, Italy). An intravenous catheter was placed in either the right or left cephalic vein. Induction to general anaesthesia (GA) was performed with titrate-to-effect propofol until endotracheal intubation was possible. General anaesthesia was maintained with isoflurane in 100% O_2. Ringer lactate solution (5 ml kg^{-1} h^{-1}) was administered throughout the procedure and cefazoline (25 mg kg^{-1}; Cefazolina Teva, Teva, Italy) was administered IV every two hours during surgery and every 8 hours postoperatively. Dogs were then randomly divided in three equal groups (n = 6): **group morphine (MOR)**, receiving IM morphine (0.3 mg kg^{-1}; Morfina cloridrato, Salf SpA, Bergamo, Italy), **group bupivacaine (BPV)** receiving locoregional block with bupivacaine (Marcaina, Aspen Pharma, Dublin, Ireland) and **group ropivacaine (RPV)** receiving locoregional block with ropivacaine (Naropina, Aspen Pharma, Dublin, Ireland). In particular, dogs between 1 and 5 kg of body received 0.5 ml per block of LA according to group assignment. Dogs weighing between 5 and 10 kg and over 10 kg received 1 and 2 ml of LA per block respectively, according to group assignment. Volumes administered contained either ropivacaine or bupivacaine at a fixed concentration of 0.5%. In the operating theatre, the patients were placed in lateral recumbency with the ear of the surgery placed upwards. Dogs were connected to a multiparameter module (GE Datex-Ohmeda S/5, Soma Technology, Inc., Bloomfield, CT, USA). All patients were mechanically ventilated to maintain PE'CO_2 between 35 and 45 mmHg. A side stream sampling capnograph was used to monitor PE'CO_2 and FE'Iso, while HR was recorded from ECG. A pulse oximeter was applied to the tongue to monitor blood oxygen saturation. Non-invasive blood pressure was recorded with a cuff placed on the posterior pedal artery. Anaesthetic plane was judged according to eye positioning, presence or absence of palpebral reflex on the contralateral eye when possible, attempts to breath spontaneously. Percentage of inspired isoflurane was adjusted accordingly, aiming to reach the lowest FE'Iso possible. Heart rate, f_R, NIBP, PE'CO_2, FE'Iso and esophageal temperature were recorded every five minutes during surgery. Whenever intraoperative nociception was detected, i.e. an acute increase in HR and blood pressure over 20% of the baseline values (Vettorato et al., 2013), a bolus of fentanyl IV (2 μg kg^{-1}; Fentadon, Dechra, Italy) was administered. If nociception was shown a second time during surgery, further 2 μg kg^{-1} of IV fentanyl was administered and constant-rate infusion (CRI) at 5 μg kg^{-1} h^{-1} was started. Discontinuation of CRI was performed at the end of surgery, before recovery from GA. A score from 0 to 2 was assigned to each dog according to the intraoperative additional analgesia received, where 0 was no analgesia received, 1 was a single bolus of fentanyl received and 2 was a second bolus of fentanyl followed by CRI receive. Both anaesthetic plane and intraoperative nociception were assessed by operators blind to treatment. Meloxicam SC (0.2 mg kg^{-1}; Metacam, Boehringer Ingelheim, Germany) was administered to all dogs at the end of surgery and ipsilateral eye was lubricated every two hours until discharge. Postoperative pain and sedation were assessed by a single observer blind to the treatment starting from extubation time (T0) and every 30 minutes up to 2 hours after T0. Assessment was then performed 3, 4, 5, 6, 7, 8, 12 and 24 hours after T0. Sedation was evaluated according to Buback et al (1996) and Wolfe et al (2006) (Table 1), while postoperative pain was assessed with the short form of the Glasgow Composite pain scale (CMPS-SF) (Reid et al., 2007). Whenever the threshold of 5 out of 24 was overcome, IM morphine (0.3 mg kg^{-1}) was administered. After the first administration of rescue analgesia, pain evaluation was stopped in the subject. Morphine was administered every 4 hours. Degree of sedation was still assessed together with general clinical assessment of the patient. Clinical assessment was performed at discharged and surgical follow up was performed 7 and 14 days after surgery.

Alert	No motor deficits, equivalent to pre-anesthesia	1
Faint sedation	Stands, walks, some ataxia, and disorientation	2
Slight sedation	Stands but ataxic, can remain sternal	3
Mild sedation	Can stay sternal, cannot stand, may struggle	4
Moderate sedation	Can raise head, usually laterally recumbent	5
Heavy sedation	Nonresponsive, cannot raise head	6

Table 1: Sedation scale according to Buback (1996) et al and Wolfe et al (2006). Sedation score was assessed in all dogs every 30 minutes up to 2 hours and every hour up to 8 hours starting from T0 and at 12 and 24 hours after T0.

<u>Block performance</u>

Dogs were placed in lateral recumbence with the ear to be blocked upwards. Hair was clipped from the lateral cantus of the eye and the labial commissure cranially, over the occipital crest dorsally, over the wig of the atlas caudally and at the level of the mandibula rami ventrally. The pinna of the ear was accurately clipped as well. The area was then surgically prepared with chlorhexidine and alcohol solution. The mandibular nerve block was performed as previously described by Carotenuto et al (2011). Briefly, the ventral aspect of the zygomatic arch was palpated in a cranio-caudal direction up to the temporomandibular joint. As a depression was felt at this level, a 22G, 50 mm insulated needle (Stimuplex A, B-Braun, Germany) connected to a neurostimulator device (Plexygon, Aryon-Vygon, Italy) set at 1 mA was inserted in a latero-medial and slightly cranio-caudal and ventro-dorsal direction (Figure 1). The needle was advanced until twitch of the masseter, digastricus and pedrigodeus medialis and lateralis muscles was evident, resulting in an opening and closing movement of the jaw. To block the auriculopalpebral branch of the facial nerve, an imaginary triangle connecting the base of the ear pinna (i.e. base of the triangle) and the external occipital protuberance (i.e. apex of the triangle) was drawn. The insulated needle was inserted in the middle of the triangle, with a dorso-ventral slightly medio-lateral direction (Figure 2). The needle was advanced until a twitch of the muscles moving the pinna and the upper eyelid was noticed. In both cases, the twitch was considered adequate when it persisted between 0.4 and 0.2 mA (Campy et al., 2008). Negative pressure was applied to the syringe and if no blood was aspirated, injection of local anaesthetic according to group assignment was performed.

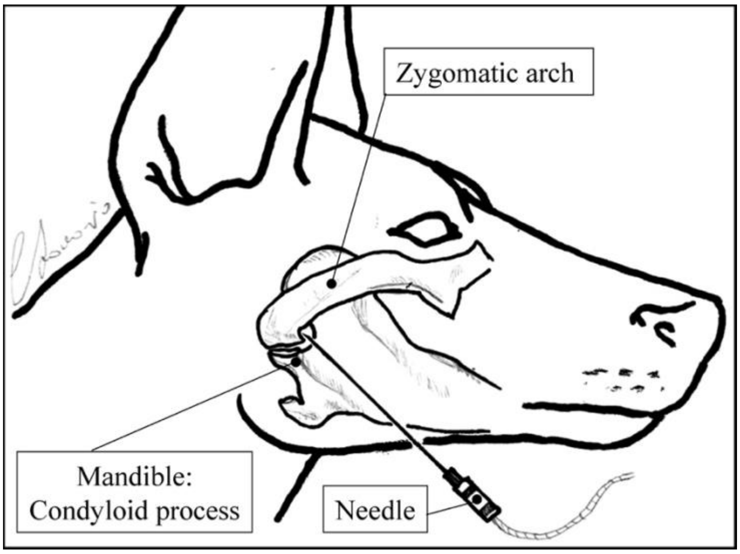

Figure 1: Mandibular block. The needle is inserted in a cranio-caudal direction at the end of the zygomatic arc.

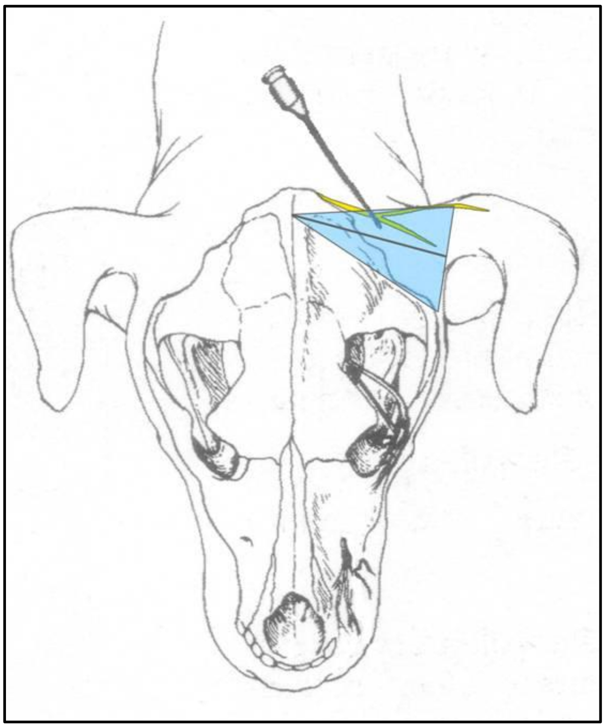

Figure 2: Auriculopalpebral (facial) block. An imaginary triangle is draw between the base of the ear pinna and the external occipital protuberance. The needle is inserted in a dorso-ventral slightly medio-lateral direction in the middle of the triangle.

STATISTICAL ANALYSIS

Data collected underwent statistical analysis using PASW 18.0 software (SPSS Inc, Chicago, USA). Intraoperative parameters such as HR, NIBP and FE'Iso, were analyzed with analysis of variance (ANOVA). Differences in pain scores were analyzed using the Wilcoxon's rank-sum test. Differences in incidence of treatment failure were analyzed using a Fisher's exact test. Statistically significative difference was set inferior to 0.05 (p value <0.05).

RESULTS

Data are presented as mean and standard deviation. Dogs were 22.8 ± 12.2 kg, 17.7 ± 9.2 kg and 26 ± 13.5 kg of mean body weight in group BPV, RPV and MOR respectively. Mean age was 7.9 ± 3.4 years, 6.4 ± 2.3 years, 10.2 ± 3.3 years in group BPV, RPV and MOR respectively (Table 2). No significative difference was found among groups concerning age and body weight. A significative difference was found in FE'Iso among groups. In group MOR, FE'Iso resulted 1.59 ± 0.27, while it resulted 1.45 ± 0.24 and 1.34 ± 0.22 in group RPV and BPV respectively (p values MOR vs RPV 0.03, MOR vs BPV 0.01, BPV vs RPV 0.03). Mean HR values showed a significative difference between group RPV (119.13 ± 14.88 bpm) and both groups MOR (111.07 ± 18.4 bpm; p = 0.04) and BPV (89.76 ± 7.9 bpm; p = 0.04), which on the contrary resulted comparable. Mean blood pressure resulted 81.97 ± 22.8 mmHg in group MOR, 80.52 ± 10.7 mmHg in group RPV and 73.69 ± 8.8 mmHg in group BPV. Four out of six dogs in group MOR required intraoperative rescue analgesia. One dog showed nociception when the external ear canal was removed and lateral bulla osteotomy was started. A single bolus of fentanyl was administered and provided analgesia until the end of surgery (score = 1). Of the remaining, three showed nociception during external ear canal dissection and a second time during bulla osteotomy. After a first bolus administration, fentanyl CRI was started (score = 2). A dog in RPV required intraoperative rescue analgesia at skin incision and received a single bolus of fentanyl (score = 1). No significative difference was found between group BPV and RPV, while a significative difference was found between group MOR and both group BPV (p = 0.017) and group RPV (p = 0.02). No dogs in BPV and RPV group required postoperative rescue analgesia, as scores assigned according to the CMPS-SF did not overcome the threshold of 5/24. All subjects in group MOR received rescue analgesia. One dog received morphine 30 minutes after T0 (CMPS-SF 8/24). Two dogs received morphine 90 minutes after T0 (CMPS-SF 6/24 in both) and three dogs received morphine 2 hours after T0 (CMPS-SF 6/24 in two of them and 5/24 in one). No patient in RPV and BPV groups received postoperative rescue analgesia. Sedation score resulted significative higher in group MOR (3.39 ± 1.31) compared to group RPV (2.58 ± 1.0) and BPV (2.42 ± 1.03). No significative difference was found between group RPV and BPV. Two out of six dogs in group BPV, one out of six dogs in group

RPV and two out of six dogs in group MOR showed facial nerve paralysis at discharge. Additional follow up at a month was scheduled. By that time all dogs showed resolution of the paralysis.

	Breed	*Gender*	*Age (years)*	*Weight (Kg)*
RPV	Mixed-breed	Male	5	20
	Mixed-breed	Male	7	12
	Cocker Spaniel	Female	8.5	14.5
	Cocker Spaniel	Female	2.5	9.5
	American Cocker	Female	8.5	15
	German Shepherd	Male	7	35
BPV	German Shepherd	Male	5	35
	Mixed-breed	Female	9	8
	Labrador R.	Female	10	37
	Mixed-breed	Male	10	22
	Mixed-breed	Male	11	25
	French Bulldog	Male	2.5	10
RPV	Mixed-breed	Female	14	30.5
	German Shepherd	Male	12	28
	Pitbull	Male	11	46
	West Highland WT	Female	4.5	8.5
	Mixed-breed	Male	9	30
	American Cocker	Male	10.5	13

Table 2: Breed, gender, age and weight of dogs in group RPV, BPV AND MOR

DISCUSSION

The innervation of the ear in dogs is provided by the trigeminus and the facial nerves. In particular, the auriculotemporal nerve leaves the mandibular branch of the trigeminus at the level of the oval foramen and runs caudally to the retroarticular process of the temporal bone. It provides sensory innervation to the external acoustic meatus and the tympanic membrane as well as to the skin of the ear (Whalen & Kitchell, 1983a; Whalen & Kitchell, 1983b). Landmarks and site of injection were chosen so to take advantage of the mixed nature of the mandibular nerve and elicit the muscular twitch of the masticatory muscles. On the other hand, the injection of LA close to the trigeminal ganglion would allow to block the mandibular nerve before the auriculotemporal nerve takes off. The auriculopalpebral branch of the facial nerve courses dorsally from the base of the ear providing both sensory and motor fibers the rostral auricular, nasolabial, palpebral and scutuloauricularis muscles. Further branches of the facial nerve (caudal auricular and internal auricular nerves) provide innervation of the external ear canal (Whalen & Kitchell, 1983a; Whalen & Kitchell, 1983b). Nonetheless, their block was not performed in this trial, seen their emergence from the stylomastoid foramen. In this study, the association of mandibular and auriculopalpebral nerve blockade with either ropivacaine or bupivacaine seemed to effectively prevent intraoperative nociception. The choice to administer acepromazine as sole premedication agent in groups receiving the block was performed to avoid any possible confounding factor related to systemic analgesia administration. No dog in group BPV and a dog in group RPV received intraoperative rescue analgesia. The latter showed nociception only at skin incision. A partial analgesia of the skin could be hypothesized, seen the possible anastomosis of superficial cutaneous branches of other cervical nerves (Shoja et al., 2014). Four out of six dogs in group MOR received intraoperative rescue analgesia. Mean heart rate in this group was probably influenced by the fentanyl CRI administered in three out of six dogs. Fentanyl is in fact a pure μ-agonist opioid 75 to 100 times more potent than morphine (Vardanyan & Hruby, 2014) and can lead to bradycardia related to centrally mediated enhanced parasympathetic activity. Considering the dosage administered, the analgesic rather than the sole cardiovascular effect of this drug might be more likely responsible for lower heart rate. In addition, fentanyl is known to have a sparing effect on isoflurane administration (Williamson et al., 2017). Nonetheless, FE'Iso was significantly higher in group MOR compared to other groups. This suggests that the mandibular/auriculopalpebral block enhanced anaesthetic stability more than systemic opioid administration. Ropivacaine is reported to be slightly less potent than bupivacaine (Bader et al., 1989; Markham & Faulds, 1996). This might explain the significative difference in FE'Iso between groups receiving the block, as RPV FE'Iso was higher that BPV FE'Iso. Mean blood pressure appeared comparable among groups. The vasodilatory effect of acepromazine could be responsible for the small variation in blood pressure values among groups. Nonetheless, mean pressure values were over 65 mmHg at any time point during GA, which is considered to be within normal range. The comparable postoperative long-lasting analgesic effect could be due to the concentration administered. In fact, vasocontraction occurs at ropivacaine concentration equal or inferior to 0.5%, leading to a reduced absorption and increase in duration of action (Cederholm et al., 1992). No dogs in RPV and BPV groups required rescue analgesia in the postoperative period. Considering that the duration of both local anaesthetics used is from 6 to 11 hours (Rioja Garcia, 2015; Tayari et al., 2017), this result was unexpected. The administration of NSAID immediately after the surgery might have helped in reducing post-surgical inflammatory pain. Nonetheless, group MOR was administered meloxicam as well, but postoperative rescue analgesia was needed in all patients after 30 to 120 minutes from T0. A possible explanation could be the pre-emptive analgesia exerted by the perineural nerve block on the healthy tissue interested by the surgery. Sedation score appeared significantly higher in group MOR, probably as a consequence of morphine administration every 4 hours after the first rescue analgesia regardless to pain assessment. Side effects related to µ-agonists administration are reported to be more common when these drugs are administered without pain (Pascoe, 2000; Bini et al., 2018). Nonetheless, this protocol choice was due to grant an adequate and stable analgesic plane after failure of postoperative analgesia was detected, and no side effects such as nausea or vomiting were observed. Overall, our findings seem in in contrast with Buback and colleagues (1996) and Wolfe and colleagues (2006) findings. Blind blockade of the great auricular nerve and auriculotemporal branch of the trigeminus, splash block administration of LA and CRI of local lidocaine did not produce any notable analgesia or improved perioperative pain management compared to morphine administration. The use of a neurostimulated approach and volumes based on size categories might have helped in increase accuracy and efficacy of blocks. In addition, the great auricular nerve (ventral rami of the second cervical nerve) innervates the convex, lateral and concave surface of the pinna (Whalen & Kitchell, 1983a) and its role in nociception during TECA-LBO surgery does not appear crucial. This study has many limits, such as a small sample size. Quality of extubation and recovery from general anaesthesia was not assessed with a dedicated scale. Our clinical impression was nonetheless consistent with previous studies in which rough recovery after TECA-LBO surgery is reported (Buback et al., 1996). The block of auriculopalpebral nerve blocks upper and lower eyelids movement, impairing blinking. The lack of palpebral reflex of the ipsilateral eye of the surgery was not assessed perioperatively in order to keep the evaluation blind. Intraoperative anaesthetic plane was in fact based on the contralateral eye position and blinking, and eye lubricant was postoperatively applied regardless in all dogs. Data concerning loss and recovery time of palpebral reflex ipsilateral to the surgery might provide information about block onset and end and could thus be considered for future trials. Nonetheless, no short- or medium-term complication such as corneal erosion and ulcer was detected. On the other hand, among the several possible complications of the procedure (e.g. persistent drainage, fistula formation, site infection, vestibular disease, Horner's syndrome), surgical facial nerve damage is reported. Total complications rage between 29% and 82%, while facial nerve deficit due to axonotmesis, neurotmesis or neuropraxia is reported to be 48.9% with a 10.5% incidence of residual damage (Doyle et al., 2004; Spivack et al., 2013). Thus, it could be difficult to establish the exact cause of facial nerve paralysis. In our study, five out of eighteen dogs showed facial nerve paralysis that resolved within a month after the surgery. Two of the dogs belonged to group MOR, supporting the hypothesis of a surgical damage, while for those in group RPV (one dog) and BPV (two dogs) a possible transitory nerve damage due to the block cannot be excluded. In conclusion, the combination of mandibular and auriculopalpebral nerve blocks either with bupivacaine or ropivacaine 0.5% provides analgesia up to 24 hours in dogs undergoing TECA-LBO. In addition, isoflurane sparing effect, reduced sedation and faster postoperative recovery where shown in comparison to perioperative systemic opioid administration.

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Locoregional anaesthesia for perioperative pain management in rabbits undergoing Total Ear Canal Ablation and Lateral Bulla Osteotomy (TECALBO): description of two cases.

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TECALBO is an effective yet invasive surgical technique to treat end-stage internal and external otitis in rabbits (Csomos et al 2016). The aim of this study is to describe a new locoregional analgesic approach, as until now only systemic analgesia was described for this surgery in rabbits. The first patient, a 7-year-old lop-eared neutered male weighing 2.1 kg, undergoing right TECALBO due to end-stage otitis with bulla empyema, received subcutaneous dexmedetomidine (60 µg/kg) and ketamine (10 mg/kg). Propofol 0.5% (1 mg/kg) was administered for induction while titrate-to-effect isoflurane was administered for maintenance. After palpating the zygomatic arch, the mandibular nerve was blocked at the level of the temporomandibular joint. The auriculopalpebral nerve was blocked in the centre of a triangle identified between the base of the pinna and the occipital crest (Ravasio et al 2008). Ropivacaine 0.5% (1.5 mg/kg per block) was injected after neurolocation of the nerves. Neither intraoperative (mean heart and respiratory rate 175 ± 22 and 25 ± 5 respectively) nor postoperative nociception were shown as no rescue analgesia was needed. Full recovery and food intake occurred within 1 hour after awakening. The second patient, a 9-year-old Dutch-belted intact female of 1.5 kg undergoing right TECALBO for end-stage otitis, received dexmedetomidine (40 µg/kg), ketamine (7 mg/kg) and midazolam (0.3 mg/kg) subcutaneously. Propofol 0.5% (2 mg/kg) was administered for induction. Titrate-to-effect isoflurane was administered for maintenance. Mandibular and auriculopalpebral nerve blocks were performed as described. No intraoperative (mean heart and respiratory rate 195 ± 15 and 31 ± 7 respectively) nor postoperative nociception were recorded, despite delayed awakening and full recovery were shown (food intake occurred 2.5 hours after awakening) and were probably related to midazolam administration. In both cases, the combination of mandibular and auriculopalpebral blocks appeared to provide effective perioperative analgesia for TECALBO surgery in rabbits (Figure 1).

Figure 1: Neurostimulator connected to an insulated needle which is used to perform neurostimulated TECALBO blocks in rabbits.

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LOCOREGIONAL APPROACH TO PARTIAL EAR CANAL ABLATION AND LATERAL BULLA OSTEOTOMY (PECA-LBO) IN A BELIER RABBIT

Detrimental effects of untreated perioperative pain can lead to life-threatening conditions in the rabbit species, such as arrhythmias, respiratory compromise and ileus (Barter, 2011). Several surgical techniques are starting to be performed in this species and locoregional anaesthesia is evolving accordingly in order to provide perioperative analgesia, reduced supplementation in systemic opioids administration and a faster postoperative recovery and retour to normal physiologic functions (d'Ovidio et al., 2014). Partial Ear Canal Ablation and Lateral Bulla Osteotomy (PECA-LBO) is a surgical procedure performed in rabbits to treat end-stage otitis media and externa (Eatwell et al., 2013). We would like to report a neurostimulated locoregional approach to perioperative pain management in a rabbit undergoing PECA-LBO.

A 7-year-old male entire Belier rabbit weighing 1.8 kg was referred at the exotics animal service of Veterinary National School of Alfort (France) for dental problems. Blood work analysis and clinical examination were unremarkable, except for bilateral epiphora, right eye absence of palpebral reflex and facial asymmetry, suggestive of right facial nerve impairment. Heart rate (HR) was 270 bpm, respiratory rate (fR) was 40 breaths minute^{-1}. No abnormal heart sound or lung murmur was detected at chest auscultation. Computer tomography examination excluded dental problems and highlighted right mandibular osteomyelitis and median otitis. PECA-LBO was scheduled for the following day. The rabbit was premedicated with IM midazolam (Midazolam, Hospira Inc, lake Forest, USA; 0.5 mg kg^{-1}) and methadone (Synthadon, Le Vet, Oudewater, Pays-Bas; 1 mg kg^{-1}). Induction with titrate-to-effect alfaxalone (Alfaxan Vétoquinol, Paris, France; 2 mg kg^{-1} in 1 ml of saline solution) via 24G intravenous catheter placed in the left marginal auricular vein was performed. Endotracheal intubation was performed with a 3 mm uncuffed endotracheal tube under endoscopic guidance. Isoflurane in 100% oxygen was delivered via coaxial Mapleson D system, allowing the rabbit to breathe spontaneously. The patient was positioned in left lateral recumbency and the hair of the right ear was clipped from the pinna to the cranial edge of the scapula. The skin was surgically prepped with chlorhexidine and the ear laid extended. A 22 G, 50 mm insulated needle (Stimuplex Insulated Needle; BBraun Medical Inc, Helsungen, Germany) connected to a nerve stimulator (Stimuplex HNS12, BBraun Medical Inc, Helsungen, Germany) was inserted in a latero-medial direction at the level of the transverse process of the atlas, aiming for the great auricular nerve (ventral rami of the second cervical nerve). A current of 1 mA was delivered to obtain a muscular twitch leading to a medio-lateral movement of the pinna. Current intensity was decreased to 0.2 mA to avoid intraneural location, and injection of 0.4 ml of bupivacaine (Chlorhydrate de bupivacaine, Aguettant, Paris, France) dilution at 0.15% was performed with persistent myoclonus at 0.36 mA. The needle was retracted and redirected lateral to the atlas wing, with a slight caudo-cranial latero-medial direction, aiming for the lesser occipital nerve (ventral rami of the second and third cervical nerve) until a muscular twitch of the anterior portion of the pinna was elicited. The same volume of bupivacaine 0.15% was injected with persistent myoclonus at 0.38 mA. Physiological and cardiovascular parameters were recorded every five minutes during the whole procedure. No acute increases in HR (284.7 ± 6.3 bpm) nor in non-invasive blood pressure (SAP 141 ± 7.8, MAP 112.5 ± 2.7, DAP 85.8 ± 7.3 mmHg) were recorded throughout the surgery and no rescue analgesia was administered. No apnoea or tachypnoea was detected (*f*R 36.5 ± 1.9), $PE'CO_2$ was within normal range and FE´Iso was maintained between 2.2 and 1.8. The rabbit was actively warmed with a heating pad from induction to recovery from anaesthesia (38.6 ± 0.7 °C). Thirty minutes after the end of surgery, the rabbit recovered uneventfully. Meloxicam (Metacam, Boehringer Ingelheim, Paris, France) (SC, 1 mg kg^{-1}) was administered. Food intake occurred within 12 hours from extubation and no gastroenteric stasis was reported. Unfortunately, the patient died 48 hours after surgery. Necroscopy showed a severe dilated cardiomyopathy, which went undetected to clinical examination due to the absence of any clinical sign and pathological heart sounds or lung murmur. Furthermore, a left atrial thrombus was detected. The innervation of the rabbit ear and pinna appears extremely complex, as skin and muscles are supplied by ventral rami of the second and third cervical nerves (great auricular and lesser occipital), the auriculo-temporal branch of the trigeminus, the auriculopalpebral branch of the facial nerve and by Arnold's nerve (the auricular branch of the vagus) (Grant et al., 1932). Despite a lack of precise information concerning the V cranial nerve in this species, the auriculo-temporal branch of the trigeminus as well as Arnold's nerve appear to provide minor contribution to the innervation of the ear (Weddell et al., 1955; Vernau et al., 2007). Considering the right facial paralysis presented by the patient, the authors decided to perform only the second and third cervical nerve block. Despite the proximity of the two nerves, a different myoclonus was expected and observed, thus a double-injection approach was chosen. A relatively high volume (0.2 ml kg^{-1} per block) at a low concentration (0.3 mg kg^{-1} per block) was preferred to allow a good perineural spread of bupivacaine, reducing the risk of systemic toxicity. As no intraoperative nociception was shown and early postoperative recovery appeared fast and manageable with NSAIDs administration, we assumed that great auricular and lesser occipital nerve block provided good intraoperative pain management in this patient. The sudden death of the rabbit two days after the surgery, due to unrelated causes, did not allow to perform a proper late postoperative assessment. Nonetheless, normal food intake and bowel function were shown 12 hours from extubation in the postoperative period. These are considered major indicators of wellbeing in rabbits (Weaver et al., 2010), thus we can presume that good postoperative analgesia was provided. Further prospective studies will focus on the role of trigeminal innervation of the rabbit ear.

CELIAC PLEXUS BLOCK FOR THE TREATMENT OF ACUTE PANCREATITIS IN A DOG

INTRODUCTION

The celiac plexus block has long been used to provide analgesia for upper abdominal pain in human patients. In particular, neurolytic celiac plexus block (NCBP) has been advocated for pancreatic cancer pain management, as it provides persistent augmented analgesia when used as an adjunct to systemic opiates. In addition, NCBP may prolong survival, despite supporting data remain controversial.
The term “celiac plexus block” refers to the interruption of innervation at the celiac plexus itself or alternatively to the splanchnic nerves. Although Max Kappis was credited for popularizing the celiac plexus block in 1914, he actually described splanchnic nerves block (Fujita & Sari, 1997). The celiac plexus contains autonomic efferent nerves, innervating the upper abdominal viscera, and visceral afferent fibres, innervating the abdominal viscera from the distal oesophagus to the transverse colon. From the celiac plexus, afferent visceral nociceptive signals return to the spinal cord along the splanchnic nerves. Consequently, analgesia may be obtained with blockade of the celiac plexus itself, i.e. “neurolytic celiac plexus block”, or the nearby exiting splanchnic nerves. In either case, abdominal visceral anaesthesia and sympatholyis are provided. Thus, NCBP has been advocated in human medicine both as a form of regional anaesthesia for intra-abdominal procedures (Beck et al., 2005) and as a procedure to palliate chronic pain when performed with neurolytic solutions. Furthermore, NCPB has been widely studied for the relief of pancreatic pain (Leung et al., 1983).
In the authors’ knowledge, NCBP has never been reported in companion animal. On the basis of these bibliographic results, we hypothesized that NCBP might be feasible and effective in a dog to manage acute-pancreatitis-related pain and to promote return to normal gastro-intestinal motility through the inhibition of sympathetic pathways as obtained in rats (Fukuda et al., 2007).

CASE DESCRIPTION

A seven-year-old mixed breed male dog weighting 17 kg was presented with signs of anorexia vomiting and severe weakness of over 48-hour duration. The dog presented a medical history of several episodes of abdominal pain, vomiting and diarrhoea. On initial presentation, the dog appeared extremely lethargic and weak. Physical examination revealed respiratory effort with increased respiratory rate (*f*R; 55 bpm); oral mucous membranes appeared congested with prolonged capillary refill time (>3”). The dog was estimated to be 7% dehydrated. Tachycardia (heart rate, HR, 170 beats min^{-1}) and mild hypothermia (37.1 C°) were noted. A mildly distended and extremely painful abdomen was detected at abdominal palpation. Pain assessment was then carried out with the short form of the Glasgow composite pain scale (CMPS-SF) (Reid et al., 2007) and resulted to be 8 out of 24.

Preliminary diagnostic tests included abdominal radiographs and ultrasonography, complete blood count (CBC), full biochemical profile, and canine pancreatic lipase SNAP test (Canine SNAP cPL; IDEXX Laboratories). Ventro-dorsal and right-lateral abdominal radiographs were normal. Abdominal ultrasonography showed a marked thickening and parenchymal abnormality of both the left lobe and the body of pancreas and the absence of bowel movements. CBC revealed leucocytosis with mature neutrophilia (15.2x 109 cell L^{-1}; reference interval, RI, 3.3-12.0 109 cell L^{-1}), high normal haematocrit (55%; RI 37-55%) and globulin (45 g L^{-1}; RI 25-45 g L^{-1}) values. The biochemical profile showed hypochloraemia (85 mmol L^{-1}; RI 109-122 mmol L^{-1}), hyperphosphatemia (2.50 mmol L^{-1}; RI 0.81-2.19 mmol L^{-1}) and hypercholesterolemia (1502 mg dl^{-1}), a mild increase in urea (12 mmol L^{-1}) and high creatinine value within normal range (158 µmol L^{-1}; RI 44-159 µmol L^{-1}). A severe increase in alkaline phosphatase (ALKP, 1790 U L^{-1}; RI 23-212 U L^{-1}), abnormally high amylase (4868 IU L^{-1}) and lipase (4530 IU L^{-1}) activities, together with severe hyperglycaemia (469 mg dl^{-1}) and severe increase in serum triglyceride concentration (6.85 mg dl^{-1}) were present as well as hypoalbuminemia (2.3 g dl^{-1}). Coagulation test was within normal limits. The Canine SNAP cPL test returned a positive result and this combined with the dog's history, physical examination, radiographic and ultrasonography results, blood and biochemistry profile, established a preliminary diagnosis of acute pancreatitis. Initial treatment consisted of crystalloid intravenous fluids administration (ad hoc solution based on electrolytic alteration) at a rate of 73 ml h^{-1} for 24 hours (maintenance plus 7% dehydration), analgesic therapy with methadone (0.2 mg kg^{-1} q 4 h IM), antibiotic therapy with cefazolin (25 mg kg^{-1} q 8 h IV) and enrofloxacin (10 mg kg^{-1} q 24 h IV), maropitant (1 mg kg^{-1} q 24 h IV) and metoclopramide (0.2 mg kg^{-1} q 12 h SC) as antiemetics. Food was withheld for 24 hours.

Dog was reassessed after 2 and 4 hours: severe abdominal pain was still present without any clinical improvements (severe abdominal pain, tachycardia and hypertension, multiple vomiting and diarreha episodes) and CMPS-SF scores were above 8. Lidocaine (Lidocaina, Ati Srl, Bologna, Italy) constant rate infusion (CRI) was added to therapy (3 mg $kg^{-1}h^{-1}$) associated with ketamine (Lobotor, Acme Drugs Srl, Reggio Emilia, Italy; 0.5 mg kg^{-1} h^{-1}). During lidocaine and ketamine administration (Mansfield & Beths, 2015), the dog did not show any dysphoria but appeared deeply sedated without no important improvement of abdominal pain. Blood gas analysis showed metabolic acidosis (pH 7.23) and hyperlactatemia (6 mmol L^{-1}), despite electrolytes returned within normal range. In accordance with pet owner and after obtaining informed written consent, celiac plexus block with a blind approach was carried out. The dog was kept under CRI and to deepen the sedation, a bolus of alfaxalone (Alfaxan, Dechra Srl, Torino, Italy; 1.1 mg kg^{-1} IV) was administered. The patient was then placed in right lateral recumbency and hair was clipped in the lumbo-dorsal region of the back between T11 and L5 and the area was aseptically prepped with chlorhexidine 4%. The spinous processes of L1 and L2 were identified by palpation, and then marked to simplify the following procedures. A 7 cm, 22 G spinal needle, was then slowly inserted between the vertebral bodies of L1 and L2, oriented parallel to the spinous processes in dorsal-ventral direction. If the needle, during insertion, reached the vertebral body of L1 or L2 or the intervertebral disc between the two vertebrae, the needle was retracted and redirected.

Once the passage of the needle tip through the peritoneum was felt as a "loss of resistance", the needle was slightly retracted and connected to the syringe containing the local anesthetics. To perform the celiac ganglia block 5 ml of ropivacaine 0.75% were used diluted with NaCl 0.9% solution to reach a total volume of 10 ml (Ravasio et al., 2015).

The injection was performed in about 2 minutes, retracting and reinserting the needle several times, in dorso-ventral direction.

The patient was evaluated 1 hour later: abdominal pain was decreased, with CMPS-SF score inferior to 4. Lidocaine-ketamine CRI was then discontinued. Physical evaluation performed 2 hours after celiac plexus block, did not show signs of abdominal pain: the patient did not show any reaction to deep abdomen palpation, and cardiovascular parameters remained stable (HR 120 bpm, *f*R 21 bpm, blood pressure measured with doppler within normal range). Wet canned canine gastro-intestinal food was offered and the patient showed good appetite and ate a small amount of food without vomiting. Ultrasonography examination, performed 3 hours after celiac plexus block, highlighted the recovery of peristalsis. The dog appeared progressively more active and, encouraged by his owner, was able to walk. Blood gas analysis presented a reduction of blood lactate value (3 mmol L^{-1}) and a progressive resolution of metabolic acidosis. Physical examinations performed twice a day until discharge, showed total absence of abdominal pain, full return to gastro-intestinal function with good motility and semi-solid feces production. The results of CBC and biochemical profile, performed four days after admission, revealed that blood values started to normalize. That day, the dog was discharged with antalgic (carprofen, Rimadyl, Zoetis Italia Srl, Roma, Italy, 1 mg kg^{-1} q 12 h OS; tramadol hydrochloride, Formevet Srl, Milano, Italy, 2 mg kg^{-1} q 12 h OS, for 3 days) and antibiotic therapy (erofloxacin, Baytril, Bayer SpA, Milan, Italy; 10 mg kg-^{1}, for 20 days).

DISCUSSION

In the authors' knowledge, this case report describes the first clinical use of the celiac plexus block for the treatment of pain related to acute pancreatitis in dogs. Acute pancreatic inflammation is a common, challenging and frustrating problem in canine internal medicine and critical care. Effective management of severe pain in acute pancreatitis is one of the most important issues in therapy, as pain reflexes contribute to the development of life-threatening secondary complications, while a good pain control promotes a faster and more effective recovery (Rykowski & Hilgier, 1995). Nonetheless, effective analgesia could be difficult to provide, as this condition does not respond to common antalgic therapy. In the present case, systemic therapies for pain control, i.e. methadone and lidocaine-ketamine CRI for 10 hours, did not cause any improvement. In human medicine, NCBP is commonly adopted for pain treatment associated to chronic pancreatitis (Gress et al., 1999) but it is also reported for the treatment of acute conditions whenever conventional antalgic methods fail to give proper pain relief

(Fusaroli & Carletti, 2015). Performing a regional block with local anaesthetics does not only provide pain management but leads also to the block of sympathetic ganglion, causing the prevalence of parasympathetic tone. This result, which is generally considered a side effect, presents a therapeutic advantage if applied to the gastrointestinal compartment. In particular, the lysis of sympathetic stimulation and the consequent prevalence of parasympathetic tone restores gastroenteric motility. In fact, peristalsis restoration with an important reduction in gastro-oesophageal reflux is reported in human beings undergoing neurosurgery procedures (Yuen et al., 2002). The central role of the celiac plexus in gastro-intestinal motility regulation was also confirmed by Fukuda and colleagues in an experimental study performed in 2007. They showed that ganglionectomy performed in rats before intestinal manipulation highly reduced the onset of post-operative paralytic ileus. Post-operative paralytic ileus seems to be strictly related to the increase of sympathetic tone consequent to surgery insult. The activation of sympathetic pathways activates α-adrenoreceptors that causes inhibition of cholinergic nerve terminals. As a consequence, muscle contraction of the small intestine and stomach is impaired leading to a reduction in gastrointestinal motility. Ganglionectomy removes the peripheral component of adrenergic neurons, improving thus gastrointestinal motility (Li et al., 2010).

The celiac plexus block plays not only a role in motility control, but also in blood perfusion regulation of gastro-intestinal compartment and this could represent another potential benefit of this therapeutic approach. Li et al. (2010) performed ganglionectomy in rats, demonstrating that this procedure is effective in removing sympathetic-related vasoconstriction of the splanchnic bed.

Anatomical landmarks, patient positioning, volume of local anaesthetics and possible side effects (i.e. intraperitoneal diffusion), as well as equipment and feasibility were previously explored in a cadaveric study (Ravasio et al., 2015). In the present report, the operator did not find any difficulty in performing the procedure and this method seems to be safe and quick to perform allowing this procedure to be useful also in compromised patient. The procedure required only mild sedation, achieved with a low dose of alfaxalone (1.1 mg kg^{-1}) added to lidocaine-ketamine infusion, already required for therapeutic reasons. In human medicine, ultrasonography has become the gold standard to perform the celiac plexus block, as this technique increases safety and efficacy of the procedure. In veterinary medicine it might, as well, found application, especially in small breeds where the direct visualisation of the needle and the anatomical landmarks might reduce the risk in intraperitoneal injection. Intraperiotenal diffusion of anaesthetic solution was detected in cadaveric study (Ravasio et al., 2015). This possible complication might not be too concerning, as it could probably lead to supplemental analgesia, due to local action of ropivacaine on peritoneum and abdominal organs (Zilberstein et al., 2008). Ropivacaine dosage is commonly used safety in clinical veterinary clinical setting.

In the present case report, the celiac plexus block completely abolished pain in a dog with acute pancreatitis unresponsive to systemic analgesic drugs. This allowed to suspend opioids therapy and improved return to gastrointestinal motility, opening the possibility for further studies to investigate the opioid-sparing effect, the outcome and the survival in a wider sample of canine patients affected by acute pancreatitis and ileum.

GENERAL DISCUSSION

Locoregional blocks developed and assessed during the three years of PhD course in dogs and rabbits, and described in this dissertation, are new and could contribute to fill a gap for soft tissue surgeries in companion animals that currently lack a locoregional approach.

Works presented are the result of a training of a year and a half of both in neurostimulated and US-guided block already described in literature. For the reasons abovementioned, neurostimulated blocks were performed in a clinical setting under supervision in companion animals referred to undergo orthopaedic surgery, whenever a PNB was necessary as part of a multimodal analgesic approach. Blocks of the thoracic and pelvic limb and of the thorax were performed. Clinical practice allowed not only to develop the manual skill but also to learn how to perioperatively manage locoregional blocks, i.e. the choice of local anaesthetic, assessment of correct needle positioning and muscular twitch evoked, intra- and postoperative pain assessment and rescue analgesia administration. These abilities appeared fundamental during the clinical trials proposed.
The training in the use of ultrasound was performed on cadavers. Block described in literature were performed with methylene blue, followed by anatomical dissection so as to evaluate the result of block performance. This training period allowed the development of eye-to-hand coordination necessary to perform *in*- and *out-of-plane* ultrasound blocks, the ability to assess ultrasonographic anatomy (sonoanatomy) and its modification related to diseases or subjective anatomical alteration, the different approach to local anaesthetic use in terms of volumes and distribution compared to neurostimulated blocks.
The knowledge and skill developed, allowed to evaluate pros and cons of both approaches and to choose the best one for each block developed and described in this dissertation. Planning of volumes, assessment of pain and design of studies could be considered a result as well.
Ultrasound-guidance could be considered the gold standard to perform PNB, thanks to the possibility to have a real time visualization of the needle, the spread of LA and the targeted and adjacent structures. On the other hand, we felt that neurostimulated blocks still find an application in companion animals' daily practice. An intensive training and quite expensive equipment are required to perform US-guided block, especially when it comes to small size patients such as rabbits.

The choice of the approach and the type of study to perform was then decided according to the region and surgery involved.
Ultrasound-guidance was the preferential approach used, due the possibility to visualise the tip of the needle and the injection together with the surrounding structure and vessels. In addition, block of pure sensory nerves and fascial block could be carried out. Nonetheless, the training period led us to modify the initial approach, as every clinical trial assessing US-guided block should be preceded by a

cadaveric study. In particular, we noticed that in some regions a lack of vessels visualisation in cadavers could prevent correct block performance. In particular, US-guided spermatic cord block was evaluated on cadavers, but no useful results were retrieved and a lack of an effective landmark (spermatic vein and artery). Neurostimulated block were chosen over US-guidance whenever the advantage to evoke a highly specific muscular twitch was shown compared with an ultrasonographic exploration of the region. Lack of connective tissue around nerves increases the difficulty in ultrasonography use, as the nerve tissue appears anechoic and cannot be visualized without a surrounding echoic or hyperechoic structure. Cadaveric studies prior clinical neurostimulated block assessment was not planned, since impulse conduction is lost in cadavers.

Four new locoregional approaches are described in dogs and two in rabbits. Case reports were included in this dissertation due to particular characteristics of patients involved and what we believed to be important statements on locoregional anaesthesia. The difficulties in perform a prospective study was related to the inclusion criteria, in particular rabbits undergoing TECA-LBO or PECA-LBO and dogs suffering from pain related to acute pancreatitis unresponsive to medical treatment. Nonetheless seen the novelty of the block proposed that could both increase safety of general anaesthesia in rabbits and give a useful tool in acute pancreatitis-related pain management, the decision to include them in this dissertation was taken.

Regions involved in studies and case reports reported were the head and neck in dogs and rabbits, and the inguinal region and splanchnic compartment in dogs.

A more detailed description of the choice between ultrasound- and neurostimulated techniques together with the type of study will follow hereafter.

Ultrasound-guided locoregional nerve blocks were assessed in the neck and inguinal region in dogs. The ultrasound-guided cervical plexus block in dogs is described for the first time in veterinary medicine. According to literature, this block can be defined as a compartmental block (Egan et al., 2013). The advantage of compartmental blocks is related to the possibility to block several nerves innervating a specific region relying on intrafascial spread of a single or double injection of local anaesthetic (Portela et al., 2014). As such, the use of ultrasonography appeared mandatory in order to perform the injection within the cervical fascia which is otherwise impossible to identify. A preliminary cadaveric study was performed for two main reasons. First of all, the region considered contains several delicate structures, such as the laryngeal recurrent nerve and the diaphragmatic nerve, whose impairment could be deleterious. Thus, a cadaveric study was planned the need to define adequate volumes of injectate in order to achieve an adequate spread to stain the targeted nerves without excessive cranial, caudal or ventral spread that could cause significant side effects. Excessive cranial spread was retrieved only in the smallest size dog. Excessive cranial spread is associated in human beings with transitory Horner syndrome (Flores et al., 2015), while ventral spread in the visceral space of the neck should not occur considering the preferential cranio-caudal distribution showed by the dye that creates hydrodissection of the facia, thus avoiding the risk of laryngeal nerve impairment. Caudal spread was not found in any cadaver. Diaphragmatic nerve impairment seems thus unlikely with the volume administered, considering its emergence from C5 and C6 in dogs (Evans, 1993). Furthermore, the second reason to perform a preliminary cadaveric assessment of the block were the landmarks, i.e. bony and connective structures that could be easily identified in cadavers. No vessels or easily perishable structures were identified during the planning of the study that could justify a clinical trial. As a result, the wig of the atlas appeared an important anatomical and ultrasonographic landmark, while the transverse process of C4 and the cervical fascia useful ultrasonographic landmarks. They were all easily-identified and allowed successful block performance.

On the contrary, ultrasonographic block assessment of intrafunicular spermatic cord was performed as a clinical study. The choice to rely on an US-technique was based on previous attempts to evaluate blind spermatic cord block. Spermatic cord in dogs is easily identified at palpation. Nonetheless, the most common consequence of blind block performance was venous puncture, leading to hematoma detected intraoperatively by the surgeon. As blood aspiration before injection always resulted negative and hematoma formation went undetected to anaesthetists, we suspected that further vascular events such as intravascular injection (Campoy, 2006) might occur and be hard to prevent as well. A cadaveric ultrasound evaluation of the region was attempted. Small vessels resulted scarcely visible with ultrasound in cadavers and the identification of the spermatic cord was difficult to perform, lacking of consistent landmarks. In addition, inadvertent venous puncture in cadavers could not be evident. We decided thus to perform a preliminary clinical assessment of US-guided ISCB instead of a cadaveric study. As expected, the testicular artery and vein (derived from the pampiniform plexus) resulted not only anatomical structures to be avoided, but also important landmarks and appeared easy to identify. Results, i.e. all blocks correctly performed and no hematoma detected, support this assumption. Furthermore, the US-guided approach allowed intrafunicular spermatic cord block up to an inguinal level, which could be applied patients undergoing orchiectomy due to testicular neoplasia that cannot receive the classic intratesticular block (McMillan et al., 2012).

<u>Neurostimulated locoregional nerve blocks</u> were performed in the region of the head and in particular for TECA-LBO surgeries in dogs and rabbits. The choice to rely on neurostimulation was due to the anatomical region involved and to the type of disease presented by the patients. The bony structure of the head creates several artefacts that should be overcome to visualise the target structure. Furthermore, neither the mandibular nor the auriculopalpebral nerves are visible under US-guidance. The first one lacks a dense connective structure as it is blocked at the exit of the skull (oval foramen) and the mandibular artery could be the only landmark for injection (Viscasillas & Ter Haar, 2017). The second appears to be too superficial and thin. Lastly, calcification of the external auricular canal frequently occurs in patients with end-stage otitis scheduled for TECA-LBO. This condition could create further artefacts and anatomical alterations. Landmarks described and the characteristic

muscular twitch elicited by the stimulation of each nerve allowed correct needle position and block performance.

A cadaveric study before the clinical trial was not performed as neurostimulated approaches cannot be assessed on cadavers. Studies reported in literature are experimental. Animals are anaesthetized and euthanized after block performance. Considering Italian legislation and risk-benefit ration of the type of approach assessed, no attempt was made in this direction. Furthermore, several blind, neurostimulated and blind blocks of the region of the head are reported in veterinary medicine and used in the clinical practice (Otero & Portela, 2017), allowing to predict possible side effects without directing evaluate the spread of the injectate.

The technique was modified in the rabbit undergoing PECA-LBO according to the different type of surgery. As in TECA-LBO the entire ear canal is removed, in PECA-LBO the cartilaginous support of the medial part of the ear canal is maintained in order to prevent ptosis of the pinna in lop-eared rabbits (Eatwell et al., 2013). Furthermore, the rabbit who underwent PECA-LBO presented a facial paralysis, thus this nerve was not considered to be blocked. The use of neurostimulator was related mostly to technical difficulties. It was in fact not possible to find a transducer small enough to be used on the rabbit head and neck.

Blind locoregional nerve blocks still play a role in companion animals in veterinary medicine. An attempt of blind intrafunicular cord block was performed during these three years of PhD course. Orchiectomy is a commonly performed procedure in veterinary medicine, and intrafunicular block aimed to implement pain control which is sometimes underestimate in this routine procedure. As already stated, the spermatic cord can be identified through palpation. A blind approach could result easy and not time-consuming, thus ideal in a busy operating schedule. Inadvertent venous puncture and hematomas occurred is a frequent side effect in blind blocks (Campoy, 2006) and occurred frequently also during our trial. The decision to switch to a more advanced and possibly safer technique, i.e. US-guidance, was taken. In this dissertation, only a case report of a blind celiac plexus block in a dog suffering from acute pancreatitis irresponsive to medical treatment is reported. The choice of the technique was based on previous data of an anatomical study. Landmarks and reference point resulted clinically adequate to provide analgesia. The introduction of ultrasound-guidance was planned but clinical limitations did not allow to further develop this type of block during the last year of PhD course, as the main inclusion criteria was the presentation of acute pancreatitis unresponsive to standard medical treatment.

All blocks reported have never been described in companion animals and also in veterinary medicine. In US-guided CPB effective spread, i.e. staining of ventral rami of C2, C3 and C4 for over 2 cm, was achieved in all 12 blocks. Considering the region involved in the block and the need to better define the volumes of injectate (i.e. pro kg dosage instead of volumes based on size categories), we felt that further studies on dog cadavers should be performed before a clinical trial.

The US-guided ISCB resulted correctly performed in all 16 blocks. Time to block performance by an anaesthetist with intermediate skills in US-guided block performance decreased from 8 to 3 minutes to perform bilateral spermatic cord block. This block thus appears to compatible with a busy operating schedule, considering also the type of surgery involved which is highly standardized and routinely performed.

In both US-guided ISCB and TECA-LBO block in dogs, opioid-free anaesthesia was performed. In the first case, the choice of dexmedetomidine CRI might have provided cardiovascular stability that resulted in a confounding factor. In the second case, intraoperative nociception and postoperative pain evaluation showed a significative difference in terms of rescue analgesia needed perioperatively between subjects receiving systemic opioid (morphine) and those receiving the block with two different types of local anaesthetics (ropivacaine and bupivacaine). Furthermore, subjects belonging to the two groups receiving the block were not given any systemic analgesia prior surgery and appeared less sedated in the postoperative period compared to subject of morphine group. The choice to administer acepromazine as sole premedication agent highlighted the efficacy of the block. The approach described supports the possibility to perform opioid-free anaesthesia in our patients, which is a recent acquisition in human medicine and whose implications are still to be assessed in veterinary medicine (White et al., 2017; Forget, 2018). Attempts to provide locoregional analgesia during TECA-LBO were carried out from 1996 till nowadays (Buback et al., 1996; Stathopoulou et al., 2018) and no effective block was described yet. The neurostimulated approach to facial and mandibular block presented in this dissertation is a new approach to this surgery that could be filling the abovementioned gap, seen its effectiveness in providing opioid-free anaesthesia and long-lasting perioperative analgesia.

In recent years, a significant increase in the number of housed rabbits was shown, along with a change in the attitude of the owners who now expect a high standard of veterinary care for their pets. Thus, application of surgical techniques generally reserved to dogs and cats became more frequent, as well as modification according to the particular anatomy of this species. Once again, an attempt of the veterinary anaesthetist to provide the best possible perioperative pain management in a species particularly prone to life-threatening postoperative pain-related complication is necessary. A preliminary assessment of new TECA-LBO and PECA-LBO blocks in rabbits is reported. As in dogs, these highly painful procedures were generally managed with systemic opioids in rabbits. The possibility to provide effective analgesia with PNB as suggests by the outcome of case reports described, allows a possible future development of opioid-free anaesthesia in a species which is particularly sensitive to opioid-related side effects. Partial ear canal ablation block on a single rabbit was performed several months after TECA-LBO blocks. The choice to focus on cervical nerves, i.e. lesser occipital and great auricular, derives from a more extensive study of literature (Grant et al., 1932; Weddell et al., 1955) and the doubt of the role in trigeminal nerve in innervation of the ear in this species. Unfortunately, data currently reported in literature are not sufficient to clarify the issue (Vernau et al., 2007).

Locoregional anaesthesia in veterinary medicine is traditionally related to surgery and to surgical pain. The last case report of this thesis, i.e. blind celiac plexus block in a dog with severe pancreatitis, is meant to stress how locoregional anaesthesia should be considered as part of a multimodal analgesic approach to manage also pain related to medical conditions. Thus, apart from the intrinsic aim of the case report, a wider purpose to set the consideration for a new role of PNB in veterinary medicine is stated.

Limits of the project are the small sample size of works. The main reasons are the learning curve in performing locoregional blocks with a neurostimulated and especially a US-guided approach. Furthermore, the training period was performed on defrosted cadavers, while for the CPB cadaveric study we decided to rely on fresh dog cadavers in order to reduce artefacts and improve the quality of results. On the other hand, clinical studies were strongly related to the clinical activity. Blocks in rabbits are presented as case reports in relation to the difficulty to enroll subjects in a prospective clinical trial. In fact, despite the improvement in rabbit care and management of recent years, several types of surgery are seldomly performed. One of the main reasons is the high mortality related to surgery and anaesthesia. The risk of death appears to be seven times greater in rabbits than in dogs (Brodbelt et al., 2008). Seen the nature of these reports, results only allow to consider the block successful in each particular case described. On the other hand, the description of locoregional blocks, even if in form of case reports, appears even more important in this species as it could increase the safety of general anaesthesia, enhancing the degree of care provided.
Celiac plexus block was presented as a single case report for a similar reason, related to the difficult enrolment in our clinical setting. Nonetheless, as already said, we considered important insert this result. It represents a work in progress that opens the possibility to consider locoregional analgesia not exclusively related to surgery, but as part of multimodal analgesia also in patients with medical conditions that need a higher degree of pain management.

CONCLUSION AND FUTURE PERSPECTIVES

According to the cadaveric or clinical evaluation performed, these techniques appeared effective and can implement the use of locoregional anaesthesia as effective part of multimodal analgesia in veterinary medicine. In particular, the application to soft tissues surgery can contribute to lessen the lack of PNB retrieved in literature and experienced in the clinical practice.
Locoregional anaesthesia could lead not only to a reduction in systemic drug supplementation, but also to perform opioid-free surgeries. Opioid-free anaesthesia is a topic of recent acquisition in human medicine, while the debate is at a very early stage in veterinary medicine. Clinical results of the TECA-LBO block described could contribute to this debate, particularly in species sensitive to opioid-related side effects such as rabbits.

Despite ultrasound-guidance in block performance has the characteristics to be set as a gold standard in veterinary anaesthesia, studies performed and reported suggest that neurostimulation still plays an important role. Nonetheless, as the debate of the superiority of US-guidance over nerve stimulation is still open even in human medicine, further retrospective multicentre studies should be performed in veterinary medicine as well.
Study, training and practice appeared fundamental, being locoregional anaesthesia strongly skill- and knowledge-related. Block performance should in fact being considered both a result of manual ability and knowledge, as the anaesthetist is required to have a precise knowledge of anatomy and anatomophysiology. Furthermore, ultrasonography requires additional knowledge of sonoanatomy and eye-to-hand coordination.

Future perspectives include further assessment of the analgesic effect of US-guided intrafunicular spermatic cord block in dogs, increasing the sample size. Further evaluation of TECA-LBO and PECA-LBO blocks in rabbits on a wider sample size will be performed, focusing in particular on the role of the trigeminus in the innervation of the rabbit ear. A second cadaveric study of cervical plexus block in dogs is planned to define a dosage based on body weight of local anaesthetic, after which clinical assessment on surgeries of the region of the neck will be carried out. Locoregional anaesthesia applied to painful medical conditions in order to implement pain management appears a field to explore that could modify the traditional consideration of PNB as only surgical-related.

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