UNIVERSITE DE LIEGE
FACULTE DE MEDECINE VETERINAIRE
DEPARTEMENT DES SCIENCES CLINIQUES
SERVICE D'ANESTHESIOLOGIE

Raffinement des méthodes d'anesthésie locorégionale échoguidée du membre postérieur chez le chien

Refinement of ultrasound-guided locoregional anaesthesia techniques of the pelvic limb in dogs

Vincent Danny Marolf

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Abbreviations

C	Control, study 1
CON	Control group, study 4
DEX	Dexmedetomidine
DEX0PN	Control group receiving ropivacaine combined to saline solution, study 3
DEX1PN	Group receiving 1 µg/kg of perineural DEX combined to ropivacaine, study 3
DEX2PN	Group receiving 2 µg/kg of perineural DEX combined to ropivacaine, study 3
DEX1IV	Group receiving 1 µg/kg of intravenous DEX combined to ropivacaine, study 3
E	Epidural group, study 2
F	Femoral nerve block group, study 2
L	Levobupivacaine, study 1
L4	Fourth Lumbar spinal nerve
L6	Sixth Lumbar spinal nerve
L7	Seventh Lumbar spinal nerve
LAA	Local Anaesthetic Agent(s)
RCT	Randomised Controlled Trial
S	Sciatic nerve block group, study 2
S2	Second Sacral spinal nerve
SF	Combined Sciatic and Femoral nerve block group, study 2
TPLO	Tibial Plateau Levelling Osteotomy
US	Ultrasound
VAS	Visual Analogue Scale

Résumé - Summary

Résumé

L'anesthésie locorégionale est efficace pour réduire la douleur peropératoire lors de la chirurgie des membres pelviens chez le chien. Il existe différentes techniques d'anesthésie locorégionale telles que l'anesthésie épidurale ou l'injection périneurale d'anesthésiques locaux à proximité des nerfs périphériques. Parmi ces nerfs, les nerfs fémoraux et sciatiques sont des endroits stratégiques car ils innervent la majeure partie du membre pelvien chez le chien.

L'approche parasacrale est une technique qui peut être utilisée pour bloquer le nerf sciatique à un endroit proximal sur le membre pelvien du chien. L'approche parasacrale guidée par stimulateur nerveux est connue et décrite (Portela *et al.* 2010), mais elle ne permet pas de visualiser les structures anatomiques telles que le nerf lui-même lors d'une anesthésie locorégionale. Il s'agit d'une technique moins fiable que la technique échoguidée. Celle-ci est également connue et décrite, mais le taux de réussite n'est que de 67 % (Shilo *et al.* 2010). L'objectif de la première étude était donc de modifier l'approche parasacrale échoguidée du nerf sciatique afin d'augmenter son taux de réussite. Une vue transverse du nerf combinée à une projection de l'aiguille dans le plan et l'injection d'un volume de 0,2 mL/kg de lévobupivacaïne 0,5 % ont permis d'augmenter le taux de réussite à 86 % chez des chiens de la race beagle.

Les techniques échoguidées des nerfs périphériques sont décrites principalement sur des cadavres ou de façon expérimentale. Peu d'études évaluent l'efficacité clinique de l'anesthésie locorégionale chez le chien. Une façon courante d'évaluer le taux de réussite de l'anesthésie locorégionale en pratique est de comptabiliser la quantité d'opioïdes nécessaire pour traiter les douleurs. Ceci est pertinent car l'administration d'opioïdes chez les chiens peut induire des effets secondaires tels que des vomissements ou une diminution de la prise alimentaire postopératoire (Bini *et al.* 2018). Comme alternative à l'anesthésie épidurale, l'approche mi-fémorale du nerf sciatique et l'approche inguinale du nerf fémoral (Campoy *et al.* 2010) peuvent être appliquées pour effectuer une anesthésie locorégionale chez les chiens qui subissent une ostéotomie de nivellement du plateau tibial. Dans la seconde étude, nous avons comparé l'efficacité de différentes techniques d'anesthésie locorégionale pour réduire la consommation peropératoire d'opioïdes dans un modèle clinique de chiens subissant une chirurgie de nivellement du plateau tibial. Les techniques des blocs échoguidés combinés du nerf sciatique et fémoral et la technique d'anesthésie épidurale ont toutes deux réduites les besoins peropératoires totaux en opioïdes en comparaison au bloc de nerf fémoral ou sciatique réalisés seuls.

L'injection périneurale d'anesthésiques locaux interrompt temporairement la conduction nerveuse. Grâce à ce mécanisme, la chirurgie peut être effectuée sans que le patient ne perçoive de douleur, car les signaux nerveux ne sont plus transmis. Les blocs nerveux périphériques effectués pendant la période

préopératoire fournissent, dans une certaine mesure, une analgésie postopératoire. Malheureusement, la durée de l'analgésie postopératoire est déterminée par la durée du bloc nerveux sensoriel, qui est lui-même déterminé par la durée d'action de l'anesthésique local utilisé. Ces effets bénéfiques sont de courte durée, même en cas d'utilisation d'anesthésiques locaux à longue durée d'action. En médecine humaine, il a été prouvé que l'ajout de dexmédétomidine, un puissant alpha-2 agoniste, aux agents anesthésiques locaux prolonge la durée du bloc nerveux sensoriel (Vorobeichik *et al.* 2017). Dans la troisième étude, nous avons donc évalué le potentiel de la dexmédétomidine comme adjuvant à la ropivacaïne pour prolonger les blocs nerveux périphériques sensoriels chez des chiens de race beagle. Nous avons identifié que l'injection périneurale de 1 µg/kg de dexmédétomidine était efficace. Dans un deuxième temps, l'efficacité de la dexmédétomidine en vue de réduire les besoins postopératoires en méthadone a été évaluée dans un cadre clinique. Dans la quatrième étude, nous avons prouvé que l'injection périneurale de dexmédétomidine combinée à la ropivacaïne lors d'une anesthésie locorégionale échoguidée réduisait le nombre de doses postopératoires de méthadone nécessaires pour contrôler la douleur chez les chiens pendant les 24 premières heures postopératoires.

Le perfectionnement des techniques échoguidées et leur application pour les blocs nerveux périphériques dans un contexte clinique améliorent le confort peropératoire des chiens qui subissent une chirurgie élective du membre pelvien. La combinaison de dexmédétomidine et de ropivacaïne semble également bénéfique aux chiens subissant une ostéotomie de nivellement du plateau tibial, possiblement induit par une prolongation de la durée du bloc nerveux sensoriel.

Summary

Locoregional anaesthesia is effective in reducing perioperative pain during orthopedic surgery. There are different techniques of locoregional anaesthesia available such as injection of local anaesthetic agents in the epidural space or to specific target nerves for pelvic limb surgery in dogs. Among these nerves, the femoral and sciatic nerves are the most important anatomical locations because they innervate most of the dog's pelvic limb.

The parasacral approach can be applied to block the sciatic nerve at a proximal location on the dog's pelvic limb. The parasacral approach guided by electrical nerve stimulator is known and described (Portela *et al.* 2010) but electrical nerve stimulator does not allow visualisation of anatomical structures and the nerve itself during locoregional anaesthesia. It is a less reliable technique compared to the ultrasound-guided technique. That approach is known and described, but its success rate is only 67% (Shilo *et al.* 2010). The objective of study one was therefore to modify the ultrasound-guided parasacral approach to the sciatic nerve in order to increase its success rate. A transverse view of the nerve with an in-plane needle approach and the injection of a volume of 0.2 mL/kg of levobupivacaine 0.5% increased the success rate to 86% in Beagle dogs.

Many ultrasound-guided peripheral nerve block techniques are described in cadavers or experimental dogs. Only a few studies evaluate the clinical efficacy of locoregional anaesthesia in dogs. A common way to evaluate the success rate of locoregional anaesthesia in clinical practice is to assess the amount of opioids required to control nociception and postoperative pain. This is of particular interest as the administration of opioids in dogs might induce side effects such as vomiting or decreased postoperative food intake (Bini *et al.* 2018). As alternatives to epidural anaesthesia, the ultrasound-guided mid-femoral approach to the sciatic nerve and the inguinal approach to the femoral nerve (Campoy *et al.* 2010) can be applied to perform locoregional anaesthesia in dogs undergoing tibial plateau levelling osteotomy. In study two, we compared the efficacy of different locoregional anesthesia techniques in reducing perioperative consumption of opioids in a clinical model of dogs undergoing elective invasive stifle surgery. The combined ultrasound-guided sciatic and femoral nerve block and the epidural anaesthesia technique both decreased the total perioperative opioid requirements compared to single ultrasound-guided femoral or sciatic nerve block.

The perineural injection of local anaesthetic agents will temporarily interrupt nerve conduction. Through this mechanism, surgery can be carried out without the patient perceiving pain because nerve transmission signals are no longer active. Peripheral nerve blocks performed during the preoperative period provide postoperative analgesia to some extent. Unfortunately, the duration of postoperative

analgesia is determined by the duration of sensory nerve block, which in turn is determined by the duration of action of the local anaesthetic agent. Postoperative analgesia is short-lived even when using long-lasting local anaesthetic agents. In human medicine, it has been proven that the addition of the potent alpha-2 agonist dexmedetomidine to local anaesthetic agents significantly prolongs sensory nerve block duration (Vorobeichik *et al.* 2017). In study three, we evaluated the potential of dexmedetomidine as adjuvant to ropivacaine to prolong sensory peripheral nerve block in Beagle dogs. We identified that the perineural injection of 1 µg/kg of dexmedetomidine was effective. As a next step, the efficacy of dexmedetomidine to reduce postoperative requirements of methadone was evaluated in a clinical setting. In study four, we proved that perineural dexmedetomidine and ropivacaine combined to ultrasound-guided locoregional anaesthesia reduced the number of postoperative doses of methadone required to control pain in dogs during the first 24 postoperative hours.

The refinement of ultrasound-guided techniques and their application for peripheral nerve blocks in the clinical setting improve the perioperative comfort of dogs undergoing elective pelvic limb surgery. The combination of dexmedetomidine with ropivacaine might also benefit dogs undergoing tibial plateau levelling osteotomy, possibly through prolonged sensory nerve blocks.

Introduction

1. Locoregional anaesthesia

Locoregional anaesthesia is nowadays a cornerstone of a balanced anaesthetic protocol. Locoregional anaesthesia consists in the perineural administration of local anaesthetic agents (LAA). The LAA will diffuse through the nerve sheaths to produce a reversible blockade of the action potentials formation and the nerve transmission. This will desensitize the specific body area innervated by the target nerve. This allows to perform invasive surgery without pain or nociception perception. The incorporation of locoregional anaesthesia techniques to the anaesthetic protocol improves patient comfort. A locoregional anaesthesia should be considered and performed whenever it is possible to contribute to the reduction of pain perception or when painful surgical or interventional procedures are planned. The pelvic limb is innervated by the lumbosacral plexus, which main components are the femoral and sciatic nerves. Theses nerves are often the target of locoregional anaesthetics agents whenever pelvic limb surgery is performed.

1.1 Locoregional anaesthesia in human medicine over time

Locoregional anaesthesia is performed daily in clinical practice since decades. Locoregional techniques are in constant evolution. Regarding the history of locoregional analgesia, there is no clear consensus about "the discovery of locoregional anaesthesia" (Deschner *et al.* 2007). A progressive discovery of different techniques and agents would best describe the evolution of locoregional anaesthesia. However, some key dates and names should be mentioned. Nerve compression were already performed centuries ago in 1564 by Paré. He reported the possibility to "provide" locoregional anaesthesia for the first time. James Young Simpson published in 1848 the first reports that locoregional anaesthesia might be superior to general anaesthesia in terms of pain management and safety (Deschner *et al.* 2007). Sigmund Freud and Karl Köller are also considered as fathers of regional anaesthesia. They introduced and studied cocaine as the first LAA. Karl Köller demonstrated the ability to perform ophthalmic surgery under local treatment with cocaine in 1884 (Goerig *et al.* 2012).

Several techniques are nowadays available to perform locoregional anaesthesia at the pelvic limb and promote pain free surgery and a great patient satisfaction. Lumbar plexus, sciatic and femoral nerve blocks are examples of target nerves where the anaesthesiologist aims to deposit LAA nearby the nerve prior to surgery. A specific target nerve is usually preferred as it will provide analgesia to the entire anatomical area innervated by the nerve. Reviews and meta-analysis have proven that the analgesia provided by combined sciatic and femoral nerve blocks is superior to administration of a sciatic nerve block combined with a local infiltration or femoral nerve block combined with a local infiltration in human patients undergoing total knee arthroplasty (Ma *et al.* 2017, Zhang *et al.* 2017). Blockade of a nerve innervating a specific target area is superior to infiltration of the surgical site with LAA. Human medicine aims to develop new techniques of locoregional anaesthesia.

1.2 History of locoregional anaesthesia in veterinary medicine

Locoregional anaesthesia in veterinary medicine has already been described in the first veterinary anaesthesia book written by Hobday in 1915. The different types of LAA available at that time and the practice of spinal anaesthesia are described with many details (Hobday 1915). This illustrates that the benefits of locoregional anaesthesia in veterinary medicine have been identified very early in the course of veterinary anaesthesiology. Things have evolved since that time and techniques and equipment available to perform locoregional anaesthesia are becoming more and more sophisticated.

1.3 The role of locoregional anaesthesia in pain management

The definition of pain has been described by the International Association for the Study of Pain in 1979. The definition reads as follow: "*pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage*". Recently, the International Association for the Study of pain and experts in pain medicine proposed a revised definition: Pain is *"an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage"* (Raja *et al.* 2020). There are absolutely no doubts that mammals, due to the evolved nature of their nervous system, and consequently dogs, are capable of feeling pain. It should be the aim of any veterinary anaesthetist to treat this condition with an adequate analgesic plan. The International Association for the Study of Pain have added six key notes to the revised definition. One of them is of particular interest to veterinary medicine: "*Verbal description is only one of several behaviors to express pain; inability to communicate does not negate the possibility that a human or a nonhuman animal experiences pain".* A difference should still be noted between the term pain and nociception. Pain include an emotional component according to the definition. In humans, many operational procedures to the pelvic limb can be performed under locoregional analgesia only. The practice of locoregional anaesthesia is often combined with general anaesthesia in dogs. This is justified by the inability of dogs to voluntary stay motionless for a defined period of time while the operation is been completed. Under these circumstances, we speak about nociception rather than pain because the emotional component of pain is not applicable while an animal is unconscious during general anaesthesia. The practice of locoregional anaesthesia to the pelvic limb of dogs will provide effective analgesia while surgery is performed under general anaesthesia. Local anaesthetic agents are the only drug class capable of providing complete analgesia (Epstein *et al.* 2015). Consequently, locoregional analgesia is the analgesic regimen of choice for surgical procedures in dogs. It should be applied to every patient whenever possible.

2. Anatomy: Innervation of the pelvic limb in the dog

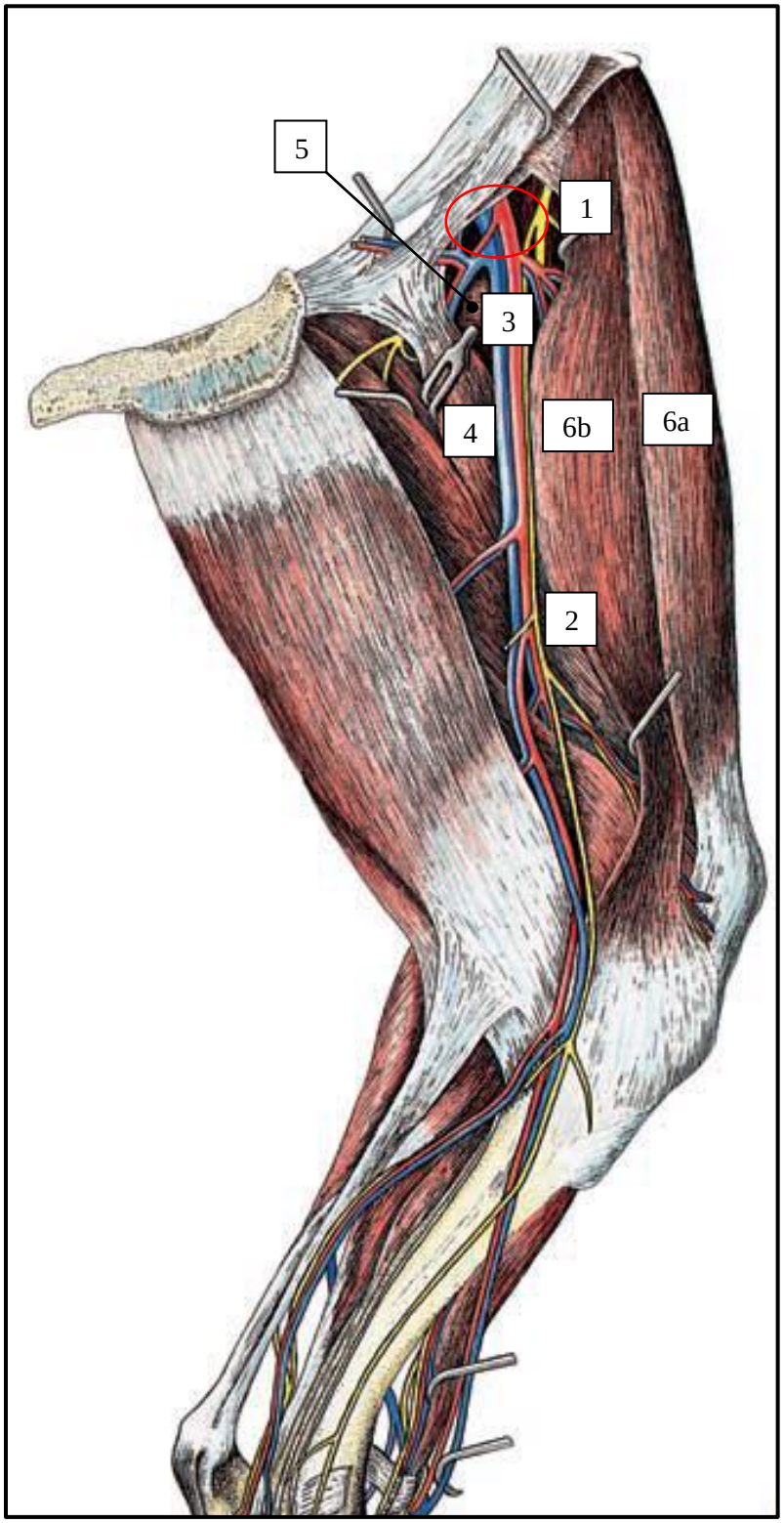
Before performing locoregional anaesthesia at the pelvic limb of the dog, a detailed revision of the innervation is necessary. The innervation of the pelvic limb of dogs originates from fourth lumbar spinal nerve (L4) up to the second sacral spinal nerve (S2). Some individual variations are possible (Dyce *et*

al. 1997, Kitchell 2013). The dorsal and ventral branches of the spinal nerves join to form the lumbar and sacral plexus. They send nerve fibres to various anatomical regions of the pelvic limb. The two main nerves, which are the main targets for locoregional anaesthesia, are the femoral and the sciatic nerve. Other main nerves arising from the lumbosacral plexus and innervating proximal parts of the pelvic limb of the dog include the cranial and caudal gluteal nerves and the obturator nerve. Those nerves will provide innervation of the *gluteal* muscles as well as *adductor* muscles. The caudal gluteal nerve will additionally provide innervation to the cranial portion of the *biceps femoris* muscle as well as the proximal part of the *semimembranosus* and *semitendinosus* muscles (Dyce *et al.* 1997, Kitchell 2013). These nerves might be relevant whenever surgery is performed in the region of the hip in dogs.

2.1. Femoral nerve

The femoral nerve is an important target nerve to provide locoregional anaesthesia to the pelvic limb. The femoral nerve originates from L4 to L6. It courses through the *psoas* muscles, parallel to the external iliac artery, before entering the *lacuna vasorum* (Dyce *et al.* 1997, Kitchell 2013). The femoral nerve provides innervation to the *quadriceps* musculature which allows the dog to stabilise the knee. In the inguinal region, the femoral nerve runs together with the femoral artery and vein within the femoral triangle. This location is important for US-guided locoregional anaesthesia as the nerve runs superficially and is easily accessible (Campoy *et al.* 2010). At the proximal aspect of the femur, the nerve is located between the *sartorius* and *pectineus* muscles and then continues as the saphenous nerve. It is important to note that the saphenous nerve is a pure sensory nerve, except for the *sartorius* muscle to which it provides motor fibres (Dyce *et al.* 1997). The figure (Figure 1) illustrates the course of the femoral and saphenous nerve.

Figure 1.

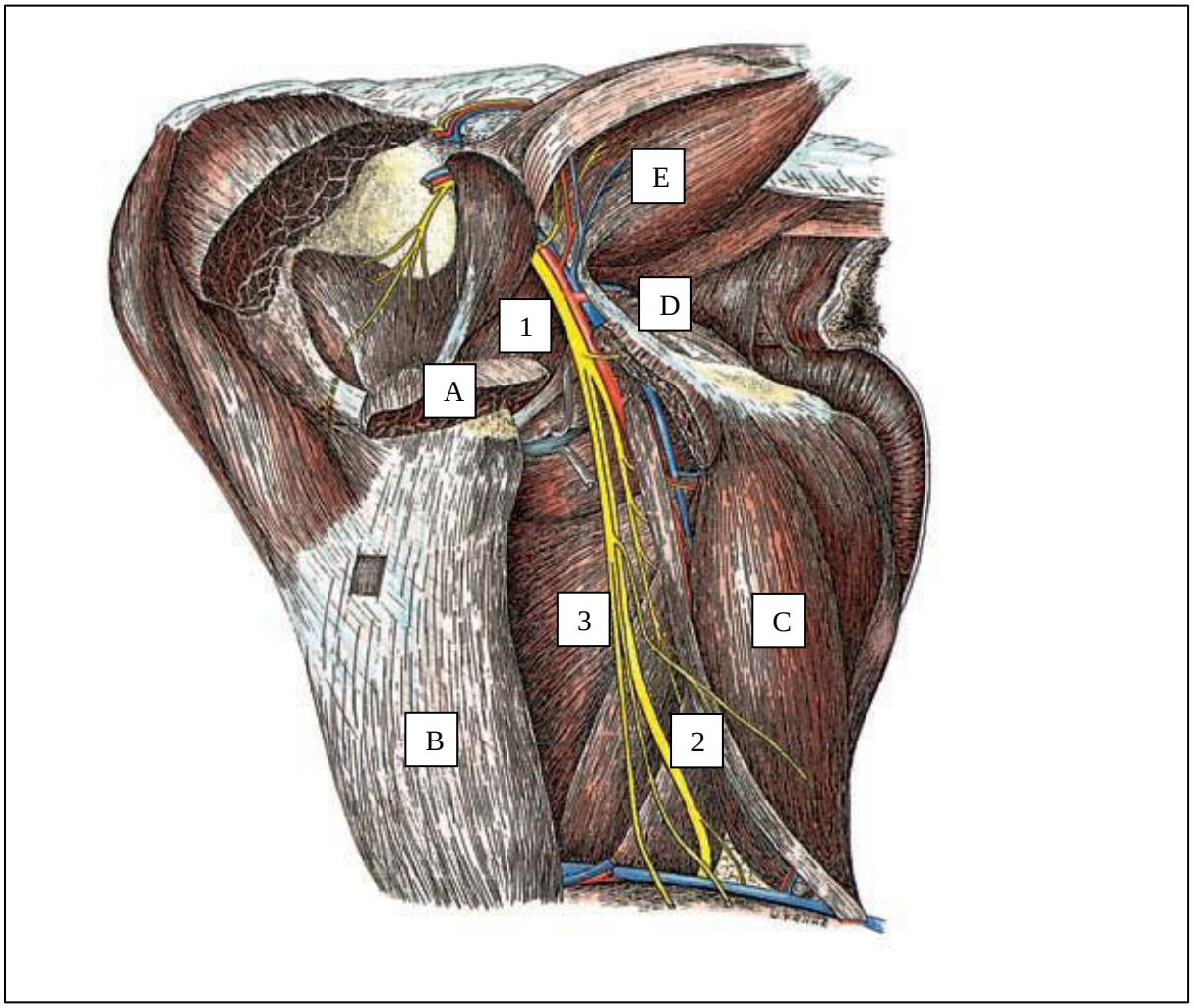


Medial view of the left pelvic limb of the dog illustrate the course of the femoral nerve (1). The femoral nerve continues distally as saphenous nerve (2). The nerves are illustrated in yellow. Femoral artery and vein (3), *lacuna vasorum* (red circled structure), *pectineus* muscle (4), *vastus medialis* of the *quadriceps* muscle (5), cranial (6a) and caudal (6b) part of the *sartorius* muscle (Budras *et al.* 2007).

2.2 Sciatic nerve

The sciatic nerve is the largest peripheral nerve of the dog. It arises mainly from L6 to the first sacral spinal nerve with occasionally nerve fibres coming from S2 (Kitchell 2013). This nerve represents an important target for peripheral nerve block as it provides sensory and motor fibres to the main pelvic musculature such as the *quadratus femoris*, the *obturator internus* or the *gemelli* muscles (Dyce *et al.* 1997). This nerve runs on the pelvis before crossing over the *incisura ischiadica major* and continues between the *gluteus profundus* and *medius* in caudal direction. It is also reported that the sciatic nerve might send nerve branches to the hip joint (Huang *et al.* 2013). It continues caudal to the major trochanter before running distally between the *biceps femoris* and *semitendinosus* muscles. In that portion, the nerve divides into the tibial and common fibular nerves. The exact anatomical location where the sciatic nerve divides into tibial and common fibular nerve strongly varies from one dog to another. The tibial nerve then passes between the two bellies of the *gastrocnemius* muscle and finally provides innervation to *tarsal extensor* muscles. The common fibular nerve runs laterally to the knee joint before dividing into a superficial and profound branch. Both branches run cranially over the distal portion of the tibial bone. They send nerve fibres to the *tarsal flexor* muscles (Dyce *et al.* 1997). The following figure (Figure 2) illustrates the course of the sciatic nerve of the dog at the level of the hip.

Figure 2.



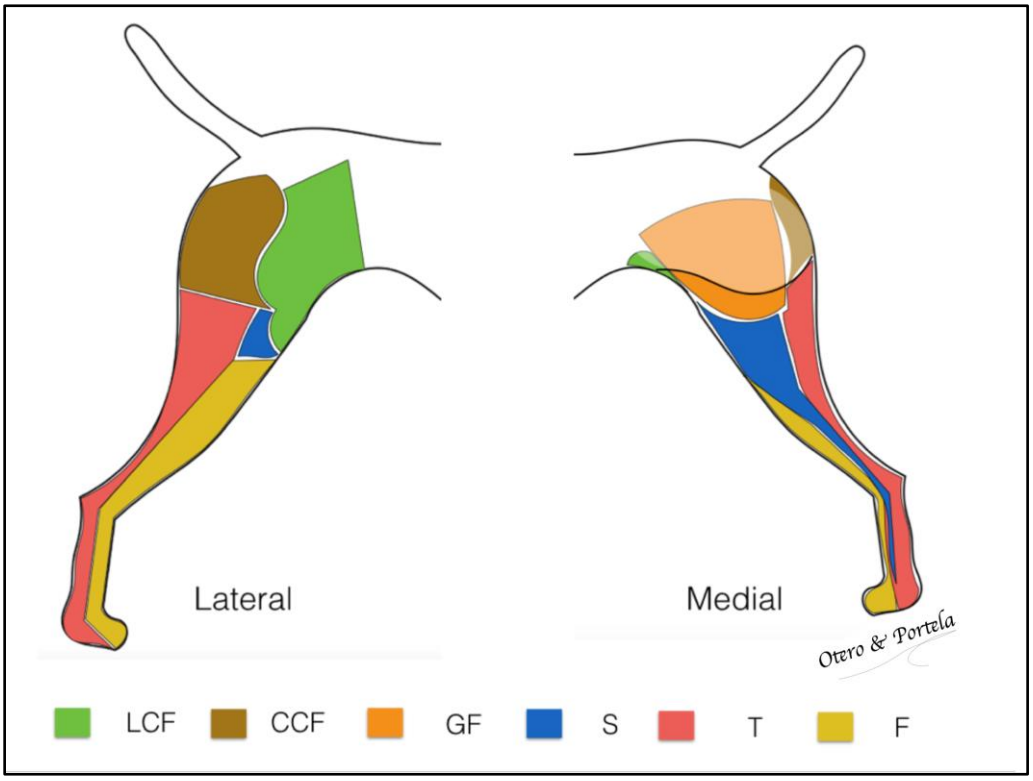
Lateral view of the anatomy of the of the left hip of the dog to illustrate the course of the sciatic nerve (1) which then divides into tibial (2) and common fibular nerve (3). The nerves are illustrated in yellow. *Gluteus medius* muscle (A), *vastus lateralis* of the *quadriceps femoris* muscle (B), *semimembranosus* muscle (C), *sacrotuberale* ligament (D). The *biceps femoris* muscle (E) has been detached from its attachment point to illustrate the sciatic nerve (Budras *et al.* 2007).

2.3 Innervation of the skin

The skin of the proximal part of the pelvic limb of the dog is innervated laterally by the cutaneous femoral nerves. The caudal part is innervated by the caudal cutaneous femoral nerve and the cranial part by the lateral cutaneous femoral nerve. The medial part of the proximal part of the pelvic limb is innervated by the genitofemoral nerve (Portela *et al.* 2019). The skin of the distal portion of the pelvic limb are innervated by different branches of the nerves described previously. The sciatic nerve innervates the cutaneous zone of the caudal thigh. The saphenous nerve is responsible of the innervation of the skin on the medial aspect of the knee joint. The fibular nerve innervates the dorsolateral aspect of the pelvic limb while the tibial nerve innervates the cutaneous zone (Levine *et al.* 2007). A thorough

knowledge of the different dermatomes will help the clinician to evaluate nerve blockade after locoregional anaesthesia. Skin sensation on the different parts of the pelvic limb can be evaluated by skin clamping and reaction in the awake dog after locoregional anaesthesia of a specific target nerve. Figure 3 illustrates the different dermatomes of the pelvic limb in the dog.

Figure 3.

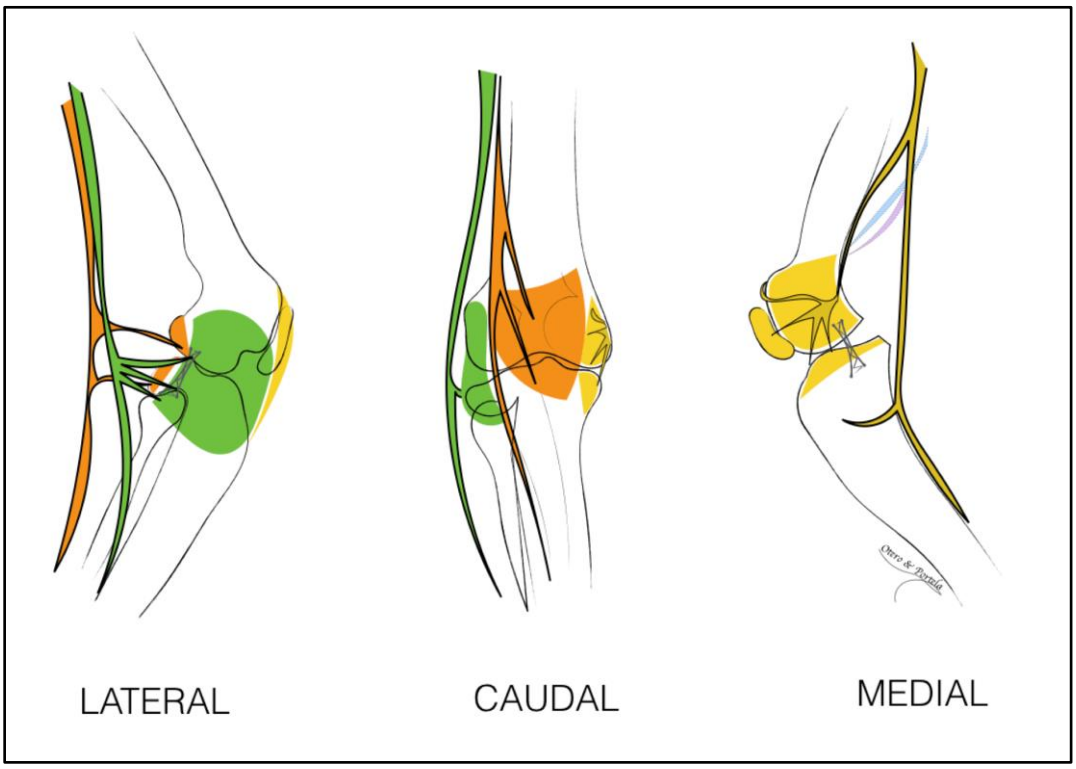


The cutaneous zones of the pelvic limb of the dog are innervated by the lateral cutaneous femoral nerve (LCF), the caudal cutaneous femoral nerve (CCF), the genitofemoral nerve (GF), the saphenous nerve (S), the tibial nerve (T) and the fibular nerve (F) (used with permission, Portela *et al.* 2019).

2.4 Innervation of the knee joint

The canine knee joint is innervated by the medial, the posterior and the lateral articular nerves. The medial articular nerve is a branch of the saphenous nerve which originates from the femoral nerve. In some dogs, the medial articular nerve might receive additional separated muscular branches of the femoral or obturator nerve (O'Connor & Woodburry 1982). The posterior articular nerve might not be present in all dogs. When present, it will provide fibres to the posterior aspect of the joint capsule. The posterior articular nerve is a branch of the tibial nerve (O'Connor & Woodburry 1982). The lateral articular nerve is a branch of the common fibular nerve. It divides into one to six branches to innervate the superior tibiofibular joint, and/or the lateral collateral ligament, and/or the lateral or posterolateral joint capsule (O'Connor & Woodburry 1982). Figure 4 shows the innervation of the canine knee joint

Figure 4



The medial articular nerve (yellow), the lateral articular nerve (green) and branches of the peroneal nerve (orange) contribute to the innervation of the canine knee joint. Some dogs might receive additional muscular branches from the femoral (blue) or the obturator (purple) nerve (used with permission, Portela *et al.* 2019).

3. Techniques for locoregional anaesthesia of the pelvic limb in dogs

Locoregional anaesthesia has evolved considerably and new techniques have brought about major improvements. Different techniques can be used to allow the LAA injection as close to the target nerve as possible. These different techniques for locoregional anaesthesia include the "blind" technique, the use of electrical nerve stimulator, or the ultrasound (US)-guided technique.

3.1. "Blind" technique and epidural anaesthesia

The definition "blind" technique can be misleading. The operator performing nerve blockade is not literally "blind". Nerve blocks towards specific nerves are performed based on surface anatomical landmarks. The epidural injection of LAA illustrates a "blind" locoregional anaesthetic technique. The injection of LAA into the epidural space is commonly performed based on palpation of the spinal process of the seventh lumbar vertebra (L7) and the wings of the ilium. Correct needle placement can be verified using simple methods such as the loss of resistance or the hanging drop technique (Adami & Gendron 2017). Epidural anaesthesia is simple and practical to perform and satisfying success rate is reported (Sarotti *et al.* 2015). Unfortunately, epidural anaesthesia induces paralysis of both pelvic limbs, which

can affect early postoperative movements in dogs. This type of procedure should not be performed in patients suffering from hypovolemia, sepsis, shock, coagulopathies, infection at the injection site or pelvic or sacral fractures. This limits the number of patients, who might benefit from epidural anaesthesia. Other "blind" techniques for locoregional anaesthetic technique of the pelvic limbs in dogs have been evaluated. Blockade of the saphenous, obturator and lateral cutaneous femoral nerves might be an effective and inexpensive method (Echeverry-Bonilla *et al.* 2017). This technique has only been evaluated in cadavers and its application to clinical cases is limited. The great trochanter and the ischiatic tuberosity are very useful anatomical landmarks to localise the sciatic nerve, which runs nearby those bony structures. A technique for saphenous, tibial and common fibular nerve block seemed promising in dog cadavers (Rasmussen *et al.* 2006a) but appeared ineffective for clinical cases (Rasmussen *et al.* 2006b). This outlines the limitation of the "blind" techniques for clinical use. Nerve blocks performed with the "blind" technique are subjective because it remains impossible to certify that the injection of LAA has been performed close to the target nerve. Nerve damage or damaging of vital structures are more frequent. This technique typically requires a higher volume of LAA to compensate for the lack of precision of injection.

3.2. Electrical nerve stimulation technique

The electrical nerve stimulator is a very useful tool to estimate the location of nerves that contains motor fibres. The introduction of this device has brought considerable improvement in the field of locoregional anaesthesia. The electrical nerve stimulator generates an electrical current and depolarises the target nerve when applied close to it. This elicits a contraction of the muscles innervated by the target nerve. This allows a more accurate localisation of a nerve and LAA can be injected perineurally. The nerve stimulator can only be used if the target nerve contains both, sensory and motor fibres. The motor fibres will allow localisation of the nerve for perineural injection of LAA. After diffusion of LAA through the nerve sheaths, anaesthesia of the sensory fibres will induce analgesia for a certain period of time. An elicited motor response at ≤ 0.5 mA indicates that the tip of the needle is close enough to the target nerve and typically results in a successful nerve block and increases nerve block success compared to the traditional "blind" technique (Klein *et al.* 2012).

In humans, it has been shown that intraneural injection is observed in 66% of patients during sciatic nerve block performed under electrical nerve stimulation guidance (Sala Blanch *et al.* 2009). The absence of motor response does not exclude intraneural needle placement and nerve stimulation with low current might even increase the risk of intraneural needle placement (Robards *et al.* 2009). Intraneural injection is usually not associated with neurological complications (Sala Blanch *et al.* 2009) but nerve puncture commonly leads to inflammation and is best avoided (Wiesmann *et al.* 2017).

The peripheral nerve stimulator increases the chance for successful nerve block also in dogs (Mahler & Adogwa 2008) compared to the "blind" technique. Several studies have proven the efficacy of this technique for locoregional anaesthesia in canine patients (Caniglia *et al.* 2012, Palomba *et al.* 2020). A

study has shown that the absence of motor response at a current of ≤ 0.3mA could not rule out a contact between the needle and the epineurium (Portela *et al.* 2013a). This might lead to intraneural injection of LAA. The absence of motor response at low current during electrical nerve stimulation should not be interpreted as a safety feature to exclude intraneural injection in dogs.

3.3. Ultrasound-guided technique

The ultrasound is a non-invasive imaging technique to precisely localise anatomical structures. This medical imaging method is based on high frequency sound waves and their reflection on anatomical tissue. The ultrasound transducer contains crystals made of piezoelectric material. It serves as sound transmitter and receiver for medical imaging. The ultrasound provides a real-time dynamic picture of the scanned structures, which has revolutionised the discipline of locoregional anaesthesia. The introduction of the US-guided technique for peripheral nerve blocks into clinical practice enabled precise needle positioning in relation to the target nerve and the spread of LAA can be observed in real time. The importance of this tool has gained popularity in the veterinary field in the last decades. Further research identifying new approaches and the development of new techniques using the US for locoregional anaesthesia needs to be perform to contribute to the well-being of the animal during the perioperative period.

3.4. Comparison of ultrasound-guided and electrical nerve stimulation techniques

In human medicine, the nerve block success rate is lower with the electrical nerve stimulator compared to the US-guided technique (Abrahams *et al.* 2009). The same may be true in veterinary medicine. Shorter onset times and longer duration of action are reported for brachial plexus block guided by ultrasound compared to electrical nerve stimulator (Akasaka & Shimizu 2017). When combining the nerve stimulator with the US-guided technique in dogs, it is more important to assess the correct position of the needle with the ultrasound rather than trying to elicit a motor response (Portela *et al.* 2013a). This might increase the number of needle pass and lead to damages of anatomical structures. A study performed in cats suggested that femoral nerve block success was higher when performed with the US-guided technique compared to the electrical nerve stimulator-guided technique (Haro *et al.* 2016). The US-guided technique appears superior to the electrical nerve stimulator technique. It is important to refine US-guided nerve block approaches with a low reported success rate. The US-guided parasacral approach to the sciatic nerve has a low reported success rate (Shilo *et al.* 2010). This technique needs to be refined to increase its success rate in dogs.

4. Ultrasound-guided approaches to the femoral and sciatic nerves

The sciatic and femoral nerves can be visualised with the ultrasound along their course as they originate from the lumbosacral plexus, until their continuation as tibial and fibular or saphenous nerves, respectively. The ultrasonographic approach of the sciatic nerve along its course has been described and the usefulness of this technique has been outlined (Benigni *et al.* 2007). However, Benigni and co-

workers (2007) could not visualize the most cranial part of the lumbosacral trunk due to its position ventral to the sacroiliac joint but were able to visualize the origin of the sciatic nerve using a window caudal to the sacrum. On the ultrasound image, nerves can be visualised as rounded, triangular or elongated hypoechoic structures surrounded by hyperechoic borders (Benigni *et al.* 2017). The appearance of the nerve also depends on the scanned anatomical region. The nerve sometimes appears as a hyperechoic structure without hypoechoic centre (Fornage 1993). High resolution ultrasounds enable to distinguish the nerve as a "honeycomb-like" structure separated by hyperechoic septae which illustrate the presence of the epineurium (Ali *et al.* 2016). Vessels are important hypo- or anechoic anatomical structures observed on the ultrasound screen which need to be differentiated from the nerve. The dynamic pulsatile characteristic of arteries can be observed in real-time and is helpful to differentiate this structure from a nerve. Colour Doppler, if available, could help to distinguish both structures. Vessels are useful anatomical landmarks for ultrasound identification of nerves. This is particularly relevant for the femoral nerve as it is located close to arteries along its course in dogs (Campoy *et al.* 2010, Garcia-Pereira *et al.* 2018).

Different proximal US-guided approaches of nerves of the pelvic limb in dogs have been described. Approaches using solely nerve stimulator guidance for nerve blocks are not described.

4.1. Femoral nerve

4.1.1. Lumbar plexus block and femoral nerve block within the iliopsoas muscle

The femoral nerve is formed at the lumbar plexus. A lumbar plexus nerve block will consequently induce a sensory blockade of the area innervated by the femoral nerve. Three different US-guided approaches to the lumbar plexus have been compared in 29 dog cadavers of different breeds: a dorsal pre-iliac, a lateral paravertebral at mid-L6 and a lateral paravertebral at mid-L7 were evaluated (Graff *et al.* 2015). A volume of 0.1 mL/kg of iodine-based solution mixed with methylene blue was injected under US-guidance. Computer Tomography was performed to evaluate the spread of the solution around the nerves. The authors concluded that all three approaches were accurate and easy to perform. To ensure diffusion of injectate around both; the femoral nerve and the obturator nerve, the lateral paravertebral approach at mid-L7 should be preferred.

The femoral nerve can be located with the ultrasound as it passes through the iliopsoas muscle (Mahler 2012, Echeverry *et al.* 2012a, Mogicato *et al.* 2015). Those approaches are sometimes reported as psoas compartment block (Tayari *et al.* 2017, Portela *et al.* 2018). Some of these approaches are also reported as ventral suprainguinal approaches (Echeverry *et al.* 2012a, Echeverry *et al.* 2012b, Shimada et al. 2017). These different approaches all described an US-guided localisation of the femoral nerve as it passes within the iliopsoas muscle.

Preliminary results to approach the femoral nerve in the iliopsoas muscle in experimental dogs have been described (Mahler 2012). The study was conducted in three phases: first, computer tomography of the anatomical region was performed to identify the nerve and its roots; second, the femoral nerve was

successfully located with the ultrasound in 82% of dogs; third, the nerve block was performed in dogs scheduled for pelvic limb surgery.

Results by Mahler (2012) enabled to refine and describe the technique with more details in healthy beagle dogs and cats (Mogicato *et al.* 2015). The authors could visualise the nerve as a rounded hypoechoic structure surrounded by a hyperechoic rim. They also identified the external iliac artery as a rounded anechoic structure which was located at approximately 5.4-14.1 mm of the femoral nerve in Beagle dogs.

The ventral suprainguinal approaches also locate the nerves as it passes through the iliopsoas. Echeverry *et al.* (2012a) described and evaluated this technique in dogs' cadavers and experimental Beagle dogs. The authors concluded that their approach appeared efficient as an alternative approach to the traditional inguinal approach.

Echeverry *et al.* (2012b) evaluated the spread of three different volumes of dye of the ventral suprainguinal approach to the femoral nerve as it passes through the iliopsoas. The lowest volume of dye (0.2 mL/kg) injected in Mongrel dog's cadavers revealed that it was sufficient to stain the femoral and obturator nerves. This US-guided approach was a good alternative to the described techniques using the electrical nerve stimulator.

The obturator and femoral nerves could both be visualised with an US-guided approach as they are located in the psoas compartment (Tayari *et al.* 2017). This technique has first been tested is dogs' cadavers. This approach is particularly interesting because its efficacy has been evaluated in 20 dogs undergoing tibial plateau levelling osteotomy (TPLO). A volume of 0.1 mL/kg of ropivacaine 0.3% or 0.5% has been injected to perform femoral and obturator nerve block in clinical cases. A minimal constant rate infusion of fentanyl (0.0-2.2 µg/kg/hr) was required in both groups to control nociception. This outlines the efficacy of this technique.

Finally, a study evaluated the effects of two different volume of bupivacaine 0.5% using the ventral suprainguinal approach to the femoral nerve (Shimada *et al.* 2017). This technique has been evaluated in sedated beagle dogs. The two different volumes of bupivacaine 0.5% (0.2 *versus* 0.4 mL/kg) induced similar nerve block duration. The motor and sensory nerve block duration was approximately 10 hours in both groups.

4.1.2. Inguinal approach and adductor canal nerve block

The approach used for femoral nerve block varies between institutions and preference of the operator. The inguinal approach is reported is regularly applied for canine patients undergoing pelvic limb surgery (Campoy *et al.* 2012a, Campoy *et al.* 2012b, Bartel *et al.* 2016).

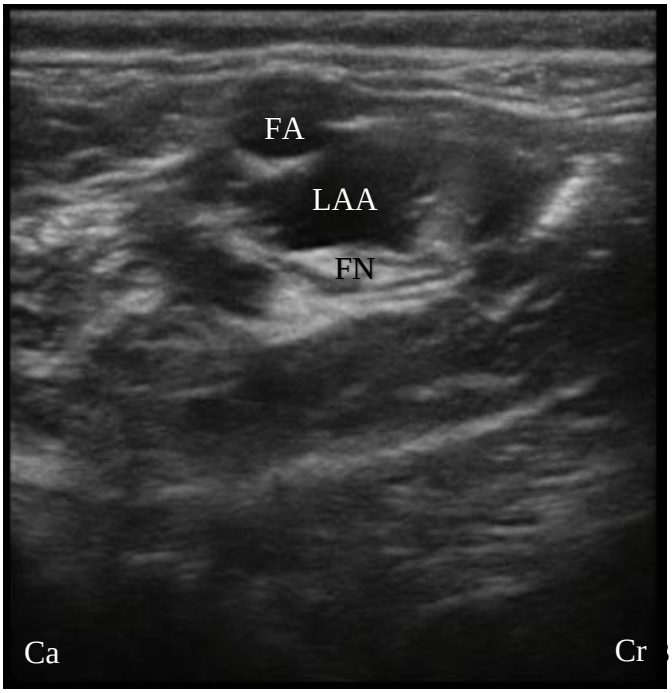
Campoy *et al.* (2010) could localise the femoral nerve using an inguinal approach. They could localise the femoral nerve in all dogs and performed a perineural injection of 0.15 mL/kg of methylene blue mixed with lidocaine. The dogs were positioned in lateral recumbency and the nerve block was performed on the uppermost limb elevated in an abducted position. They observed that the femoral

artery and vein were useful anatomical structure to identify the femoral nerve. The nerve was located caudal to the fascia of the rectus femoris muscle. The nerve structure is located superficially and it was easy to visualise and perform a perineural injection. Anatomical dissection revealed consistent staining of the nerve. Figure 4 illustrates an ultrasound image after perineural femoral nerve injection of LAA using the above-mentioned inguinal technique.

Echevery *et al.* (2010) used a similar approach but performed the femoral nerve block on the undermost pelvic limb with the dogs positioned in lateral recumbency. The study included anatomical dissection of pelvic limb cadavers, performance of the nerve block on cadavers and perineural injection of 0.3mL/kg of lidocaine 1% in sedated dogs. The US-guided identification of the femoral nerve was difficult in vitro and could not be identified in 50% of cases in vivo.

The femoral nerve courses distally as the saphenous nerve and passes within the adductor canal. An adductor canal nerve block technique has been evaluated under US-guidance in dog cadavers' (Castro *et al.* 2018). The US-transducer was placed in the longitudinal plane of the pectineus muscle. Methylene blue injection of 0.3 mL/kg within the adductor canal was obtained in 100% of cases but stained the saphenous nerve > 2cm only in 55% of cases. The success rate of this technique seems low and refinement of the technique and clinical efficacy need further investigations.

Figure 4.



Transverse Ultrasound image of LAA seen as an anechoic pocket of fluid next the femoral nerve (FN), seen as a thin hypoechoic structure surrounded by a hyperechoic rim. The femoral artery (FA) can be observed on the image as an anechoic structure. The femoral artery (FA) is an important anatomical structure to identify the femoral nerve as those two structures are located close to each other. Ca: caudal; Cr: Caudal.

4.2. Sciatic nerve

4.2.1. Proximal approaches to the sciatic nerve

The ultrasonographic approach of the sciatic nerve as it crosses the ilium and along its entire course of the pelvic limb in dogs has been described (Benigni *et al.* 2007). An US-guided parasacral approach of the sciatic nerve is reported (Shilo *et al.* 2010). They scanned the sciatic nerve in a longitudinal plane. They compared the efficacy of perineural injection of 0.03 mL/kg, 0.06 mL/kg or 0.13 mL/kg of bupivacaine 0.5% with perineural injection of saline solution. Unfortunately, the sciatic sensory nerve block after bupivacaine injection was only effective in four out of the six Hound dogs (67%) included in the study. This suggest that either the technique or the volume of LAA should be modified.

An alternative US-guided approach has been described as the nerve passes between the great trochanter and the ischiatic tuberosity (Costa-Farré *et al.* 2011). The nerve could be visualised using a transverse view as a hypoechoic structure surrounded by a hyperechoic rim caudal to the great trochanter and caudal to the ischiatic tuberosity. The caudal gluteal artery was observed caudal to the nerve. A perineural sciatic nerve injection of 0.1 mL/kg of lidocaine 2% was performed in five sedated dogs. The nerve block components were evaluated during three hours after LAA injection. Complete motor block

was only obtained in three dogs. The sensory nerve block was complete for the peroneal component of the sciatic nerve but was only partial for the tibial component of the sciatic nerve in all dogs. This technique can be used to perform US guidance of the sciatic nerve between the great trochanter and the ischiatic tuberosity but refinement of the technique is needed to obtain complete sensory nerve block.

4.2.2. Mid-femoral approach

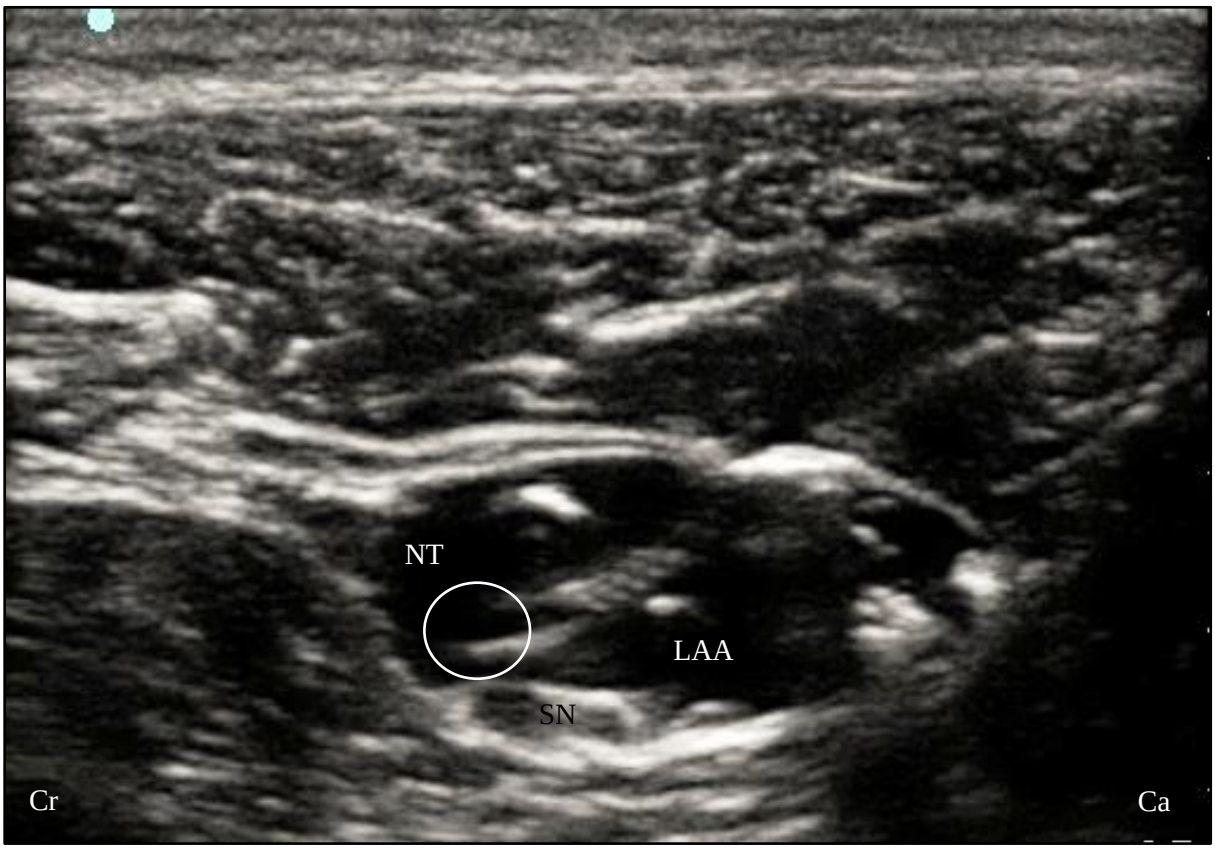
Descriptive studies using the US-guided technique to block the sciatic nerve frequently use a more distal approach. The nerve is localised distal to the ischiatic tuberosity at the level of the caudal thigh (Campoy *et al.* 2010; Echeverry *et al.* 2010).

Campoy *et al.* (2010) evaluated a mid-femoral US-guided approach to block the sciatic nerve in four experimental Beagle dogs. A volume of 0.05 mL/kg of lidocaine and methylene blue solution was injected perineurally. Dogs were euthanised and anatomical dissection was performed. Sciatic nerve staining was adequate in three dogs. The US image of the sciatic nerve was adequate. Muscles surrounding the sciatic nerve which can be visualised on the ultrasound include: the *biceps femoris muscle* (craniodorsal), the *abductor muscle* (cranioventral) and the *semimembranosus muscle* (caudal). This study was the first to describe an US-guided sciatic nerve block in dogs.

Echeverry *et al.* (2010) also described an US-guided sciatic nerve block using a mid-femoral acoustic window. They first performed anatomical dissection of eight pelvic limbs to localise the sciatic nerve precisely; second, they evaluated the ultrasonographic appearance of the sciatic nerve in another eight pelvic limbs; third, they performed US-guided sciatic nerve block in eight sedated dogs. The sciatic nerves were easily identified during the second phase of the study and nerve blocks were performed successfully during the third phase of the study. The practicability of the mid-femoral US-guided sciatic nerve block could be validated. The mid-femoral approach described by Campoy *et al.* (2010) and Echeverry *et al.* (2010) are now widely applied in clinical practice and are often referenced in clinical studies (Arnholz *et al.* 2017; Warrit *et al.* 2019a; Warrit *et al.* 2019b).

Those techniques, combined with a femoral nerve block, provide efficient analgesia distal to mid femur. They are particularly useful for elective knee surgery such as TPLO, tibial tuberosity advancement or the extracapsular repair technique, which are surgical procedures routinely performed in dogs. Figure 5 illustrates an US image using a mid-femoral approach for sciatic nerve blockade.

Figure 5.



Transverse ultrasound image of LAA seen as an anechoic pocket of fluid next to the sciatic nerve (SN). The nerve can be observed on the image as an elongated structure surrounded by a hyperechoic rim with two hypoechoic rounded structures in the middle. The circled structure represents the needle tip (NT). Cr: cranial, Ca: caudal.

5. Risks and benefits of locoregional anaesthesia

Every medical procedure is associated with its own risks. The right balance between the benefits and risks needs to be outweighed thoroughly. The benefits of locoregional anaesthesia outweigh its risks and it is therefore routinely performed in clinical practice every day. Locoregional anaesthesia has the potential to reduce perioperative mortality in human patients undergoing vascular surgery compared to general anaesthesia (Hajibandeh *et al.* 2018; Bennett *et al.* 2019). Locoregional anaesthesia performed under US-guidance might possibly contribute to reduce the risks of neurological complications after peripheral nerve block. This chapter reviews the benefits and risks of locoregional anaesthesia and focuses on the advantages and disadvantages of the US-guided technique.

5.1. Benefits of locoregional anaesthesia

The advantages of nerve blocks are numerous. The main benefit is without any doubt the pain control provided by the LAA as the nerve transmission is reversibly interrupted. This enables the reduction of pain in the postoperative phase as long as the LAA exert its effects. A Cochrane database review highlighted the evidence of better pain control in human patients undergoing elective hip replacement after peripheral nerve block or neuraxial anaesthesia compared to systemic analgesia (Guay *et al.* 2017). A femoral nerve block provides better pain control than patient-controlled analgesia for total knee replacement (Chan *et al.* 2014) and might also reduce the incidence of postoperative nausea and vomiting. The addition of locoregional anaesthesia to the multimodal anaesthetic regimen might also reduce the perioperative need of opioids.

In dogs, locoregional anaesthesia is usually combined with general anaesthesia as dogs will not stay still during surgery. Pelvic limb peripheral nerve blocks and epidural anaesthesia have shown to reduce the minimum alveolar concentration in dogs (Campoy *et al.* 2012a, Portela *et al.* 2013b, Romano *et al.* 2016). Lately, a "zero pain philosophy" website (https://www.zeropainphilosophy.com/) to "achieve analgesic excellence" in dogs has been created. Many information and support for veterinarians are available online. To achieve this goal, locoregional anaesthesia clearly needs to be provided in dogs before invasive surgeries.

5.1.1. Benefits of the ultrasound-guided technique

The benefits of the US-guided technique are real. This method enables precise localisation of neuronal structures and their adjacent anatomical structures, anatomical variation might be identified, the needle and the exact administration and spread of LAA can be observed in real-time (Marhofer 2010). Other benefits of the US-guided technique include: lower amount of needle pass, shorter time to perform the nerve block, shorter onset time of nerve blockade and a lower dose of LAA might be needed (Koscielniak-Nielsen 2008). The different anatomical structures such as blood vessels, nerves, muscles, bones and tendons can all be visualised and the risk of intraneural or intravascular injection is reduced with the use of US (Marhofer *et al.* 2005). Hematoma or iatrogenic puncture of a vessel might be reduced

when the US technique is used because direct visualisation of vessels is possible.All thoses advantages illustrate the usefulness of this technique for clincial practice.

5.2. Risks & complications of locoregional anaesthesia

Risks and complications of locoregional can be classified as major or minor complications. Under major complications we understand that consequences can be irreversible, catastrophic or have long-term consequences for the patient. Minor complications are usually self-limiting without long-term complications for the patients.

A publication has reported the incidence of major complications in France to be 3.5/10'000 in human patients. The list of complications was reported as follows: ‹‹ (1) Cardiac arrest requiring cardiac massage and/or epinephrine; (2) acute respiratory failure requiring tracheal intubation and/or assisted ventilation; (3) seizures; (4) peripheral nerve injury, defined as a sensory and/or motor deficit with clinical and/or electrophysiological abnormalities suggesting a peripheral site of injury and no evidence of spinal cord lesion; (5) cauda equina syndrome; (6) paraplegia; (7) cerebral complication; (8) meningeal syndrome; and (9) death ›› (Auroy *et al.* 2002). A review has analysed the rate of neuropathy after central (spinal or epidural) anaesthesia and after peripheral nerve block (Brull *et al.* 2007). The rate of reported neurological complication is 0.04% after central anaesthesia and 3% after peripheral nerve block. There was only one reported case of permanent neuropathy after peripheral nerve block in the studies analysed in this review. The risk of neurological complication after peripheral nerve blockade remains rare. Those complications were reported in humans and reports on the incidence of major complications in dogs are lacking.

Tingling or bruising sensations, infections or vascular trauma with subsequent hematoma formation are examples of minor complications without long-term consequences for the patient. An excessive duration of motor blockade (>24 hours) can sometimes be observed. This might happen when large volume and concentrations of LAA are used. In that case, self-injury is possible and regular monitoring of the dog is advised. This is best avoided by judicious use of appropriate volumes and concentration of LAA, especially in large dogs.

To minimise the risks of nerve trauma, it is recommended to use a needle with a short-bevelled tip. Needles with special shape will help to visualise the needle tip with precision to reduce the risk of traumatic puncture of the nerve (Schafhalter-Zoppoth *et al.* 2004). Severe nerve lesions usually happen when injection or puncture of the endoneurium occurs. Lesions of the epineurium are commonly mild and transient (Neal *et al.* 2015). Hypodermic sharp needles are best avoided to perform locoregional anaesthesia to avoid intraneural injection. The risk of infection can be minimised by using a strict aseptic technique. A nerve block should not be performed whenever signs of infection or pyoderma are observed after shaving of the dog's skin. Additionally, locoregional anaesthesia should not be performed in dogs suffering from thrombocytopenia or coagulopathies. The syringe should also be aspirated and the needle hub observed for signs of blood before LAA injection to avoid intravascular injection (Grubb &

Lobprise 2020). Applying the above-mentioned recommendations will help to reduce the risk of complications in locoregional anaesthesia

5.2.1. Risks and disadvantages of the US-guided technique

The risk of the ultrasound-guided technique depends on the operator performing for peripheral nerve blocks. Special skills and adequate knowledges of the anatomy are mandatory to guide a needle under direct visualisation to prevent iatrogenic damage. Unfortunately, there is a lack of large randomised clinical trial comparing the risk of complications of the US-guided technique compared with other techniques in human and veterinary medicine (Marhofer *et al.* 2010). The question about the increased margin of saftey with the US technique still creates a debate within the regional anesthesia world. A study found that patients still had neurological symptoms such as numbness or tingling ten days (8.2%), one month (3.7%) or 6 month (0.6%) after US- guided nerve blocks (Fredrickson *et al.* 2009). A recent review suggested that the US-guided technique in children seems to reduce the risk of failed block but does not or only minimally reduce the risk of minor complications (Guay *et al.* 2019). However, it seems evident that if the technique is used correctly, the margin of saftey might be increased. There is little evidence to draw strong conclusions in dogs. It is likely that results from human medicine might be extrapolated to veterinary medicine. Interestingly, a study in dogs concluded that costs of US-guided sciatic and femoral nerve block might be increased due to the equipment required but that the anesthesia costs related to pain management and complications migth be decreased (Warrit *et al.* 2019a). The cost of the initial investment for the purchase of the US equipment in a clinical practice might be a disadvantage. The learning curve of the operator for successful block is usually slow. The success of an US-guided nerve block greatly varies with the skills of the operator for a given technique (Marhofer *et al.* 2005). Those might also be considered as disadvantages of the technique.

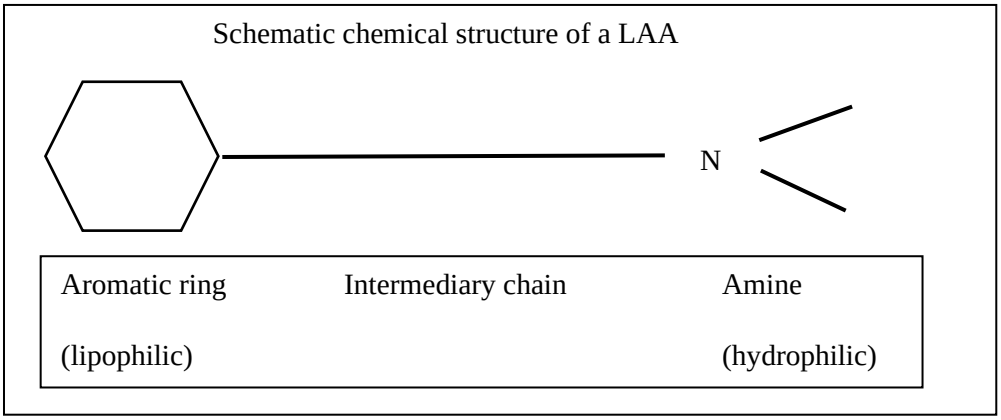
6. Local anaesthetic agents

Various types of LAA are available for clinical use. Commonly used LAA include lidocaine, ropivacaine or bupivacaine, among others. These LAA all vary by their duration of action, their potency and their onset. The duration of action of LAA is primarily determined by their protein affinity. The stronger the affinity, the longer the duration of action (Becker & Reed 2012). The duration of action is of particular interest. The longer the duration of action, the longer the duration of analgesia will be. Unfortunately, even when using long acting LAA such as ropivacaine, levobupivacaine or bupivacaine, the duration of action rarely exceeds 6 hours (Rioja Garcia 2015, Lemke & Dawson 2000). This duration of action is short and research should aim on developing strategies to prolong the analgesia provided by LAA. The combination of adjuvants to LAA might be a possible approach (see Chapter 7). The anaesthetic potency is dependent on the lipid solubility of the agent. This affects the diffusion through the nerve sheath. As an example, ropivacaine is more lipid soluble than lidocaine and therefore more potent than lidocaine. This explains that ropivacaine is usually commercialised as a 0.5% solution and lidocaine as a 2%

solution. The onset time of a LAA is also dependent of the lipid solubility of the LAA. Another important factor for the onset time is the ionisation constant (pKa) of the LAA. According to the Henderson-Hasselbach equation, the pKa of a LAA molecule will determine which proportion of the drug is in the ionised and the non-ionised form, which is determinant for penetration of the nerve tissue and is responsible for the onset time of the LAA. Local anaesthetic agents can be classified using different classification schemes. The classification by their duration of action is one of them. Local anaesthetic agents are also commonly classified by their chemical structure as ester-linked or amide-linked LAA.

6.1. Classification

LAA are formed by a lipophilic aromatic ring structure, an intermediary chain and a hydrophilic amino group at the end of the chain. The nature of the intermediary chain is determinant for their classification according to the chemical structure.



6.1.1. Ester-linked local anaesthetic agents

The intermediary chain of Ester-linked LAA is formed of an ester group (COO-CH2). They are Amino-ester LA. They are not commonly used in clinical practice because of their usual shorter duration of action. This is due to their rapid hydrolysation by plasma choline esterases. Cocaine, procaine, tetracaine or benzocaine are examples of amino-ester linked LAA.

6.1.2. Amide-linked local anaesthetic agents

The intermediary chain of Amide linked LAA is composed of an amide group (NH-CO-CH2). The LAA of this group are commonly used in clinical practice due to their chemical nature, which is suitable for clinical use. Lidocaine, ropivacaine or bupivacaine are examples of LAA of this group.

6.2. Mechanism of action

After being in contact and diffusion into nerve tissue, LAA will block nerve conduction by blocking the formation of action potentials. This means that the nerve signal transduction is unable to take place. This action is mediated by reversibly blocking the sodium (Na^+) voltage-gated channels. The ionised form of the LAA will bind to the sodium channel. This will prevent the receptor to function properly. No further action potential can be formed because the inactive state of the receptor prevents depolarisation. The

sodium channels are closed at resting membrane potential. Whenever a depolarisation occurs, they rapidly open to upstroke the action potential before closing again. Depolarisation is therefore essential to form an action potential (Becker & Reed 2012, Lagan & McClure 2004).

6.3. Examples of local anaesthetic agents

Ropivacaine is commonly used in veterinary clinical practice for peripheral nerve block. Levobupivacaine is used to a lesser extent compared to ropivacaine but is also regularly applied for locoregional anaesthesia in dogs. The profile of ropivacaine and levobupivacaine are briefly described as both LAA were used for perineural injections in this thesis.

6.3.1. Ropivacaine

Ropivacaine is an amino-amide LAA with a duration of action of up to 6 hours (Rioja Garcia 2015). The interesting pharmacokinetic profile of ropivacaine is that it is less lipophilic than other LAA, less than bupivacaine for example. Ropivacaine might less likely penetrate larger motor fibers than bupivacaine. This could possibly reduce motor blockade compared to sensory blockade. This might be of advantage when motor blockade needs to be reduced. This might promote early postoperative locomotion while pain sensation might still be absent. However, this is not always as straight forward when ropivacaine is used in clinical practice (Kuthiala & Chaudhary 2011). The profile of ropivacaine regarding its toxicity in case of inadvertent overdose or intravascular injection makes it a safe and suitable LAA for clinical use. Ropivacaine is less cardiotoxic and neurotoxic than bupivacaine (Kuthiala & Chaudhary 2011).

6.3.2. Levobupivacaine

Levobupivacaine has an interesting clinical and pharmacokinetic profile. Levobupivacaine is an enantiomer preparation and does not contain the R (+) isomer of the racemic mixture of bupivacaine. The R (+) isomer had been associated with central nervous system and cardiovascular complications after injections (Heppolette *et al.* 2020). Levobupivacine has therefore a larger therapeutic index than bupivacaine (Bajwa & Kaur 2013). Levobupivacaine conserves the advantages of bupivacaine such as a long duration of action up to 240-360 minutes (Lemke & Dawson 2000) and makes it one of the LAA of choice for clinical and experimental projects involving locoregional analgesia in dogs. Interestingly, a PRISMA meta-analysis in human medicine has recently concluded that levobupivacaine seems to be more potent than ropivacaine when used for peripheral nerve block (Li *et al.* 2017).

7. Adjuvants for locoregional anaesthesia

Local anaesthetic agents exert their function through their physico-chemical properties. Different agents have been combined with LAA to modify those properties in an attempt to prolong the duration of action or to hasten the onset time. Adrenaline is the most common adjuvant combined to LAA. Recently, the effects of dexamethasone and dexmedetomidine combined to LAA have been studied. Other agents such

as bicarbonate, opioids (e.g., buprenorphine) or midazolam can be combined to LAA but are not commonly used in a clinical setting for peripheral nerve blockade (Brummett & Williams 2011).

7.1. Adrenaline

Adrenaline has been combined to LAA since decades. The drug causes vasoconstriction and will slow the systemic reabsorption of the LAA. It will therefore prolong the duration of the nerve block. The recommended ratio of adrenaline to LAA for clinical use is 1:400'000 to 1:200'000 (Rioja Garcia 2015). Adrenaline is often combined to short acting LAA such as lidocaine. The action of adrenaline is not as effective when it is combined to LAA with a longer duration of action. Some authors therefore do not recommend its use with ropivacaine or bupivacaine for example (Brummett & Williams 2011). The prolonged duration is usually self-limited and the prolonged motor blockade is not superior to 60 minutes (Tschopp *et al.* 2018).

7.1.1. Complications with adrenaline

The combinations related to the use of adrenaline with LAA are not always harmless. Adrenaline is a potent catecholamine acting on adrenergic receptors and interact directly with the cardiovascular system. The use of adrenaline should be used with caution in patients at risk of arrhythmia or cardiac ischemia. A decreased blood flow to the spinal cord is possible when adrenaline is injected intrathecally. It should also be used with caution in patients prone to neurotoxicity as a consequence of decreased blood flow (Niemi 2005). Tissue necrosis at the injection site because of vasoconstriction should be considered (Hartzell *et al.* 2010).

7.2. Bicarbonate

Local anaesthetic agents can also be combined with sodium bicarbonate 8.4%. The rationale behind is to increase the pH of the solution to speed the onset time and duration of action of the LAA. The literature remains controversial on this topic (Rioja Garcia 2015). Bicarbonate will also decrease pain on injection as it is known that LAA injection might be painful especially if the solution is injected quickly. However, it seems to have little effect when combined to ropivacaine or bupivacaine. The combination of bicarbonate with bupivacaine might cause precipitation of the solution (Bourget *et al.* 1990). The solution should be inspected before perineural or epidural injection and bicarbonate should be used with caution.

7.3. Dexamethasone

Dexamethasone is a synthetic anti-inflammatory corticosteroid. It is commonly used to treat inflammatory pain (Zhou *et al.* 2018). Its efficiency as perineural adjuvant has been tested in several clinical trials in humans. A Cochrane review has proven that perineural dexamethasone combined with LAA can prolong sensory nerve block by six and a half hours compared to LAA alone in humans but the quality of evidence was low (Pehora *et al.* 2017). Dexamethasone is thought to negatively affect wound healing due to its effect on fibroblasts, collagenisation and epithelisation (Mahmut *et al.* 2003). In humans, "a single dose of dexamethasone probably does not increase the risk of postoperative

infection" (Polderman *et al.* 2018). This suggest that a single dose might probably be used safely for peripheral nerve blocks. A meta-analysis compared the clinical efficacy of dexmedetomidine and dexamethasone as adjuvants to LAA for supraclavicular brachial plexus block (Albrecht *et al.* 2019). Dexamethasone significantly prolonged the duration of analgesia by 2.5 hours compared to dexmedetomidine. The scientific evidence was low and research focussing on direct comparison of both drugs used as perineural adjuncts was recommended. The combination of ropivacaine and dexamethasone should be used with caution because the mixture of both solutions might cause precipitation (Watkins *et al.* 2015). The effects of perineural dexamethasone have not been studied in dogs to date.

7.4. Opioids

Opioids are very effective to control pain. They are often administered systemically by intramuscular or intravenous injection. This can induce systemic side effects such as panting, nausea, vomiting or bradycardia. Opioids such as morphine or buprenorphine can be combined with LAA and administered in the epidural space to provide effective pain control in dogs (Smith & Kwang-An Yu 2001, Bartel *et al.* 2016). The efficacy of perineural buprenorphine, fentanyl or morphine has been evaluated in different clinical trials in humans (Kirksey *et al.* 2015). Buprenorphine seems the most promising opioid to prolong sensory nerve block in humans. Sensory nerve block is prolonged by 6 hours after combination with bupivacaine compared to nerve block without buprenorphine (Candido *et al.* 2010). Morphine and fentanyl do not seem to prolong nerve block effectively (Kirksey *et al.* 2015). Systemic side effects such as postoperative nausea and vomiting are commonly reported when opioids are used as adjuvants (Kirksey *et al.* 2015). This might be due to the reabsorption of opioids after perineural injection.

7.5. Dexmedetomidine

Dexmedetomidine is one of the most promising adjuvants used for locoregional anaesthesia. Dexmedetomidine is a potent alpha 2 adrenoceptor agonist that exerts analgesic properties (Grosu & Lavand'homme 2015). It has been extensively studied in human medicine due to its potential to effectively prolong peripheral nerve blocks. The results of several clinical trials have been supporting the use of long-acting amide-type LAA combined with dexmedetomidine in clinical settings (Fritsch *et al.* 2014; Keplinger *et al.* 2015). It has been proven that perineural dexmedetomidine reduces postoperative pain, enhances patients' satisfaction and decreases postoperative oral morphine consumption (Vorobeichik *et al.* 2017). The amount of dexmedetomidine required to prolong locoregional anaesthesia in different studies varies. Doses of 100 µg per nerve block, 0.75 µg/kg and 2 µg/kg have been suggested (Keplinger *et al.* 2015, Bisui *et al.* 2017, Jung *et al.* 2018). The minimal dose of dexmedetomidine to efficaciously prolong sensory nerve block remains to be determined. Whether the effect of dexmedetomidine on local nerve block is linked to the local or systemic action of the drug remains unknown but a study in healthy volunteers suggested that prolonged analgesia provided

by ropivacaine and dexmedetomidine at the saphenous nerve was possibly mediated by a peripheral mechanism (Andersen *et al.* 2017).

Dexmedetomidine is routinely used as a sedative agent in dogs but it is not commonly used as perineural LAA adjuvant. To date, only two studies regarding the perineural injection of dexmedetomidine with LAA have been published in dogs (Bartel *et al.* 2016, Trein *et al.* 2017). The analgesic efficacy of perineural bupivacaine combined with dexmedetomidine is comparable to epidural bupivacaine with buprenorphine in dogs after stifle arthroplasty (Bartel *et al.* 2016). The addition of 0.2 µg/kg of dexmedetomidine to ropivacaine seems insufficient to prolong sensory sciatic and femoral nerve block in experimental dogs and a higher dose of dexmedetomidine is probably necessary (Trein *et al.* 2017). The analgesic potential of perineural dexmedetomidine in dogs needs to be further studied. This was one of the aims of this thesis.

Goals

The field of US-guided locoregional anaesthesia is constantly expanding. The development of new techniques, new US-guided approaches, new combinations of LAA are contributing to the well-being of canine patients. Research in locoregional anaesthesia in human medicine is growing and efforts should be made to expand the knowledge of locoregional anaesthesia in veterinary medicine as well. The US-guided technique seems to be the best available tool for good clinical practice. The published US-guided nerve blocks techniques were reviewed. We identified that some US-guided peripheral nerve blocks techniques, such as the parasacral approach to the sciatic nerve, had an unsatisfactory reported success rate. The main goals of this thesis were to improve the success rate of the parasacral approach by modifying the existing technique and to evaluate the opioid requirements after the US-guided mid-femoral sciatic nerve block and inguinal femoral nerve block approaches in a clinical setting. Unfortunately, the duration of action of LAA used alone is short. Therefore, we aimed to evaluate the efficacy of dexmedetomidine combined to ropivacaine to prolong sensory nerve blocks and to evaluate the potential of this combination to reduce postoperative opioids requirements in dogs undergoing TPLO. In summary, the thesis aims to refine US-guidance for peripheral nerve blocks of the pelvic limb in dogs.

Study 1: The aim of study 1 was to modify and improve an existing US-guided technique in dogs to achieve a better success rate than previously described (Shilo *et al.* 2010). The previously described US-guided parasacral approach to the sciatic nerve in dogs used a longitudinal US view of the sciatic nerve and a low volume of LAA. The success rate of the technique reported by Shilo *et al.* (2010) was unsatisfying. The parasacral approach has been modified and the volume of LAA adapted to improve the success rate of this technique.

Study 2: The goal of study 2 was to evaluate the intraoperative and early postoperative opioid requirements in dogs undergoing TPLO. The differences of intraoperative fentanyl and postoperative methadone consumption after different techniques of locoregional anaesthesia were recorded for this purpose. The consumption of opioids after US-guided combined sciatic and femoral nerve block using the technique described by Campoy *et al.* (2010) was compared to single US-guided femoral or sciatic nerve block and to the epidural technique using surface anatomical landmark palpation.

Study 3: Study 3 aimed to compare different doses and routes of administration of dexmedetomidine for locoregional anaesthesia in experimental Beagle dogs. The nerve block duration of an US-guided sciatic and femoral nerve block with ropivacaine 0.5% combined with perineural or IV dexmedetomidine at different doses was determined. The second goal of this study was to measure plasma levels of dexmedetomidine after perineural and IV injections to demonstrate if the effect of perineural dexmedetomidine results from a perineural rather than a systemic mechanism of action.

Study 4: The study 4 was the clinical application of the findings of study 3. Perineural dexmedetomidine at 1 µg/kg or the same volume of perineural saline solution was combined to ropivacaine 0.5% for US-guided sciatic and femoral nerve block in dogs undergoing TPLO. The goal of this study was to determine the amount of methadone required to treat pain in the postoperative phase between both groups. The hypothesis was that the need for postoperative rescue analgesia with methadone would be reduced in the dexmedetomidine group compared to the control group receiving saline solution. The study was designed as a two-centre clinical trial, which allowed for a recruitment of a larger number of participants and a wider range of population groups, and the ability to compare results among centres, all of which increase the generalisability of the study results.

Experimental section

Experimental section

Study 1:

Sciatic nerve block in dogs: description and evaluation of a modified ultrasound-guided parasacral approach

Veterinary Anaesthesia and Analgesia
2019, 46(1):106-155

Vincent Marolf, Helene Rohrbach, Géraldine Bolen, Anne-Sophie Van Wijnsberghe & Charlotte Sandersen

Abstract

Objective To develop a modified ultrasound-guided parasacral approach to the sciatic nerve and compare the effects of a volume of 0.2 mL kg^{-1} of 0.5% levobupivacaine to an equivalent volume of 0.9% saline injected near the sciatic nerve.

Study design Cadaveric and experimental, blinded, randomized study.

Animals Seven canine cadavers and seven experimental Beagle dogs.

Methods Both sciatic nerves of seven cadavers were identified using a modified in-plane ultrasound-guided approach. Methylene blue solution (0.2 mL) was injected perineurally and success was evaluated through dissection. The same approach was repeated in seven Beagle dogs sedated with dexmedetomidine (50 µg kg^{-1}) injected intramuscularly (IM). After randomization, 0.2 mL kg^{-1} of 0.5% levobupivacaine (limb L) and 0.2 mL kg^{-1} of 0.9% saline (limb C) were injected perineurally on either right or left limb. Block success was determined by sensory deficits every hour for 8 hours after an atipamezole injection (0.2 mg kg^{-1}) IM. Reaction to pinprick (binary score) over the course of the sciatic nerve (4 locations) and locomotion were assessed.

Results The overall sciatic nerve block success was 93% in cadavers and 86% in sedated dogs. It was impossible to localize the sciatic nerves in one obese sedated dog. Significant differences between limb L and limb C were observed for pinprick at great trochanter, caudal thigh and lateral tarsal joint ($p < 0.0001$). Reaction to pinprick was absent in all dogs at great trochanter and caudal thigh up to at least 3 hours on limb L. Locomotion was impaired in all but one dog for 60 (30–210) minutes (median; interquartile range). No complications were observed.

Conclusion and clinical relevance A volume of ≥ 0.2 mL kg^{-1} and a concentration of 0.5% levobupivacaine can be recommended when using a modified ultrasound-guided parasacral approach to the sciatic nerve in dogs.

Introduction

The sciatic nerve provides sensory and motor fibres to the pelvic limb. Sciatic nerve block has traditionally been performed using anatomical landmarks or electrical nerve stimulators. The ultrasonographic approach to this nerve has been described in dogs (Benigni *et al*. 2007). The usefulness of ultrasound (US)-guided sciatic nerve block has been demonstrated by several clinical studies (Costa-Farré *et al.* 2011; Arnholz *et al.* 2017). US-guided technique enables precise needle positioning in relation to the target nerve and the spread of local anaesthetic can be observed in real time. Improved peripheral nerve block success rate using the US-guided technique compared with the electrolocalization technique has been reported by a meta-analysis performed in human medicine (Abrahams *et al.* 2009). A similar improved success rate was demonstrated in a study performed in cats for producing femoral nerve block (Haro *et al.* 2016).

Descriptive studies using the US-guided technique for the sciatic nerve have used a mid-femoral approach in dogs (Campoy *et al.* 2010; Echeverry *et al.* 2010). Alternatively, an approach to the nerve as it crosses the femur between the great trochanter and the sciatic tuberosity has been described (Costa-Farré *et al.* 2011). These techniques, combined with a femoral nerve block, appear to provide effective analgesia distal to the femur. Analgesia of a proximal area, such as the hip joint, has traditionally been provided using epidural anaesthesia (Wetmore & Glowaski 2000). A disadvantage of epidural anaesthesia is that motor blockade of both pelvic limbs is achieved, preventing early postoperative ambulation, whereas a nerve block of the sciatic nerve will provide analgesia for the surgical limb without affecting the opposite limb.

The parasacral approach for injection of local anaesthetic around the sciatic nerve in dogs has been reported using electrolocalization (Portela *et al.* 2010) or US-guided technique using a long axis view (longitudinal plane) (Shilo *et al.* 2010). The authors suggested to modify either the dose or the US-guided technique before applying it to clinical cases.

Objectives of this study were first, to develop, describe and evaluate a modified technique to block the sciatic nerve at the parasacral level using an US-guided cross-section view (transverse plane) in Beagle cadavers. Second, to compare levobupivacaine (0.2 mL kg^{-1}; 0.5%) with an equivalent volume of 0.9% saline solution when injected adjacent to the sciatic nerve. We hypothesized that locomotion, proprioception and sensory functions would be normal in the control pelvic limb (saline) but significantly reduced or absent in the treatment limb (levobupivacaine).

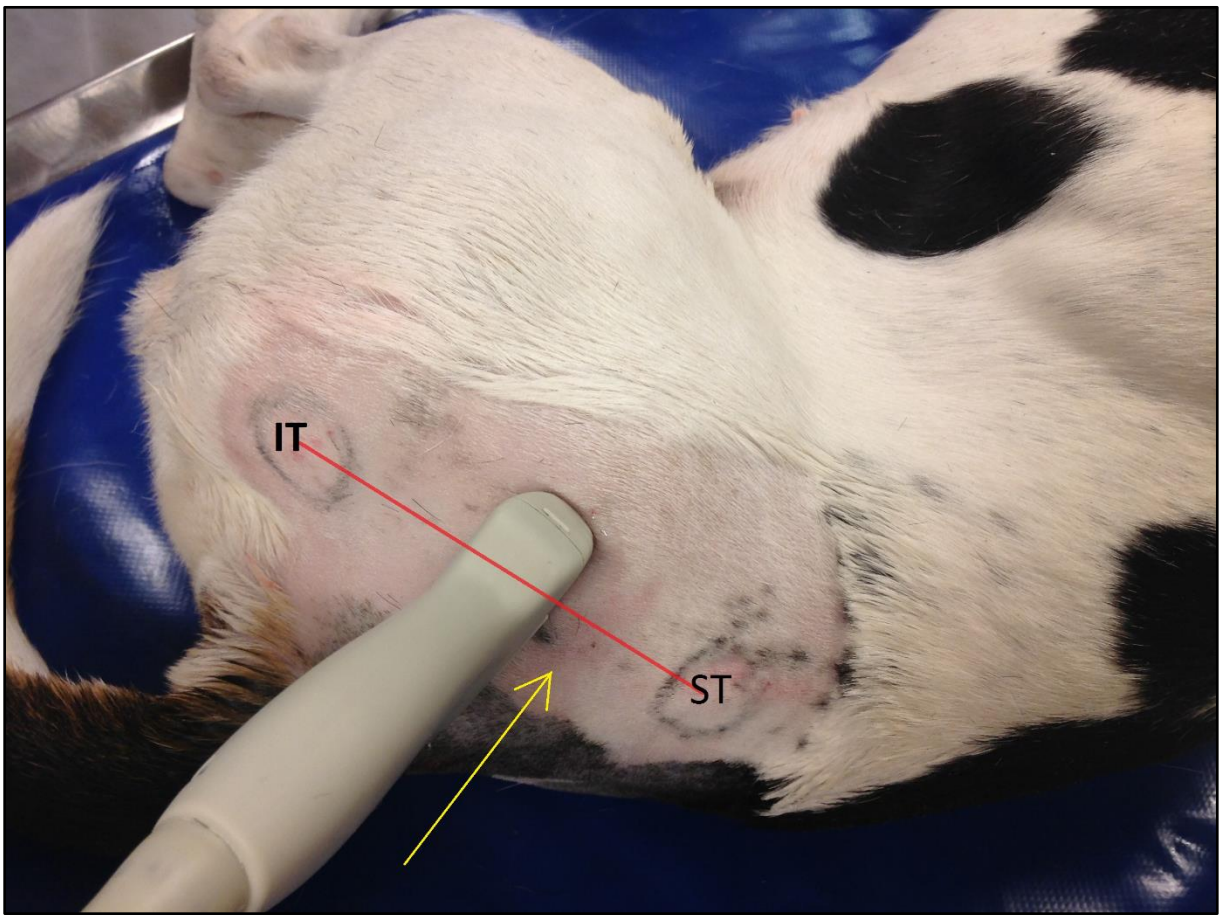
Materials and Methods

An ethical approval was obtained for the experimental part of the study. The authorization was delivered by the University of Liège, Belgium, approval for animal experimentation (Commission d'éthique animale; no. 1770).

Cadaver study. Anatomical dissections were performed in canine cadavers after localization of the sciatic nerve at the level of the iliac crest using ultrasound and injection of methylene blue dye. Seven adult female Beagle cadavers, euthanized for reasons unrelated to the study, were studied. The cadavers were thawed, placed in any lateral recumbency and the hair clipped over the entire pelvic area. The site was cleaned; first with a chlorhexidine-based solution, then with alcohol based medical solutions. The sciatic nerve was localized using a portable US unit (M-Turbo; SonoSite Inc., WA, USA) and a high frequency 8–13 MHz linear probe. The observer was positioned dorsally to the cadaver and contact transmission gel was applied on the area. The US probe was placed perpendicular to the skin surface at the centre point of a line between the ischiatic tuberosity and the sacral tuberosity. The ultrasound position represents the starting point to localize the sciatic nerve in transverse section at the modified parasacral level (Fig. 1). The probe was then rotated from this point towards the first coccygeal vertebra, until an angle of 60–90° between the ultrasound and the vertebral column was obtained. The nerve could be localized as a hypoechogenic rounded structure at the surface of the iliac spine visible as a convex hyperechoic interface associated with distal acoustic shadowing (Fig. 2). The probe was glided cranially to follow the sciatic nerve in transverse plane until it was overshadowed by the iliac spine and could no longer be visualized (Fig. 3). An echogenic stimulation needle (21 gauge, 10 cm, SonoPlex Stim cannula; Pajunk USA, GA, USA) was inserted in a caudolaterodorsal to craniomedioventral direction under US-guidance using the in-plane technique. The needle was carefully advanced until the tip could be visualized near the target nerve and methylene blue (0.2 mL) was injected. The cadaver was turned to the opposite lateral recumbency and same procedure was repeated for a total of 14 injections in seven cadavers. The skin and subcutaneous fat was incised in a craniodorsal to caudoventral

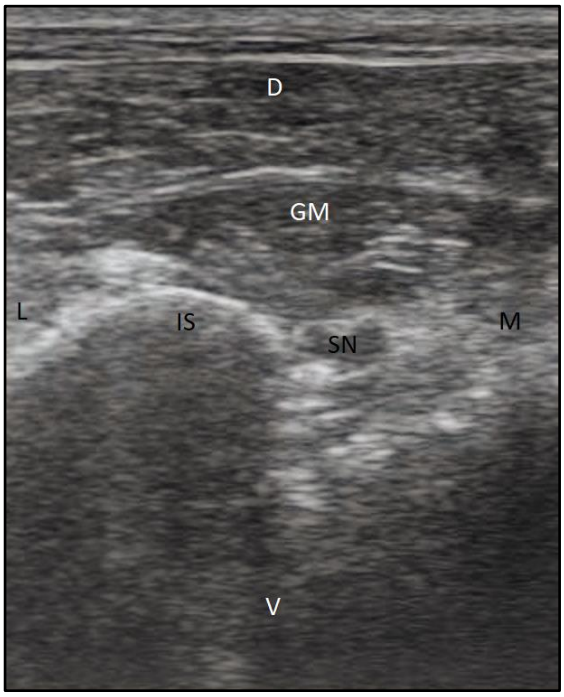
direction over the iliac crest, and skin and subcutaneous fat was removed. The gluteal musculature was dissected and abducted to evaluate the distribution of the dye. A perineural injection was considered successful when dye stained perineural connective tissue in direct contact with the sciatic nerve and/or the nerve itself (Fig. 4). The perineural placement of dye was considered a failure if no stained tissue was in direct contact with the nerve.

Figure 1.



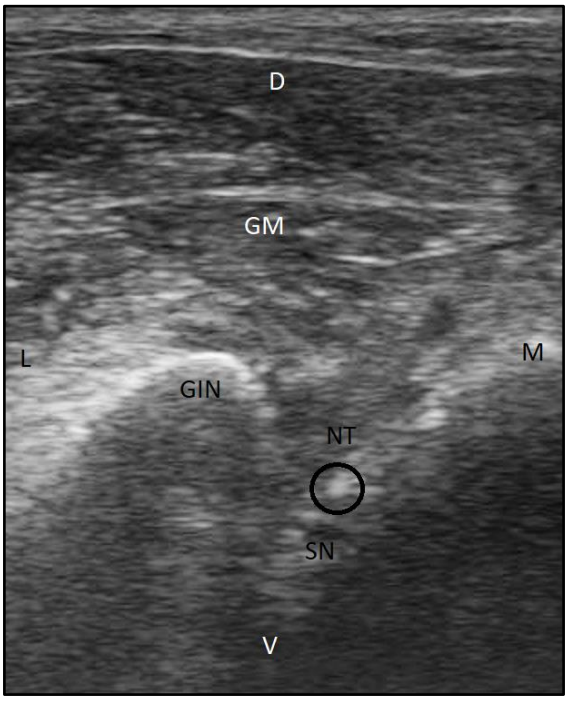
Ultrasound probe positioned at the centre of a line connecting the sacral tuberosity (ST) and the ischial tuberosity (IT) in a dog positioned in right lateral recumbency. Probe is rotated towards the tail base. Arrow indicates the needle insertion point, using the red line as a reference point.

Figure 2.



Ultrasound image of the sciatic nerve (SN) in transverse section as a round hypoechoic structure at the surface of the ischiatic spine (IS) visible as a convex hyperechoic interface associated with distal acoustic shadowing. GM, gluteal musculature; V, ventral; D, dorsal; L, lateral, M, medial.

Figure 3.



Ultrasound image of the sciatic nerve (SN) passing under the greater ischiatic notch (GIN). The needle tip (NT, circled structure) is positioned near the SN for local anaesthetic injection. The sciatic nerve localization in this image is deeper (approximately 2 cm) and more cranial (approximately 3 cm) compared with Figure 2. GM, gluteal musculature; V, ventral; D, dorsal; L, lateral, M, medial.

Figure 4. Staining of the sciatic nerves in different dogs after an ultrasound-guided injection of methylene blue (0.2 mL) using a modified parasacral approach. Arrows indicate areas of staining. Ca, caudal; Cr, cranial; D, dorsal; GIN, greater ischiatic notch; SN, sciatic nerve; V, ventral.

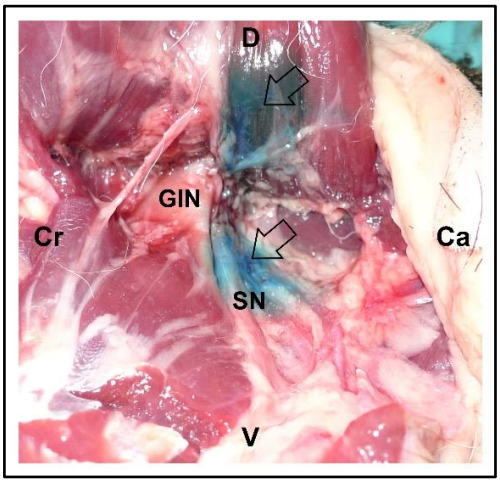


Figure 4a.

(a) positive perineural staining

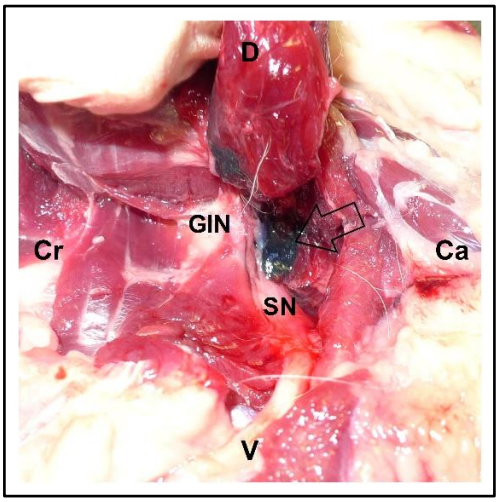


Figure 4b.

(b) partial staining

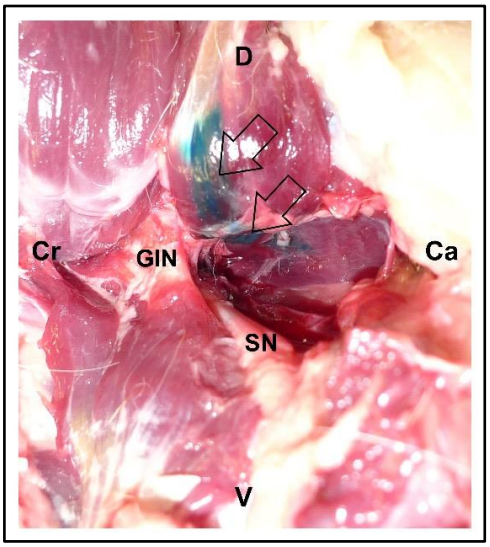


Figure 4c.

(c) negative staining

***In vivo* experimental study.** A group of seven research Beagle dogs (four males, three females) aged 5.1 ± 4.0 years were used to perform locoregional anaesthesia of the sciatic nerve. The experiments were carried out in an equipped research room located in the same building as the kennel to which the dogs had already been acclimatized. All dogs were fed dry commercial food once daily but were fasted for 24 hours prior to the experiments. They were fed in the late morning on the day of the experiment after fully recovering from sedation. Water was provided *ad libitum*. No abnormalities were detected during physical examination (including neurologic and orthopaedic examinations) and the dogs were classified as American Society of Anesthesiologists Class I. The dogs weighed 15.2 ± 1.5 kg and their body condition score (BCS; 1–9) was 5.9 ± 1.3 (Nestlé Purina Body Condition Score chart https://www.allpetsny.com/uploads/Body_condition_chart_dog.pdf, last accessed on 05.09.2018).

The dogs were sedated with dexmedetomidine (50 µg kg^{-1}; Dexdomitor; Zoetis, Belgium) injected intramuscularly (IM) in the triceps muscle. Oxygen was supplemented at 2 L $minute^{-1}$ throughout sedation via a circle breathing system equipped with a face mask. The anatomical site was prepared and the sciatic nerve was localized as described in the cadaveric part of the study. For each dog, levobupivacaine (1 mg kg^{-1}; 0.2 mL kg^{-1}; 0.5%, Chirocaine; AbbVie, Belgium) was injected adjacent to the sciatic nerve (treatment L) and another injection of saline (0.2 mL kg^{-1}; NaCl 0.9%) was injected on the opposite side using the same technique (treatment C). Treatment L was randomly assigned to the right or left sciatic nerve using a random program generator (www.random.org). Treatment C was injected in the contralateral limb. Perineural injection was first performed at the right sciatic nerve. The investigator (VM) performing and evaluating the nerve blocks was not aware of treatment assignment until all experiments were completed. Atipamezole (0.2 mg kg^{-1}; Antisedan; Zoetis, Belgium) was administered IM after both injections were performed.

Tests to evaluate locomotion, proprioception and sensitivity were performed on each limb. Locomotion was evaluated clinically and quantified by a motricity score (1, complete weight bearing on the limb; 2, partial weight bearing on the limb; 3, no weight bearing) and by the ability to walk in a circle (1, no missteps present; 0, missteps present). Proprioception was evaluated through reposition of

the limb after the dorsal part of the digits was positioned on the ground (knuckling test) and following scores were attributed: 1, immediate reposition of limb; 2, reduced or retarded reposition of the limb; 3, no reposition of the limb. Antinociception was evaluated by pinprick with a 22 gauge, 32 mm hypodermic needle applied at 5 locations: a) over the sacrum, b) over the great trochanter of the femur, c) at the caudal aspect of the thigh at the mid-femoral level, d) over the lateral part of the tarsal joint and e) interdigitally between the third and fourth digit. A reaction to toe clamping was assessed by clamping most lateral and most medial pads of digits with the flat portion of a mosquito forceps. A manual increase in pressure was applied for 2 seconds but the first ratchet was not closed to avoid iatrogenic tissue damage. The pressure was immediately released as soon as a positive reaction was observed. The tests to evaluate the sensory component (pinprick and toe clamping) were considered positive (score = 1) if withdrawing of the limb, crying, barking, actively looking at the stimulated area or escaping was observed and considered negative (score = 0) if none of these behaviours was observed.

A baseline measurement (T0) was performed prior to the experiment for each test. The measurements were repeated 10, 20, 30 and 60 minutes after atipamezole injection (T10, T20, T30, T60; respectively) and every hour (T120, T180, T240 etc.) until baseline values had returned in all categories. Orthopaedic and neurologic examinations were performed in all dogs 24 hours after the experiment to identify any signs of nerve damage or residual blockade.

Statistical analysis. A sample size calculation using an online calculator (sealedenvelope.com) for a binary outcome superiority trial was used. A sciatic nerve block success of 60% when using levobupivacaine against a success of 0% when using saline was considered. A total of six animals were integrated to the study to have an 80% chance with an alpha standard error of 5% to detect a significant difference. One animal has been added in case of possible loss of data. Beagle dogs, seven cadavers and seven live dogs were included in the study. A statistical analysis was performed using GraphPad Prism Version 5.00 for Windows (GraphPad Software, CA, USA). The data were analyzed for normality distribution using the Kolmogorov-Smirnov test. Parametric data are presented as mean ± standard deviation. Comparison between treatments L and C in each dog was performed with Wilcoxon matched

pairs signed rank tests and Bonferroni correction for 10 time points (T10–T420) was applied. Changes over time observed at different time points (T0–T480) between subjects were analyzed using Kruskal-Wallis test with Dunn's *post hoc* correction whenever significant differences were observed. Significance was set at $p \leq 0.05$ and at $p \leq 0.005$ with Bonferroni correction.

Results

Cadaver study. Injections were considered successful in 13 of 14 nerves (93%) (Fig. 4). In one dog, tissues lateral to the iliac crest and the deep gluteal muscle were stained and not the sciatic nerve.

***In vivo* experimental study.** The sciatic nerve could not be localized in the first dog (intact female, bodyweight 17.6 kg, BCS 8/9) and the perineural injections were not performed. Treatment L was injected on the right side in four dogs and the left in two dogs. Measurements were recorded up to T300 in one dog, to T360 in one dog, to T420 in three dogs and to T480 in one dog before return of baseline values (Figs 5 & 6). No adverse effects were observed during and after the perineural injections.

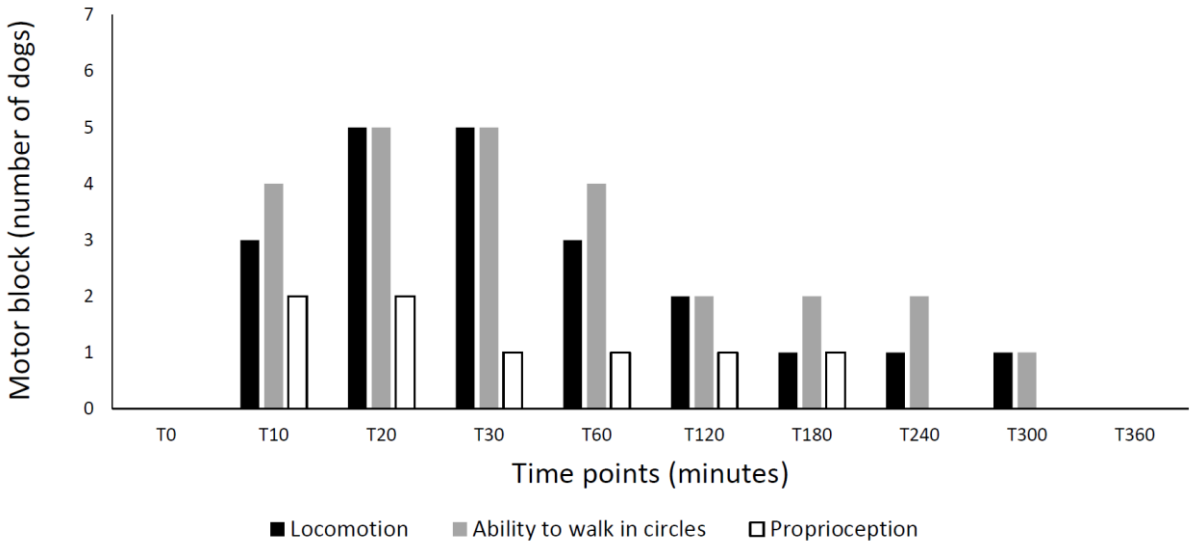
Assessment of locomotion. There was a statistical difference between limb C and limb L in the motricity score ($p < 0.0001$) and in the ability to walk in a circle ($p < 0.0001$). One dog had no locomotor dysfunction despite having modified sensory scores. Duration of motor block deficits (score = 2) was 60 (30–210) minutes (median, interquartile range). Locomotor score ($p = 0.0024$) and ability to walk in circles ($p = 0.0011$) over time did not reach significant differences after Dunn's *post hoc* correction (Fig. 5).

Assessment of proprioception. A decreased reposition of the limb (score = 2) was observed in three out of six dogs at T10 and T20 in one dog, at T10 in one dog and at T20–T180 in the last dog. The knuckling test was not significantly different between limb L and limb C ($p = 0.006$) in each dog, neither was it over time between subjects ($p = 0.395$). The orthopaedic and neurologic examinations performed 24 hours after the experiments were normal in all dogs.

Assessment of sensory component. Pinprick testing revealed a statistical difference between limb L and limb C at the great trochanter of the femur ($p < 0.0001$), at the caudal aspect of the thigh, at the mid-femoral level ($p < 0.0001$) and at the lateral part of the tarsal joint ($p < 0.0001$; Fig. 6). Reaction to pinprick at the sacrum was always observed and a score of 1 was recorded at all time points. The reaction to interdigital pinprick and to lateral toe clamping did not reach statistical difference with

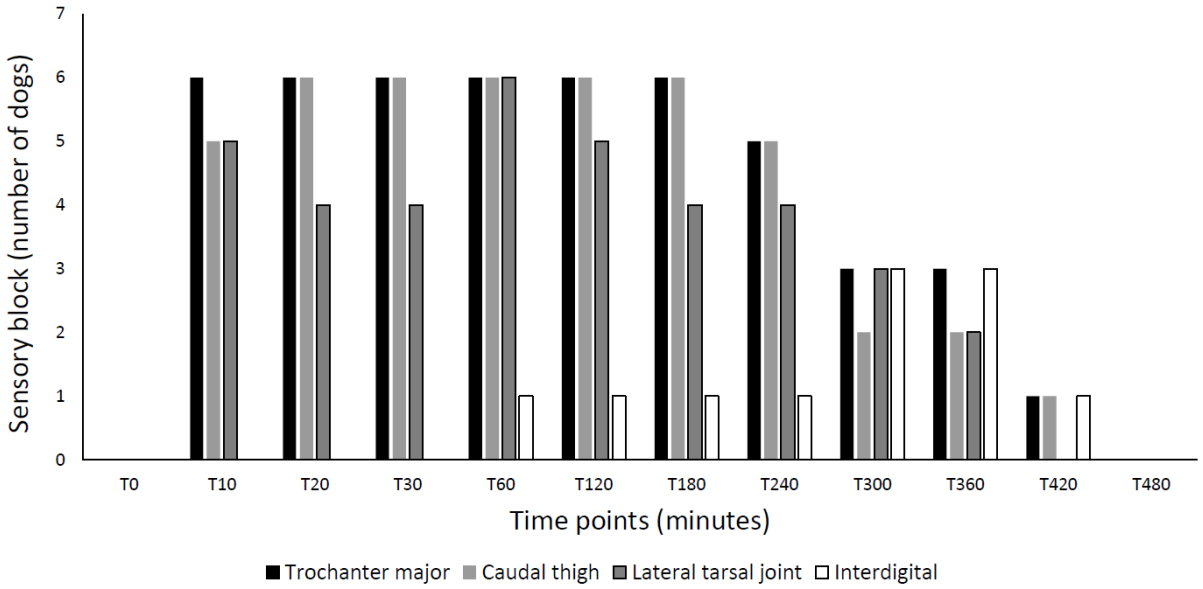
Bonferroni correction ($p = 0.0369$). Reaction to clamping of the medial pad was always observed and a score of 1 was recorded at all time points. Sensory test measurements over time were significantly different for the great trochanter and caudal thigh sites at time points T0 (baseline) *versus* T10–T180 and at time points T10–T180 *versus* T480 (both sites $p < 0.0001$). The sensory component over time at the tarsal joint site was significantly different between T0 *versus* T60 and between T60 *versus* T420–T480 ($p < 0.0004$), but not for the interdigital site and lateral toe clamping ($p = 0.7643$). The success rate of the sensory nerve block was 86% (six of seven successful perineural sciatic nerve injections) as determined by significant sensory deficits (score = 0) observed in six animals at the great trochanter, caudal thigh and lateral tarsal joint sites.

Figure 5.



Number of dogs with locomotor and proprioceptive deficits after an ultrasound-guided injection of 0.5% levobupivacaine (0.2 mL kg−1) at the parasacral level of the sciatic nerve.

Figure 6.



Number of dogs with sensory deficits assessed by pinprick with a 22-gauge hypodermic needle at different anatomical sites after an ultrasound-guided injection of 0.5% levobupivacaine (0.2 mL kg−1) at the parasacral level of the sciatic nerve.

Discussion

The first objective of the study was to develop a modified US-guided parasacral approach to the sciatic nerve evaluated by cadaver dissections. A success rate of 93% was achieved. The second objective was to compare the effect of 0.5% levobupivacaine (0.2 ml kg^{-1}) *versus* saline solution injected near the sciatic nerve using the evaluated technique. A success rate of 86% was achieved. These are higher success rates than the 67% obtained in a previous study employing a similar approach (Shilo *et al*. 2010). Differences are the volume and concentration of the local anesthetic and some modification of the technique. Shilo *et al.* (2010) divided the total volume of 0.5% bupivacaine between the saphenous (one-third) and the sciatic (two-thirds) nerves. The results suggested that the block was more complete and lasted longer when the highest volume (0.13 mL kg^{-1}) was injected near the sciatic nerve. Portela *et al.* (2010) used an electrical nerve stimulator for the parasacral approach. In that study the total volume of bupivacaine (0.25%, 0.1 mL kg^{-1} or 0.5%, 0.05 mL kg^{-1}) was divided into four equal parts and injected at the fourth, fifth and sixth lumbar nerves, and a parasacral injection. To quote a review about peripheral nerve blocks of the pelvic limb in dogs "Where low injectate volumes are used which are insufficient to surround the selected nerve, the technique is likely to fail. This appears to be the current stumbling block with the parasacral technique as reported by Shilo *et al.* (2010) and Portela *et al.* (2010)" (Gurney & Leece 2014).

Consequently, a higher volume (0.2 mL kg^{-1}) of 0.5% levobupivacaine was used in this study. The results indicate that this volume and concentration of injectate induced adequate sensory blockade at the trochanter major, caudal thigh and lateral tarsal joint sites. A previous observation during local anesthetic injection at the parasacral level was that the volume injected appeared to 'push the sciatic nerve away' instead of surrounding the nerve as is observed during a mid-femoral approach (Shilo *et al.* 2010). A similar action was observed in the present study, as the injected solution did not remain entirely around the selected nerve and spread away from the intended target site. This may explain why a larger

volume might be necessary with the parasacral approach, despite the usual assumption that US-guided techniques are associated with a reduction in volume of anaesthetic (Walker *et al.* 2009).

The parasacral sciatic nerve injection could not be performed in the first dog. The cadaver and experimental parts of the study were performed more than a year apart. The investigator required additional familiarization with the technique after this time lapse. A learning curve is observed for performing US-guided nerve blocks, and devices such as needle enhancement software have been used to improve injection technique (Viscasillas *et al.* 2013). Block failure in the first dog could also be attributed to the dog's conformation (BCS 8/9). Targeted sites are located deeper in obese animals, making visualization of the needle tip in a deeper and steeper plane more challenging. Schwemmer *et al.* (2006) suggested that the use of ultrasound helped localization of the interscalene brachial plexus in obese humans. Therefore, with the limitation of an altered perception of the anatomical structures, additional practice in overweight animals may be required to avoid block failure. In addition, the use of a lower frequency probe may facilitate visualization of the nerve in a deeper location.

The hypothesis of this study concerning differences in locomotion, proprioception and sensory components after injection of levobupivacaine was partially confirmed. All dogs had significant sensory deficits at specific sites that were not always associated with locomotion deficits. The effect on proprioception was minimal. Similar results (i.e., sensory deficits but not always motor blockade) are obtained after epidural injection of 0.5% levobupivacaine in dogs (Gomez de Segura *et al.* 2009). The discrepancies between sensory and motor blockade seem to be strongly related to the type, volume and concentration of local anesthetic used, and also fibre organization within the nerve, nerve diameters, fibre types (Aδ, Aβ, C), myelinated *versus* unmyelinated fibres or length of the nerve exposed (McDowell & Durieux 2006; Rioja Garcia 2015).

Significant differences in sensation of interdigital pinprick and lateral toe clamping were not present. Loss of sensation at these sites would be an expected consequence of sciatic nerve blockade. A possible explanation may be related to the large size of the nerve impacting diffusion, which will depend

on the volume and concentration of the local anaesthetic. There are two main innervations of the pelvic limb: the femoral nerve which originates from L4–L6 and the sciatic nerve which originates from L6–S2. The femoral nerve contributes to stabilization of the stifle by innervation of the quadriceps muscle that controls the patella. The areas chosen to test the sensory component by pinprick were selected based on the dermatome innervated by the sciatic nerve (Campoy *et al.* 2015). The skin over the sacrum is innervated by the lateral cutaneous femoral nerve, a branch of the femoral nerve. The medial toe is innervated by branches of both major nerves; the superficial fibular nerve and the saphenous nerve (Evans & de Lahunta 2013). The sacral and medial toe sites were purposely chosen as control areas because of their innervation by branches of the femoral nerve. All dogs were reacting to stimulation of these areas during sciatic nerve blockade and this may explain lack of complete proprioception and motor block.

Bupivacaine has been popular for peripheral sciatic nerve block but reports of the duration of action vary greatly. The duration of sensory sciatic nerve block after administration of bupivacaine was 145–330 minutes (Shilo *et al.* 2010) whereas the duration of complete sensory blockade at the fibular and tibial nerves was 70–268 minutes (Portela *et al.* 2010). By contrast, injection of 0.5% bupivacaine (0.15 mL kg^{-1}) using a standard sciatic nerve approach resulted in durations of action of 12 and 10 hours for motor and sensory block, respectively (Cathasaigh *et al.* 2018). A short motor and long sensory nerve block are usually preferred to promote early postoperative ambulation while providing effective pain treatment. Levobupivacaine has a larger therapeutic index than bupivacaine owing to the missing R(+) enantiomer molecule mainly responsible for the cardiovascular and central nervous system side effects (Gristwood & Greaves 1999). Unfortunately, few studies report the use of levobupivacaine for peripheral sciatic nerve block in dogs (Vettorato *et al.* 2013). Levobupivacaine 0.5% induced a longer sensory sciatic nerve block than ropivacaine 0.5% in humans (Pham Dang *et al.* 2015). Levobupivacaine was selected for the present study as the local anesthetic of choice because it has the potential to induce long sensory nerve block.

A limitation of this study is the use of a small number of dogs of similar weight and size. Future studies should include a larger number of dogs of different breeds and sizes. A further limitation is in the study protocol where evaluations were performed every 60 minutes to reduce the stress on the dogs. A shorter time span between evaluation points may have generated additional useful data. Although the sensory nerve blocks were evaluated using a binary score, a graduated score may have further classified the degree of sensory nerve blockade because dogs may not react consistently to pinprick stimuli.

No adverse side effects were observed during or after the experimental procedure. However, perineural local anesthetic injection is associated with some risk. The potential for vascular puncture or intravascular injection should be considered given the proximity of gluteal vessels to the anatomical site, although US-guided sciatic nerve block is less likely to result in vascular puncture than a technique employing electrolocalization. In humans, perforation of the rectum is listed as a risk of the parasacral approach should the needle be inadvertently directed more dorsally (Ripart *et al.* 2005; Cao *et al.* 2015). These complications of the parasacral approach have not been reported in veterinary medicine.

Innervation of the hip joint of the dog may vary among individuals. Anatomical dissections of the canine hip joint capsule have documented invariable innervation by the cranial gluteal nerve but never by the caudal gluteal nerve (Huang *et al.* 2013). Branches of the sciatic nerve often cover the hip joint capsule, but coverage by femoral and obturator nerves occurs to a lesser extent. Given the proximity of the cranial gluteal and sciatic nerves at the parasacral level, local anaesthetic intended to block the sciatic nerve might also provide cranial gluteal nerve blockade. Dissections of human cadavers have revealed parasacral sciatic nerve injections spreading to the obturator nerve and sacral roots in 82% of successful injections (Valade *et al.* 2008). This observation is in opposition to the finding that anesthesia of the obturator nerve does not occur with the parasacral approach in a clinical setting (Aissaoui *et al.* 2013). Whether this would occur in dogs remains to be determined. It is possible that the modified parasacral approach could offer an alternative to epidural anaesthesia to provide sufficient analgesia to the hip joint and surrounding muscles. Further studies involving hip surgery in dogs are required to confirm this hypothesis.

Conclusion. The modified parasacral approach is an effective alternative approach to the sciatic nerve and administration of 0.5% levobupivacaine (0.2 mL kg^{-1}) resulted in a high success rate for sensory nerve block at the level of the hip and caudal thigh in dogs.

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Experimental section

Study 2

Opioid requirements after locoregional anaesthesia in dogs undergoing tibial plateau levelling osteotomy: a pilot study.

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Vincent Marolf, Claudia Spadavecchia, Nicole Müller, Charlotte Sandersen &

Helene Rohrbach

Abstract

Objective To determine the intraoperative and early postoperative opioid requirement after sciatic and/or femoral nerve block or epidural anaesthesia in dogs undergoing tibial plateau levelling osteotomy (TPLO).

Study design Prospective, masked, pilot, randomised, clinical trial.

Animals A total of 40 client owned dogs undergoing TPLO.

Methods Each dog was randomly assigned to group SF (combined sciatic and femoral nerve block), group S (sciatic nerve block), group F (femoral nerve block) or group E (epidural anaesthesia). A total of 0.3 mL kg^{-1} of ropivacaine 0.5% was administered to each nerve or in the epidural space. Intraoperatively, fentanyl (2 µg kg^{-1}) was administered intravenously when heart rate, mean arterial pressure or respiratory rate increased >30% compared to baseline values. Postoperatively, a visual analogue scale (VAS) and a modified German version of the French pain scale (4AVet) were used to assess pain every 30 minutes for 150 minutes and again once the morning after surgery. Methadone (0.1 mg kg^{-1}) was administered intravenously if the VAS was ≥ 4 cm (maximal value 10 cm) or the composite pain score was ≥ 5 (maximal value 15).

Results Groups SF and E required less total intraoperative and early postoperative opioid doses compared to groups S and F (p = 0.031). No dogs in groups SF had a block failure or required postoperative methadone. A reduced methadone requirement was found for SF compared to all the other groups up to 150 minutes after recovery (p = 0.041).

Conclusion and clinical relevance Combined sciatic and femoral nerve block and epidural anaesthesia lead to less cumulative consumption of perioperative opioids than single nerve blockade. Sciatic or femoral nerve block alone might be insufficient to control nociception and early postoperative pain in dogs undergoing TPLO.

Introduction

Tibial Plateau Levelling Osteotomy (TPLO) is a surgical technique performed in dogs suffering from cranial cruciate ligament rupture. Thanks to the favourable postoperative outcome reported (Kowaleski *et al.* 2013), the procedure has become increasingly popular in many veterinary hospitals. However, as TPLO includes arthrotomy or arthroscopy, soft tissue elevation, osteotomy as well as bone plate application, it leads to moderate to severe postoperative pain and adequate perioperative analgesia is essential (Christopher *et al.* 2013).

For invasive orthopaedic procedures under general anaesthesia, the adjunct of locoregional anaesthesia, like perineural injections of local anaesthetics, markedly improves the wellbeing of animals during the perioperative period. Reasons for this include a higher analgesic efficacy with a lower risk of side effects (such as dsyphoria) when compared to systemic drugs (Troncy *et al.* 2002; Becker *et al.* 2013).

The duration of a peripheral nerve blockade is drug and dose dependent (Sakonju *et al.* 2009). When compared to other local anaesthetics, ropivacaine has a favourable profile for local blocks due to its long-lasting and reliable analgesic effects (Shah *et al.* 2018). The epidural injection of ropivacaine has been shown to provide adequate perioperative analgesia in dogs undergoing TPLO surgery (Adami *et al.* 2012). Peripheral nerve blocks can provide complete pain relief and reduce the amount of medication (such as halogenated agents) required to maintain general anaesthesia (Pascoe 1997; Campoy *et al.* 2012)

The usefulness of the ultrasound-guided technique for sciatic nerve block has been outlined in dogs (Echeverry *et al.* 2010). Sonographic guidance for peripheral nerve blocks improves the success rate of the block (Abrahams *et al.* 2009). In animals, the technique of ultrasound guidance for the blockade of large peripheral nerves such as the sciatic and the femoral nerves have been described in anatomical studies (Campoy *et al.* 2010; Shilo *et al.* 2010). The large majority of studies describing ultrasound guided sciatic and femoral nerve block were performed on cadavers, experimental or healthy dogs (Costa-Farré *et al.* 2001; Cathasaigh *et al.* 2018; Marolf *et al.* 2019). Ultrasound guided sciatic and

femoral nerve blocks are nowadays commonly used in clinical practice to control nociception in canine patients undergoing hindlimb surgery (Campoy *et al.* 2012). Unfortunately, clinical studies investigating the outcome of different locoregional anaesthetic techniques are sparse (Arnholz *et al.* 2017; Tayari *et al.* 2017). Recently, perioperative analgesia provided by a perineural injection of ropivacaine or saline at the lumbar plexus and sciatic nerve has been compared in dogs undergoing TPLO (Warrit *et al.* 2019b). Greater analgesia and better recovery scores were observed when ropivacaine was injected under ultrasound guidance compared to saline. Further expertise to determine the success rate and to prove the efficacy of ultrasound guided sciatic and femoral nerve block for dogs in a clinical context is essential.

The aim of the study was to evaluate perioperative opioid analgesic requirement after epidural anaesthesia, sciatic nerve block, femoral nerve block or a combined sciatic/femoral nerve block with ropivacaine in dogs undergoing TPLO. We hypothesized that an ultrasound guided combined sciatic/femoral nerve block or epidural anaesthesia would lead to lower rescue intraoperative fentanyl and/or postoperative methadone administration than a single ultrasound guided femoral or sciatic nerve block.

Materials and Methods

The study was designed as a prospective, pilot, masked, randomised, clinical trial. The experimental trial was performed with permission from the local Committee for Animal Experimentation (Canton of Bern BE 83/12, No 22523, Switzerland). The study was a pilot and consequently the number of animals per group was arbitrarily set at 10 animals per group. This would correspond to the number of animals required for a power of 80% and a standard error alpha set at 5% if two doses of opioids were required in the control group and none were required in the treatment group with a standard deviation of 1.5 doses. The study was terminated when a complete data set of 40 animals (10 per group) was available for analysis. Client-owned dogs with a cranial cruciate ligament rupture undergoing elective TPLO were enrolled in the study. Only dogs classified as ASA I or II according to the American Society of Anesthesiology (ASA) physical status grading system were included in the study. Signed owner consent was a prerequisite for a participation in the study. Dogs with concomitant systemic diseases, infectious skin diseases in the area of the blocks or bleeding disorders were excluded from the study.

Procedure. After intramuscular (IM) premedication with acepromazine (Prequillan; Arovet AG, Switzerland; 0.02 mg kg^{-1}), an intravenous (IV) catheter was placed in a cephalic vein. General anaesthesia was induced by IV injection of propofol (Propofol Lipuro 1%; B. Braun Medical, Switzerland) titrated to effect. After endotracheal intubation, isoflurane (Isoflurane; Provet, Switzerland) was delivered in 100% oxygen using a rebreathing system and an isotonic crystalloid solution was administered IV at a rate of 10 mL kg^{-1} $hour^{-1}$. Monitoring included electrocardiography, pulse oximetry, respiratory gas measurements, spirometry, invasive blood pressure measured by cannulation of the metatarsal artery and oesophageal temperature. All parameters were measured continuously and recorded every 5 minutes. Dogs were allowed to breathe spontaneously. If the end-tidal carbon dioxide ($PE´CO_2$) was higher than 50 mmHg (6.67 kPa), pressure support ventilation was started with a peak inspiratory pressure (PIP) of 10 cmH_2O. An end-tidal isoflurane concentration of 1.3% was targeted during anaesthesia. Hypotension, defined as mean arterial pressure (MAP) lower than

60 mmHg, was treated with a bolus of a colloid solution (Voluven; Fresenius Kabi AG, Switzerland; 1 or 2 mL kg^{-1} IV over 15 minutes). A second bolus of the same dose was repeated if necessary. At the time of extubation, carprofen (Rimadyl; Zoetis Schweiz GmbH, Switzerland; 4 mg kg^{-1} IV) was administered to all dogs.

Dogs were randomised by drawing a lot from an envelope indicating the treatment group: group SF (combined sciatic and femoral nerves block), group S (sciatic nerve block), group F (femoral nerve block), group E (epidural injection). The masking procedure included an identical preparation of all injection sites in all dogs. The skin was pricked at all injection sites with an injection needle to avoid group identification. The anaesthetist (NM) performing anaesthesia and postoperative pain scoring was unaware of the selected block. A volume of 0.3 mL kg^{-1} ropivacaine (Naropin 0.5%; Aspen Pharma Schweiz GmbH, Switzerland) was injected into each injection site according to the group allocation. Group SF received two injections of 0.3 mL kg^{-1}, one for each nerve. A volume of 10 mL per injection site was never exceeded.

The epidural injections as well as the sciatic and femoral nerve blocks were performed as previously described (Campoy *et al.* 2010). The epidural injection at the lumbosacral space was performed with a 75 mm 19-gauge spinal needle (Spinocan; B Braun, Switzerland) with the bevel facing cranially after palpation of anatomical landmarks. The "popping" sensation when penetrating the interarcuate ligament, the lack of resistance to injection and the hanging drop technique were applied for assessment of proper needle positioning (Adami & Gendron 2017).

The sciatic and femoral perineural injections were performed under sonographic guidance. A portable ultrasound unit (M-Turbo SonoSite; Bothell, WA, USA) with an 8-13 MHz linear probe was used to visualize the target nerve, the needle and the distribution of the local anaesthetic. After aseptic preparation of the puncture site, the nerve blocks were performed using an insulated 21-gauge 90 mm needle with facet tip and injection line (Sonostim; Pajunk GmbH, Germany). Ropivacaine 0.5% was injected under real-time ultrasound control. The person performing locoregional anaesthesia had at least 1-year clinical experience in ultrasound guidance for sciatic and femoral nerve block and epidural anaesthesia.

Assessment of nociception and pain. An increase in heart rate (HR), MAP or respiratory rate (f_R) of 30% above baseline values (defined as the mean values recorded over 15 minutes during general anaesthesia before surgical stimulation started) was considered indicative of nociception leading to administration of a fentanyl bolus (Fentanyl-Janssen; Janssen-Cilag AG, Switzerland; 0.002 µg kg^{-1} IV). A fentanyl continuous rate infusion (CRI) at a rate of 0.005 µg kg^{-1} $hour^{-1}$ was started after the second fentanyl bolus. Fentanyl boluses were repeated every 5 minutes until the physiological variables returned to baseline. The total amount of fentanyl administered per dog during surgery was recorded as the total intraoperative fentanyl dose. Intraoperative vital parameters were evaluated separately for arthroscopy and TPLO.

Pain was evaluated using a modified German version of the French pain scale (4AVet) and a 100 mm Visual Analogue Scale (VAS) with end points labelled as 0 (no pain) and 100 (worst pain imaginable for this type of surgery). The assessments were performed preoperatively (T-1), at recovery as soon as the animal was able to lift the head (T0), and 30 (T30), 60 (T60), 90 (T90), 120 (T120) and 150 (T150) minutes after extubation as well as at 8.00 hours on the day after surgery (T8AM). The evaluations were performed by an anaesthetist (NM) unaware of the treatment group. Rescue analgesia methadone (Methadon Streuli; Streuli Pharma AG, Switzerland; 0.1 mg kg^{-1} IV) was administered when the VAS was ≥ 40 mm or the multidimensional pain scale was ≥ 5 [0 (no pain), 15 (worst possible pain)]. The total number of postoperative rescue methadone doses administered were recorded for each dog. Duration of efficacy (minutes) was recorded as the time elapsed from the local anaesthetic injection to the first injection of methadone. After the evaluation at T150, all dogs received buprenorphine (Temgesic; Individor Schweiz AG, Switzerland; 0.02 mg kg^{-1} IV) every 8 hours as standard pain medication during the hospital stay. Buprenorphine was repeated after the pain evaluation at T8AM had been completed.

A block failure was declared if a dog required two or more opioid boluses during surgery and/or during the early postoperative phase (up to T150). The administration of one fentanyl bolus during surgery or one methadone bolus in the early postoperative period was not considered a block failure.

Statistical evaluation. Data analysis was performed using statistical software (Sigma Stat, Version 3.5, Systat Software, San Jose, CA). A Fisher exact test was performed using an online software calculator (https://www.socscistatistics.com/tests/fisher/default2.aspx, last accessed on 16.08.2020). Demographic data, duration of nerve block, anaesthesia and surgery were tested for normality with a Kolmogorov-Smirnov test and were equally distributed. Results are presented as mean ± standard deviation (SD). Non-parametrically distributed data are presented as median and interquartile range [IQR]. The mean values as well as the range (minimum maximum) were evaluated for HR, MAP and f_R for the arthroscopy and TPLO operative periods. Kruskal Wallis Analysis of Variance (ANOVA) on ranks (Dunn's Method) was performed to evaluate differences in intraoperative vital parameters among groups at each time point. Mann-Whitney Rank Sum Test was used to compare intraoperative vital parameters between the arthroscopy and TPLO phase within groups. Multidimensional pain scores evaluations and VAS were analysed with repeated measures ANOVA on ranks followed by Tukey test for comparison between time points within groups and with one-way ANOVA on ranks for comparison between groups at each time points and duration of nerve blocks. Rate of block failure, the number of dogs that required opioid interventions and the total number of opioid doses in each group was analysed with Fisher-exact tests. Significance was set at $p \leq 0.05$.

Results

Animals. A total of 44 dogs completed the study. Insufficient data were available in four dogs and 10 dogs per group were included in the analysis. The mean weight of the dogs was 36.8 ± 10.1 kg and the mean age was 6.0 ± 2.6 years. The mean weight of group S (45.85 ± 12.02 kg) was higher (p = 0.007) than the mean weight of the group's SF (32.9 ± 6.13 kg), F (34.55 ± 8.01 kg) and E (33.74 ± 5.96 kg).

Anaesthesia. Arthroscopy and TPLO were successfully performed in 44 dogs. All animals recovered from anaesthesia and they were discharged from the hospital 1 day after surgery. Duration of anaesthesia ranged from 210 to 350 minutes (260 ± 35 minutes) while duration of surgery (arthroscopy + TPLO) was 75 to 145 minutes (102 ± 16 minutes). The anaesthesia time was long because of the time required for surgical preparation and pre- and postoperative radiography. No difference among groups could be detected.

Intraoperative comparison of physiological variables. The median values and IQR of intraoperative physiological variables (HR, f_R, MAP) are presented in Table 1. During arthroscopy, HR was higher in group F than in group S (p = 0.02). The f_R was higher in group E than in groups SF and F (p = 0.002) while MAP was lower in group E than in all other groups (p < 0.001).

During TPLO, no difference in HR could be detected among groups (p = 0.057). Regarding f_R, group E and S showed higher values than group SF and F. The MAP was lower in group E than in all other groups (p < 0.001).

The physiological variables did not differ between arthroscopy and TPLO in groups SF and E. In group S, f_R increased (p = 0.036) while MAP decreased (p = 0.007) during TPLO compared to arthroscopy. In group F the range of HR and f_R was higher during TPLO than during arthroscopy with p = 0.004 and p = 0.039, respectively.

Table 1 Intraoperative physiological variables of 40 dogs undergoing surgery for stifle arthroscopy followed by tibia plateau levelling osteotomy (TPLO). Dogs were given 0.3 mL kg-1 of ropivacaine 0.5% per injection site (maximum 10 mL per injection) administered preoperatively. Animals were randomly assigned to one of four groups with 10 dogs per group. Ropivacaine was administered perineurally by ultrasound guidance using a mid-femoral approach to the sciatic nerve (group S), an inguinal approach to the femoral nerve (group F), a combined ultrasound-guided approach to both nerves (group SF) or by palpation of surface anatomical landmarks for injection into the lumbosacral epidural space (group E). All data are presented as median and interquartile range [IQR] or range (minimum-maximum).

Group	**Arthroscopy** Median [IQR] Range			**TPLO** Median [IQR] Range		
	HR (beats minute^{-1})	**f_R (breaths minute^{-1})**	**MAP (mmHg)**	**HR (beats minute^{-1})**	**f_R (breaths minute^{-1})**	**MAP (mmHg)**
SF	95 [81-112] 16 (11-21)	10 [9-16]* 2.5 (2-5)	74.5 [68-80]*[c] 18 (10-25)	100 [85-111] 15.5 (11-30)	12 [9-17]*§ 3.5 (3-5)	75 [70-80]* 12.5 (9-17)
S	95 [85-104]* 21.5 (11-31)	12 [11-15]¶ 3 (1-7)	80 [70-88]†# 21.5 (12-30)	95 [81-108] 30 (27-40)	13 [12-17] ‡§¶ 4 (2-6)	75 [66-82]†# 19 (15-30)
F	103 [92-108]* 13.5 (10-16)¶	10 [9-13]† 4.5 (3-8)#	78 [72-81]‡ 21 (15-23)	102 [85-111] 27.5 (22-43)¶	12 [9-13]†‡ 6 (4-10)#	78 [70-85]‡ 19.5 (16-24)
E	95 [80-105] 17.5 (10-31)	14 [12-16]*† 2.5 (2-3)	65 [61-77]*† ‡ 17.5 (8-25)	95 [80-105] 18.5 (15-53)	14 [11-16]*† 5 (3-7)	65 [61-75] *†‡ 16 (10-29)

*,†, ‡, § = significant difference (within the same column) between groups ($p < 0.05$); ¶, # = significant difference (within the same row) between arthroscopy and TPLO phase; HR, heart rate; *f*R, respiratory rate; MAP, mean arterial pressure

Intraoperative hypotension. A colloid bolus of 2 mL kg^{-1} was administered to one dog in group S and a bolus of 1 mL kg^{-1} was administered to two dogs in group E which was repeated in one dog in group E.

Rescue analgesia. During surgery, fentanyl was only administered during the TPLO phase. Block failure was declared in five dogs (3 in group S, 1 in group F, 1 in group E).

In group SF one dog received one bolus of fentanyl. In group SF, no dogs required methadone in the postoperative phase and no block failure was observed. There was no difference in the intraoperative fentanyl requirement between groups SF and E *versus* groups S and F ($p = 0.695$). Group SF required less postoperative methadone compared to the other groups ($p = 0.041$). Intraoperative fentanyl consumption of group SF did not differ from the other groups ($p = 0.653$). In group S, a fentanyl CRI was started in two dogs while one dog was given a single fentanyl bolus. One dog needed six boluses of fentanyl in addition to a fentanyl CRI during the intraoperative phase. All three dogs required methadone at T30. The nerve blocks of all three dogs were defined as a failure. In group F, a fentanyl bolus was given to two dogs, one of them was given additional methadone at T0. Perioperative analgesia was insufficient for this dog and the nerve block was defined as a block failure. In four animals, additional methadone was given in the postoperative phase, two at T120 and two at T150. In group E, one dog required fentanyl CRI during surgery and methadone at T0 and T30. For this dog, a block failure was declared. A single bolus of fentanyl was administered to one dog during surgery and another was given a single bolus of methadone at T90. Block failure between groups E and SF (complete nerve block) *versus* groups S and F (partial nerve block) was not different ($p = 0.342$) and neither was the opioid requirement (opioid yes or no) per dog between groups for the intraoperative and early postoperative phase ($p = 0.176$). There was a difference between groups E and SF *versus* single nerve block groups (groups S and F) when the total administered doses of opioids were analysed for the intraoperative and early postoperative period ($p = 0.031$). Rescue opioids administered per dog are presented in Table 2.

Table 2. Opioids administered intraoperatively to control nociceptive autonomic reflexes or postoperatively to control pain in 40 dogs undergoing surgery for stifle arthroscopy followed by tibia plateau levelling osteotomy (TPLO). Dogs were given 0.3 mL kg-1 of ropivacaine 0.5% injected preoperatively at the sciatic and femoral nerve (Group SF), the sciatic nerve (Group S), the femoral nerve (Group F) using ultrasound guidance or in the epidural space (Group E). Each dog was randomly assigned to a group and each group included 10 dogs. Each row represents one dog.

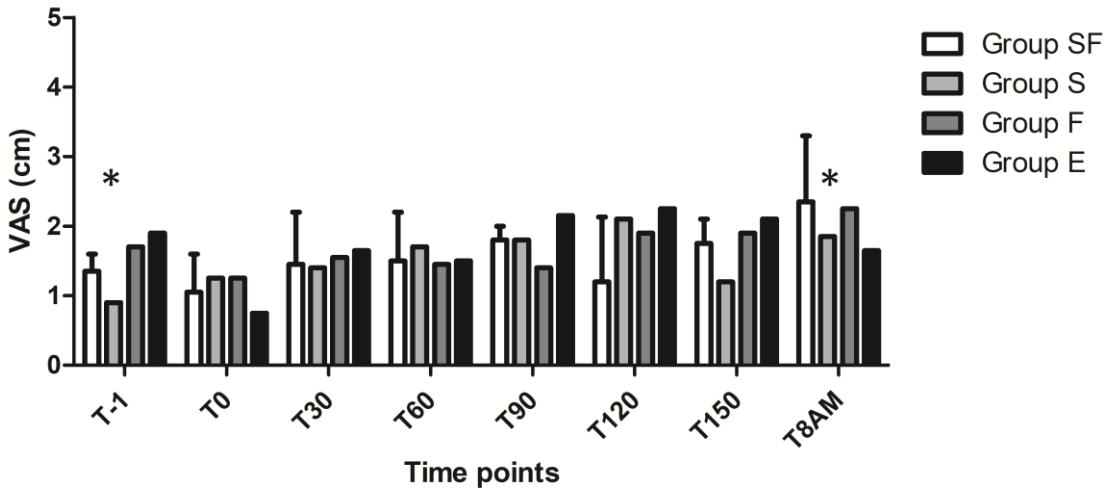
Group	Number of fentanyl boluses (2 µg kg^{-1}) administered during surgery	CRI of fentanyl (5 µg kg^{-1} $hour^{-1}$) administered during surgery	Number of methadone (0.1 mg kg^{-1}) boluses administered after surgery	Time points of methadone administration (minutes)	Block failure
SF	1	-	-	-	-
S	6	yes	2	T30, T90	yes
S	1	-	1	T30	yes
S	2	yes	1	T30	yes
F	-	-	1	T150	-
F	1	-	-	-	-
F	-	-	1	T150	-
F	1	-	1	T0	yes
F	-	-	1	T120	-
F	-	-	1	T120	-
E	3	yes	2	T0, T30	yes
E	-	-	1	T90	-
E	1	-	-	-	-

CRI: continuous rate infusion; T0, T30, T90, T120, T150: time points in minutes after recovery from surgery when a multidimensional pain score and visual analogue scale has been used to evaluate pain.

Postoperative pain evaluation. Methadone administration led to exclusion from further analyses (T30 –T150). At T8AM all animals were included as this time point was considered as an independent time point. Results for VAS are illustrated in Fig. 1. The VAS of all animals (n = 40) was higher (p = 0.025) at T8AM (2.35 [1.7-3.1]) than at T0 (1.2 [0.8-1.6]). No differences in VAS between groups at any time point could be detected. The VAS was higher in group S at T8AM than at T-1 (p = 0.033). Results of the composite pain scale are illustrated in Figure 2. The pain scores of all animals (n = 40) were higher ($p < 0.0001$) at T8AM (3 [2-4]) than at T0 (0 [0-1]), T30 (0 [0-3]) and T60 (2 [0-3]). No differences in pain scores between groups could be detected at any time point. In group S, the pain score was higher at T8AM than at T0 (p = 0.026).

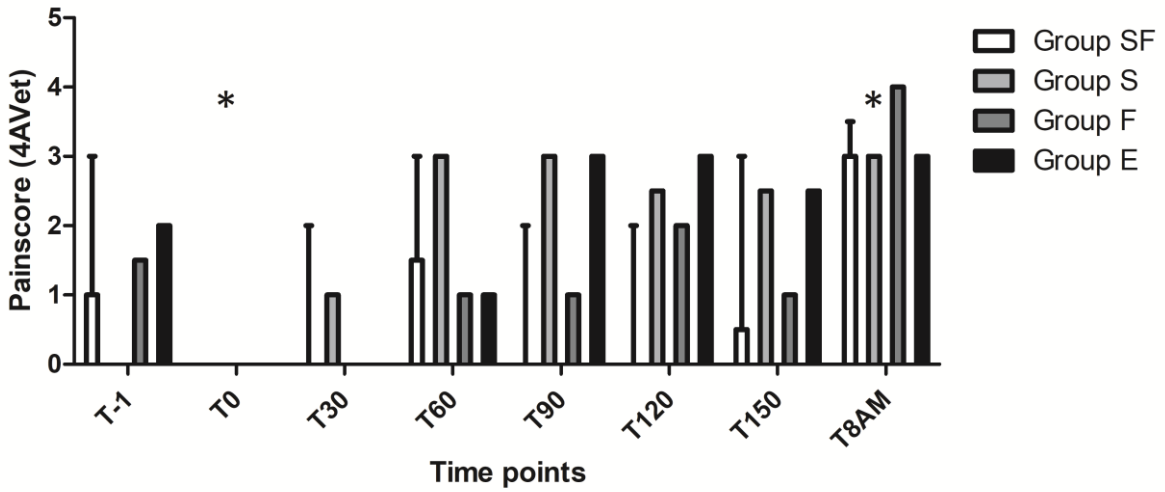
Duration of analgesia. The duration from the time locoregional anaesthesia was performed until extubation was 209 ± 29 minutes. Mean duration of analgesia was at least 349 ± 41 minutes. No difference between groups could be detected (p = 0.278). In animals that were not given methadone, the entire duration of effect could not be determined.

Figure 1.



The median value and error bar of a Visual Analogue Scale (VAS; 0 cm = no pain; 10 cm = worst pain possible) in 40 dogs undergoing surgery for stifle arthroscopy followed by tibia plateau levelling osteotomy (TPLO). Groups were randomly allocated with 10 dogs per group. For detailed legend see Table 1. The maximum value on the y-axis has been set at 5 cm for illustrative purposes. VAS was determined at various time points (T); T-1: preoperative, T0: at recovery when the dog was able to lift the head, T30-T150: minutes after extubation, T8AM: the morning following the day of surgery. * shows significant difference ($p < 0.05$) between T-1 and T8AM in group S.

Figure 2.



The median value and error bar of a Composite pain scale (4AVet; 0 = no pain; 15 = worst pain possible) evaluated in 40 dogs undergoing surgery for stifle arthroscopy followed by tibial plateau levelling osteotomy (TPLO). Groups were randomly allocated with 10 dogs per group. For detailed legend see Table 1. The maximum value on the y-axis has been set at 5 for illustrative purposes. Pain scores were evaluated at different time points (T); T-1: preoperative, T0: at recovery when the dog was able to lift the head, T30-T150: minutes after extubation, T8AM: the morning following surgery. * shows significant difference ($p < 0.05$) between T0 and T8AM in group S.

Discussion

The results of this study have shown that a combined femoral and sciatic nerve block or an epidural anesthetic lead to less cumulated perioperative consumption of opioids than a sciatic or a femoral nerve block alone. This confirms the analgesic efficacy of epidural anaesthesia and the combined sciatic and femoral nerve block for the provision of effective intraoperative antinociception and postoperative analgesia (Caniglia *et al.* 2012).

No dog given a combined sciatic and femoral nerve block received methadone during the postoperative phase, while some dogs given a single nerve block or a lumbosacral epidural anaesthetic required methadone.

In humans undergoing total knee arthroplasty, a single femoral nerve block seems to provide short-term postoperative analgesia which is superior to patient-controlled intravenous analgesia alone (Paul *et al.* 2010). However, a recent meta-analysis showed better postoperative pain control after a combined sciatic and femoral nerve block than a femoral nerve block alone for total knee arthroplasty. A reduced postoperative opioid consumption was noted in patients receiving combined nerve blocks compared to a single nerve block (Zorrilla-Vaca *et al.* 2018). In dogs undergoing TPLO, those with a single femoral nerve block were given more postoperative methadone than those given a combined sciatic and femoral nerve block (McCally *et al.* 2015). This underlines a superior postoperative analgesic efficacy provided by a combined nerve block compared to a single nerve block.

Considering the sensory innervation of the knee joint, only blockade of both nerves, the femoral and the sciatic, allows complete desensitization of this anatomical area. Indeed, the canine stifle joint is innervated by the medial articular nerve, which originates from the femoral nerve, and the lateral and occasionally posterior articular nerves, which derive from the sciatic nerve (O'Connor & Woodbury 1982).

Block failures were observed in groups E, S and F but not in group SF. In groups S and F this might be explained by insufficient analgesia resulting from anaesthesia of a single nerve despite its potential to provide some analgesia. Block failure in group E might be linked to the procedural failure

rate which has been reported to vary between 7% (Troncy *et al.* 2002) and 32% (Sarotti *et al.* 2015). The introduction of ultrasound guided techniques in clinical practice for the performance of locoregional sciatic and femoral nerve blocks has considerably increased their success rates (Perlas *et al.* 2008; Ponde *et al.* 2013). In dogs, the success rate of ultrasound guided sciatic nerve block varies between 86-93% (Marolf *et al.* 2019). An experienced clinician, sufficient practice or previous training are additional advantages associated with increased success rates (Rueda Rojas *et al.* 2019). The high success rate of ultrasound guided combined sciatic and femoral nerve blocks might explain the observation that no postoperative rescue analgesia was needed in group SF compared to groups E, S and F.

The analysis of the physiological variables recorded intraoperatively showed significant differences among groups. The lower blood pressure found in group E might be explained by the blockade of the sympathetic nervous system through local anaesthetics (Holte *et al*. 2004). The cause of hypotension of one dog in group S probably resulted from hypovolemia as blood pressure increased after a fluid bolus. During anaesthesia, modifications in physiological variables were used to estimate intraoperative nociception. There was no difference in these variables between arthroscopy and TPLO in groups SF and E while differences were observed in groups S and F. This can probably be explained by better control of nociception in groups SF and E, which might suggest that single nerve blockade provides insufficient control of nociception during TPLO.

Ropivacaine can provide effective sensory blockade of up to 6 hours duration (Feldman *et al.* 1996). The minimum duration of action of ropivacaine observed in our study is in accordance with its reported duration of action. Unfortunately, the beneficial effects of the nerve block had probably declined the day after surgery. This might explain why pain scores and VAS were higher at T8AM compared to T-1 and T0, despite the administration of buprenorphine. The administration of opioids during the perioperative period can provide effective control of nociception if nerve blocks are ineffective. However, opioids may induce potential side effects such as bradycardia, postoperative nausea, or vomiting. The strategic use of perioperative opioids seems judicious. The use of a composite pain scale during the postoperative period guides the administration of opioids. Bini *et al.* (2018) showed that the use of a composite pain scale to assess pain after TPLO decreases the amount of methadone required

when compared to its administration at fixed intervals. Furthermore, titrating analgesia to an individual's need was associated with a decreased incidence of vomiting and an increased food intake (Bini *et al.* 2018).

The present study has several limitations. The fact that pain scores where only assessed for 2.5 hours postoperatively should be considered as a major limitation. A modified German version of the French pain scale (4AVet) was used for pain evaluation. The use of a translated version of a pain scale may have affected its validation. No power calculation was performed *a priori* because data about the perioperative use of opioids after locoregional anaesthesia was unavailable at the time the study started. Finally, no confirmation method was applied to verify correct needle placement in the epidural space.

In group SF, ropivacaine injection using ultrasound guidance did not reduce the intraoperative need for fentanyl when compared to epidural or femoral or sciatic nerve block alone. However, physiological variables in group SF were more stable, suggesting better control of nociception. Analgesia provided by femoral and sciatic nerve blockade or epidural anaesthesia reduced the requirement for perioperative opioids compared to the use of single nerve blockade. Single nerve block seems insufficient to control nociception and early postoperative pain in certain dogs undergoing TPLO. The combination of sciatic and femoral nerve blockade seems the most promising technique to reduce postoperative methadone consumption during the first 2.5 postoperative hours when compared to epidural or single sciatic or femoral nerve anaesthesia.

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Experimental Section

Study 3:

Pharmacokinetics and effects of perineural or intravenous dexmedetomidine combined with ropivacaine for sciatic and saphenous nerve blocks in a canine model

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Vincent Marolf, Keila K. Ida, Danuta Siluk, Wiktoria Struck-Lewicka, Michał J. Markuszewski & Charlotte Sandersen

Abstract

Objective Determine the appropriate dose and route and the plasma level of dexmedetomidine for locoregional anesthesia in dogs.

Animals Seven experimental adult Beagle dogs.

Procedures In phase 1, dogs were randomized to receive the following three treatments in a Latin square crossover design: ultrasound-guided injection of ropivacaine 0.5% at the sciatic (0.2 mL/kg) and saphenous (0.2 mL/kg) nerve, combined with either saline (DEX0PN), dexmedetomidine at 1 µg/kg (DEX1PN) or 2 µg/kg (DEX2PN). In phase 2, nerve blocks were combined with intravenous dexmedetomidine 1 µg/kg (DEX1IV). Nerve blocks were evaluated at different timepoints. Dexmedetomidine concentrations were determined in collected plasma samples.

Results The duration of sensory nerve block was significantly longer in DEX1PN and DEX2PN compared to DEX0PN ($P < 0.05$). DEX1IV did not prolong nerve block duration compared to DEX0PN. Peak plasma concentrations after perineural administrations of dexmedetomidine were reached after 30 minutes in DEX1PN (338 ± 190 pg/mL) and DEX2PN (786 ± 549 pg/mL). Bioavailability was 54 ± 40% and 73 ± 43% in DEX1PN and DEX2PN groups, respectively. The highest plasma level of dexmedetomidine was measured in DEX1IV group (1032 ± 415 pg/mL) 5 minutes after injection.

Conclusion and clinical relevance One µg/kg of dexmedetomidine administered perineurally leads to lower plasma level compared to DEX2PN and DEX1IV, and significantly prolongs sensory sciatic and saphenous nerve blocks compared to DEX0PN and DEX1IV. One µg/kg of dexmedetomidine combined to ropivacaine seems an adequate dose for perineural injection for locoregional anesthesia in dogs.

Key words: dog; plexus; adjuvant; pain; femoral; ultrasound

Abbreviations

ASA: American Society of Anesthesiologist

BPM: Beats Per Minute

CV: Coefficient of Variation

DEX: Dexmedetomidine

HR: Heart Rate

PN: Perineural

SD: Standard Deviation

Introduction

The combination of local anesthetics and DEX, a potent alpha-2 adrenoceptor agonist, has gained popularity for locoregional anesthesia. It has been proven to prolong sensory nerve blockade, enhance patients' satisfaction, and reduce pain and postoperative oral morphine consumption (Marhofer & Brummett 2016, Abdallah & Brull 2013, Vorobeichik *et al.* 2017).

Experimental studies with high doses of DEX showed that it did not induce axon and myelin degeneration and confirmed that its use is safe when combined with local anesthetics (Marhofer & Brummett 2016, Brummett *et al.* 2008)

In humans, the results of several clinical trials have been supporting the use of long-acting amide-type local anesthetic combined with DEX in clinical settings (Akhondzadeh *et al.* 2018, Fritsch *et al.* 2014). The amount of DEX required to prolong locoregional anesthesia in different studies varies and DEX doses of 100 µg per nerve block, 0.75 µg/kg and 2 µg/kg have been suggested (Marhofer & Brummett 2008, Keplinger *et al.* 2015, Bisui *et al.* 2017, Jung *et al.* 2018). The minimal dose of DEX to efficaciously prolong sensory nerve block remains to be determined. Whether the effect of DEX on local nerve block is linked to the local or systemic action of the drug remains unknown. Intravenous administered DEX prolongs the effect of nerve block in a similar manner than when it is administered perineurally in humans (Abdallah *et al.* 2016). However, the systemic use of DEX can induce effects such as bradycardia and sedation that might be stronger after IV compared to perineural administration.

In dogs, studies about perineural dexmedetomidine for locoregional anesthesia are rare (Bartel *et al.* 2016, Trein *et al.* 2017). Ultrasound-guided sciatic and saphenous nerve blocks are performed regularly in experimental and clinical daily practice in dogs, but local anesthetics are not commonly mixed with DEX (Campoy *et al.* 2010). Plasma levels and the optimal dose of DEX for perineural injection to significantly prolong nerve block in dogs are unknown.

The aim of the study was to evaluate the effects of ropivacaine 0.5% combined with DEX for sciatic and saphenous nerve blocks in dogs. The primary objective was to compare nerve block duration after perineural injection of ropivacaine alone with ropivacaine combined with perineural or IV DEX at 1 and 2 µg/kg. The secondary objective was to determine whether the plasma levels of DEX could potentially

be associated with its clinical effects when used for the sciatic and saphenous nerve blocks. We hypothesized that combination of ropivacaine and DEX would significantly prolong nerve block duration compared to ropivacaine alone, and that this effect would not be associated with the plasma levels of DEX.

Materials and Methods

An authorisation was delivered by the commission for the ethical use of animals at the Faculty of Veterinary Medicine, University of Liège, Belgium (No 16-1887). A sample size calculation for continuous outcome and superiority trial using an online software (www.sealedenvelope.com) has been used. Power and standard error alpha were set at 80% and 5%, respectively. The mean duration (± SD) of sensory ulnar nerve block produced by 22.5 mg of ropivacaine combined with 100 µg of DEX (9.1 ± 3.3h) compared to the mean duration of sensory nerve block which might be expected after perineural injection of 0.2 mL/kg of ropivacaine 0.5% in dogs (4h) has been used for calculation (Keplinger *et al.* 2015). The calculation revealed that a total of 7 dogs per group were required.

Animals. To be included in the study, dogs needed to be healthy on physical examination, have no abnormalities after orthopaedic and neurologic examination and classified as ASA I or II for anaesthesia related risk. All dogs were 10 years old except for one female which was 6 years old. Seven intact experimental adult Beagle dogs (four males, three females) weighing 16.9 ± 2.3kg (mean ± SD) underwent first three experimental treatments with a minimum wash out period of one week between treatments (phase 1) and underwent a fourth experimental treatment eight months later (phase 2). The dogs were fed dry commercial food once daily. Dogs were fed two hours after recovery from anesthesia and water was provided ad libitum. The experiments were carried out in an experimental room located in the same building as the kennel. Dogs were kept in single cages for nerve block evaluation and were returned to the kennel after the last evaluation.

Anesthesia. A 22-gauge catheter was inserted in a cephalic vein and Ringer's lactate solution was administered at 5 mL/kg/hr. Anesthesia was induced with IV propofol[a] (4-8 mg/kg) and an adequately sized cuffed endotracheal tube was placed in the trachea. The dogs were connected to a circle breathing system and 2-3% sevoflurane[b] in 100% oxygen was used for maintenance. Monitoring included pulse oximetry, non-invasive blood pressure measurement with a size 3 paediatric cuff placed around the front limb, a 3-lead base apex electrocardiogram, end-tidal carbon dioxide and anesthetic vapor analyser using

a multiparameter monitor[c]. A jugular vein catheter[d] was placed to enable post-anesthetic stress free blood sampling.

Sciatic and saphenous nerves blockade. A DEX solution of 500 µg/mL[e] was diluted with 0.9% saline solution in a 1:10 ratio to obtain a 50 µg/mL solution. In phase 1, dogs were randomized for the first three treatments using an online randomization generator (www.random.org). The groups consisted of perineural sciatic and saphenous injections of 0.5% ropivacaine[f] (0.4 mL/kg) + 0.9% saline solution (0.04 mL/kg; DEX0PN group); 0.5% ropivacaine (0.4 mL/kg) + diluted DEX (0.02 mL/kg = 1 µg/kg) + saline solution (0.02 mL/kg; DEX1PN group); 0.5% ropivacaine (0.4 mL/kg) + diluted DEX (0.04 mL/kg = 2 µg/kg; DEX2PN group). In phase 2, all dogs were allocated to treatment with perineural 0.5% ropivacaine (0.4 mL/kg) + diluted DEX (0.02 mL/kg = 1 µg/kg) injected IV (DEX1IV group). The sciatic and saphenous nerve blocks were performed first and DEX was injected slowly over 60 seconds through the cephalic vein catheter directly thereafter and dogs were recovered. The total volume of each drug administered perineurally was divided equally between the sciatic and saphenous nerves.

The area over the saphenous and the sciatic nerves was clipped and disinfected. Sterile contact gel was applied and ultrasound guided nerve block[g] of the sciatic and saphenous nerves was completed with an insulated needle[h] using a standard approach (Campoy *et al.* 2010). An electrical nerve stimulator[i] was used to confirm right needle placement for sciatic nerve block. The volume corresponding to the content of the echogenic needle and injection line (0.7mL) was flushed with 0.7mL of saline solution after each perineural injection. The same experienced board-certified veterinary anesthetist (VM) performed all nerve blocks and was unaware of the treatments given in the first three treatment groups.

Post-anesthetic phase. Sevoflurane administration was discontinued and dogs were recovered from anesthesia. The minutes required for extubation were recorded.

Heart rates were assessed every 15 minutes by left thorax wall auscultation with a stethoscope until return of pre-anesthetic baseline measurements. The sedation scores were evaluated every 15 minutes

using a sedation scoring scheme until a score of 0 or a negative scoring was measured (negative value = awake; 0 = no sedation; 14 = maximum sedation) (Hofmeister *et al.* 2010).

Sciatic and saphenous nerve blocks were evaluated using nociception, locomotion and proprioception tests performed every 15 minutes after perineural injection until recovery from nerve blockade. The investigator was blind to the treatments during phase 1 of the experiments. Nociception was assessed by clamping the skin with a needle holder for 2 seconds over the caudal part of the thigh (to evaluate the sciatic nerve), over the dorsal part of the fourth metatarsus (to evaluate the fibular nerve), over the plantar part of the fourth metatarsus (to evaluate the tibial nerve), over the medial part of the distal femur (to evaluate the saphenous nerve). Behaviours such as barking, crying, immediate withdrawing of the limb, escaping, actively looking at the stimulated site were attributed a score of 1 (sensory feeling present), mild reactions such as slow withdrawing of the limb or slow head movement towards the stimulated area were attributed a score of 2 (sensory feeling partially present) and absence of reaction was attributed a score of 3 (sensory feeling absent).

Locomotion was assessed using the following scores to define motor block: 1, normal gait; 2, abnormal gait, missteps observed; 3, abnormal gait, dragging of the limb.

Spontaneous reposition of the limb after the dorsal part of the metatarsal phalanxes were positioned on the ground was evaluated with scores (1, immediate reposition; 2, reduced or retarded reposition; 3, no reposition) to characterize proprioception deficits.

Nociception, locomotion and proprioception tests were performed every 15 minutes (T1, T2, T3, T4, etc.) until at least two successive score of 1 were recorded for each parameter tested. The time elapsed from the perineural injection until the first score 2 or 3 (partial blockade) and score 3 (complete blockade) was defined as the onset time. The time that the scores 2 or 3 and score 3 only were observed was defined as the duration of partial and complete nerve blockade, respectively.

Pharmacokinetics. Ten mL of blood were withdrawn from the jugular catheter before two mL of blood were sampled for analysis. The 10mL were restored to avoid excessive blood loss and the jugular catheter was flushed with 2mL of saline solution. Blood samples were placed into heparinized tubes at

15, 30, 60, 90, 120, 180, 240, 360, 480 min (an additional blood sample was collected at 5 min in DEX1IV group), which were immediately centrifuged (4 min at 2200×g). Plasma samples were collected with a pipette, identified and frozen at -80C° until transport on dry ice to the Medical University of Gdańsk, Poland. Samples were analysed using reverse phase high performance liquid chromatography coupled with triple quadrupole mass spectrometry detection (RP-HPLC-QqQ/MS, Agilent Technology, Waldbronn, Germany) with the use of previously developed and validated determination method (Szerkus *et al.* 2017).

Because of the possible species differences, revalidation of the most crucial analytical method parameters (specificity, linearity, intra- and interday precision and accuracy) was performed. The calibration curves for DEX were made by spiking canine plasma with proper DEX concentrations. Each calibration curve was composed of 7 concentration levels (5, 10, 50, 100, 500, 1000, 2500 pg/mL). Precision and accuracy of the method were studied with the use of quality control plasma samples at three concentration levels (20 pg/mL – LQC, 200 pg/mL – MQC, 2000 pg/mL – HQC). Besides, the specificity of DEX was carried out by the determination of blank canine plasma extract for DEX ion transition as well as canine plasma extract fortified with DEX.

The plasma concentration of DEX was plotted against time and standard formulas were used according to the best compartmental model that fit the data to calculate the bioavailability ([(AUCPN/AUCIV) x (DOSEIV/DOSEPN)] x 100), elimination half-life, plasma clearance, and volume of distribution of DEX.

Statistical analysis. Data distribution was assessed for normality using the Kolmogorov-Smirnov test. The bodyweight, anesthesia time, time for extubation, HR, onset and duration of sensory nerve blockage, proprioception, locomotion, plasma concentration, bioavailability, and half-life were compared between groups using two-way repeated measures ANOVA and post hoc Tukey test. Sedation scores were assessed within a group using Wilcoxon Signed Rank test. All data were assessed using GraphPad Prism 5.03 and $P < 0.05$ was considered significant.

Results

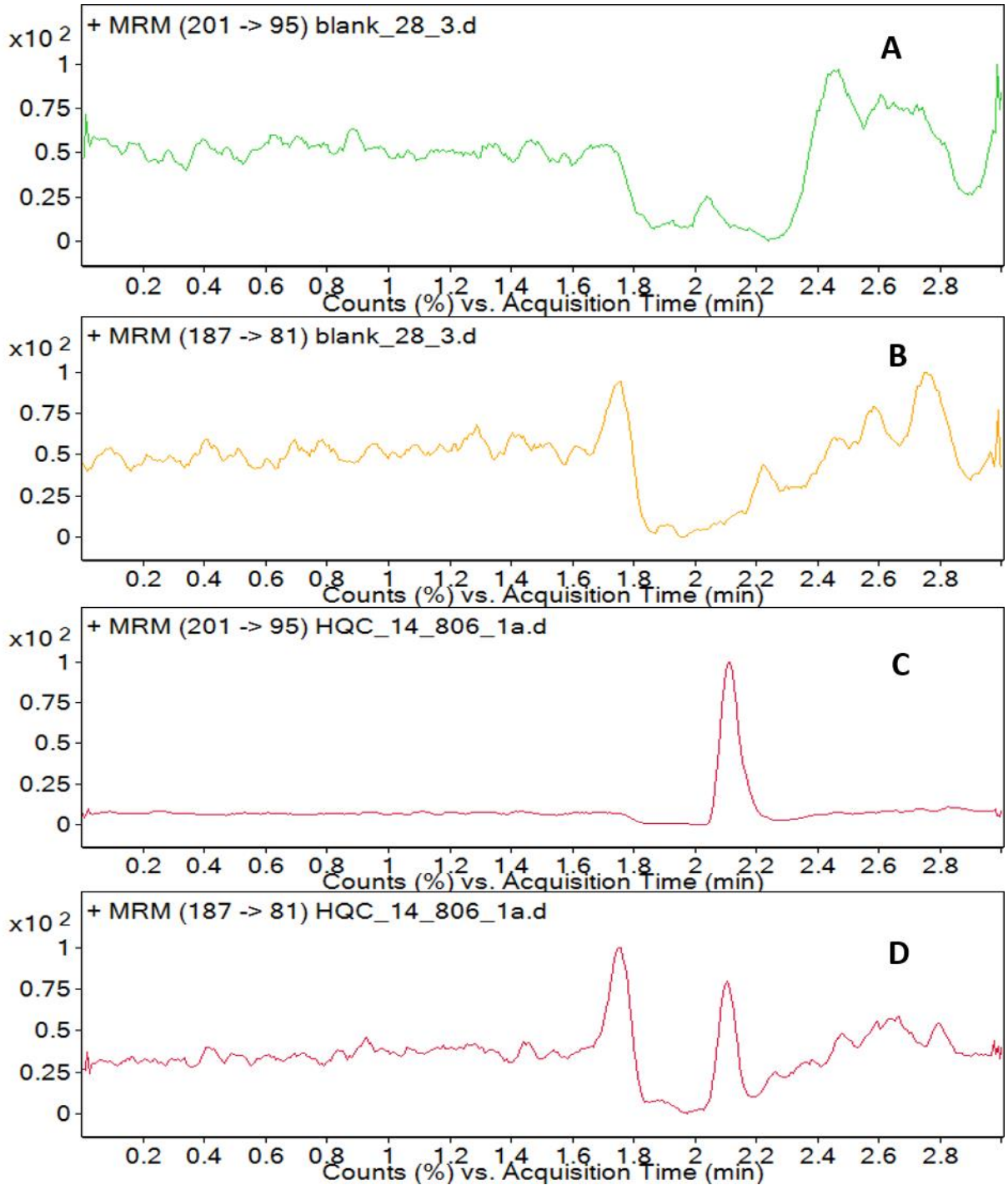
One dog was euthanized after sudden diagnosis of liver tumour metastasis. Data of this dog were obtained only for the DEX0PN and DEX1PN treatments.

No significant differences in bodyweight (DEX0PN: 16.9 ± 2.3 kg; DEX1PN: 16.9 ± 2.3 kg; DEX2PN: 16.6 ± 2.4 kg; DEX1IV: 14.5 ± 1.6 kg; P = 0.2051) and anesthesia time (DEX0PN: 26.1 ± 7.8 minutes; DEX1PN: 21.4 ± 6.1 minutes; DEX2PN: 28.2 ± 9.1 minutes; DEX1IV: 25.2 ± 8.6; P = 0.4844) were observed between groups.

Extubation time was longer in the DEX1IV group (13.0 ± 4.2 minutes) compared with the DEX1PN (5.1 ± 2.3 minutes; P = 0.0013) and DEX0PN (5.7 ± 2.3 minutes; P = 0.0022) groups. The DEX2PN group (10.5 ± 4.2 minutes) took longer to be extubated than the DEX1PN group (P = 0.0142).

Analytical method validation. The analytical determination was based on a formerly developed method, used for analysis of DEX in pediatric patients after intravenous administration of the drug (Szerkus *et al.* 2017). As a result of revalidation, the method was linear in a range from 5 pg/mL to 2500 pg/mL with correlation coefficient above 0.999. The result of specificity is illustrated in Figure 1 while the intra- and inter-day precision and accuracy results are presented in Table 1. Coefficient of variation for precision was below 10 % for both intra- and inter-day studies. Concerning accuracy, the differences between determined concentrations and nominal concentrations were less than 10 %. Based on the obtained revalidation data, the method revealed to be selective, linear in the tested concentration range, precise and accurate, as the validation parameters values fell within the bioanalytical methods criteria proposed by FDA guidance[j].

Figure 1.



Exemplary chromatograms of the blank canine plasma extract for dexmedetomidine ion transition (A) or for detomidine (IS) ion transition (B), canine plasma extract fortified with dexmedetomidine (C) or detomidine (D). Ion transitions were followed in the multiple reaction monitoring mode (MRM).

Table 1. Validation parameters (inter- and intra-day precision and accuracy) from dexmedetomidine determination with the use of LC-QqQ/MS technique in MRM mode.

INTRA-DAY PRECISION AND ACCURACY (n=6)				
Nominal concentration (pg/mL)	**Determined concentration (pg/mL)**	**Standard deviation (pg/mL)**	**Precision (CV)**	**Accuracy (%)**
20	21.6	1.8	8.54	107.82
200	219.4	11.1	5.07	109.69
2000	2077.8	57.9	2.79	103.89

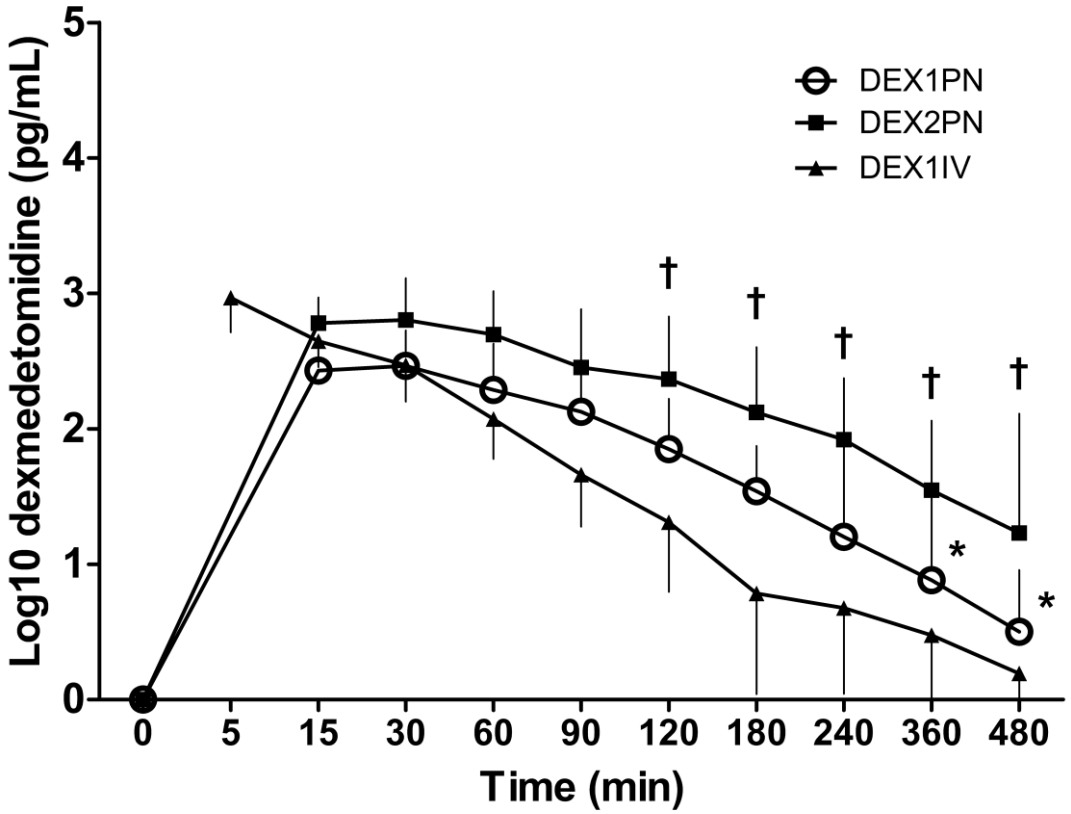
INTER-DAY PRECISION AND ACCURACY (n=20)				
Nominal concentration (pg/mL)	**Determined concentration (pg/mL)**	**Standard deviation (pg/mL)**	**Precision (CV)**	**Accuracy (%)**
20	20.5	1.8	9.01	102.51
200	215.4	14.0	6.49	107.69
2000	2110.2	118.1	5.59	105.51

CV: coefficient of variance

Pharmacokinetics. Plasma samples of one dog in group DEX1IV had to be excluded from analysis because the results were not consistent with other data (low or undetectable level of DEX). This might either be related to blood sampling errors, perivascular injection, an error in DEX doses administered or analysis dysfunction. Sufficient plasma samples were not available to repeat analysis and calibration for RP-HPLC-MS. The plasma concentration versus time curve is shown in the figure 2. The shape of the curve indicates that a two-compartmental model best fit the data.

Dexmedetomidine plasma levels were 1032 ± 415 pg/mL at first measurement (5 minutes after injection) in DEX1IV group. Peak plasma concentrations were reached at 30 minutes in the DEX1PN group (348 ± 200 pg/mL) and in the DEX2PN group (819 ± 607 pg/mL). DEX plasma concentration was significantly different in DEX1IV at 120 (P = 0.0212), 180 (P = 0.0051), 240 (P = 0.0084), 360 (P = 0.0062), and 480 (P = 0.0482) minutes compared with DEX2PN, and at 360 (P = 0.0224) and 480 (P = 0.0482) minutes compared with DEX1PN (Fig. 2). Results for bioavailability, elimination half-life, volume of distribution and plasma clearance are presented in Table 2.

Figure 2.



Mean plasma dexmedetomidine concentration in dogs that received perineural sciatic and saphenous nerve injections of 0.5% ropivacaine (0.4 mL/kg) combined with perineural injection of a lower (1 µg/kg; DEX1PN) or higher (2 µg/kg; DEX2PN) dose of dexmedetomidine or with IV administration of

dexmedetomidine (1 µg/kg; DEX1IV). For perineural injections, the dose was divided equally between the 2 sites. Error bars represent SD. *Within a time point, value for the DEX1PN treatment was significantly ($P < 0.05$) different from the value for the DEX1IV treatment. †Within a time point, value for the DEX2PN treatment was significantly ($P < 0.05$) different from the value for the DEX1IV treatment.

Table 2. Pharmacokinetic variables of dexmedetomidine in dogs administered ropivacaine for sciatic and saphenous nerve block combined with either saline (DEX0PN), 1 µg/kg dexmedetomidine (DEX1PN), or 2 µg/kg dexmedetomidine (DEX2PN) perineurally, or 1 µg/kg dexmedetomidine IV (DEX1IV).

	DEX1PN (n=7)	**DEX2PN (n=6)**	**DEX1IV (n=5)**
Bioavailability (%)	54 ± 40	73 ± 43	N/A
Half-life (minutes)	147 ± 98	258 ± 119	93 ± 50
Plasma clearance (mL/kg/min)	N/A	N/A	4.6 ± 0.3
Volume of distribution (L/kg)	N/A	N/A	0.6 ± 0.4

N/A: not applicable, n: number of dogs

Sedation. An increase in the sedation scores was observed at 15 minutes in DEX1PN (1.0 [0.0 – 7.0]; P = 0.0340), DEX2PN (2.5 [0.0 – 14.0]; P = 0.0055), and DEX1IV (13.0 [6.0 – 14.0]; P = 0.0335) groups, and at 30 minutes in DEX2PN (1.5 [0.0 – 13.0]; P = 0.0055) and DEX1IV groups (4.5 [2.0 – 9.0]; P = 0.0350) compared with baseline (score 0 in all groups). Dogs of the DEX0PN group had no significant changes in the sedation scores throughout.

In the DEX1IV group, the sedation scores were significantly higher compared with all other groups at 15 minutes (versus 0 [0 – 2] in DEX0PN, P = 0.0027; versus 1 [0 – 7] in DEX1PN, P = 0.0048; versus 2.5 [0 – 14] in DEX2PN, P = 0.0496), and were greater than DEX0PN (0 [0 – 2], P = 0.0032) and DEX1PN (0 [0 – 5], P = 0.0099) groups at 30 minutes.

Heart rate. The HR 15 minutes after discontinuation of anesthesia were 101 ± 9 BPM in DEX1PN group; 83 ± 24 BPM in DEX2PN group; 107 ± 35 BPM in DEX0PN group; 74 ±15 in DEX1IV group. A significant difference was present between baseline versus 15 minutes in the DEX1IV group ($p = 0.0021$) and between DEX0PN versus DEX1IV at 15 minutes ($p = 0.0250$).

Nociception. The onset time and duration of the sensory nerve blockade are presented in Table 3. The sensory response was present (score 1) in all dogs at baseline. No significant differences in the onset time for tibial ($P = 0.1322$), fibular ($P = 0.3848$), saphenous ($P = 0.1297$), and sciatic ($P = 0.0596$) nerves sensory blockade (scores 2 and 3) were observed among groups. The onset time for a complete sensory tibial ($P = 0.3598$), fibular ($P = 0.2739$), and saphenous ($P = 0.1811$) nerves blockage (score 3) was not significantly different between groups. The only significant difference in the beginning of a sensory blockade was observed for the complete sciatic nerve blockade that was faster in the DEX2PN group than in the DEX0PN group ($P = 0.0073$). The duration of sensory blockade (scores 2 and 3) and complete sensory blockade (score of 3) of the tibial nerve was significantly longer in DEX1PN group compared with the DEX0PN ($P = 0.0029$ and $P = 0.0030$, respectively) and DEX1IV groups ($P = 0.0121$ and $P = 0.0055$, respectively). The sensory fibular nerve block (scores 2 and 3) was significantly longer in the DEX1PN and DEX2PN compared with the DEX0PN ($P = 0.0096$ and $P = 0.0101$, respectively) and DEX1IV ($P = 0.0148$ and $P = 0.0130$, respectively) groups. The complete sensory fibular nerve block (score 3) was longer in the DEX1PN ($P = 0.0108$) and DEX2PN ($P = 0.0060$) groups compared with DEX0PN. The sensory saphenous nerve blockade (scores 2 and 3) and complete blockade (score 3) lasted significantly longer in DEX1PN group compared with DEX0PN ($P = 0.0022$ and $P = 0.0146$, respectively) and DEX1IV ($P < 0.0001$ and $P = 0.0052$, respectively). In the DEX2PN group, the duration of sensory nerve blockade (scores 2 and 3) was longer than in DEX1IV ($P = 0.0368$), and the complete block (score 3) was longer than in DEX0PN ($P = 0.0337$) and DEX1IV ($P = 0.0265$).

The sensory sciatic nerve blockade (scores 2 and 3) and complete blockade (score 3) lasted

significantly longer in DEX1PN (P = 0.0154 and P = 0.0132, respectively) and DEX2PN (P = 0.0106 and P = 0.0003, respectively) groups compared with DEX0PN.

Table 3. Median (minimum – maximal) onset times and duration of mild sensory blockade (scores 2 and 3) and complete sensory blockade (scores 3) of the tibial, fibular, saphenous, and sciatic nerves in dogs administered ropivacaine combined with either saline (DEX0PN), 1 µg/kg dexmedetomidine (DEX1PN), or 2 µg kg dexmedetomidine (DEX2PN) perineurally, or 1 µg/kg dexmedetomidine IV (DEX1IV).

	DEX0PN	**DEX1PN**	**DEX2PN**	**DEX1IV**
Tibial nerve				
Onset of blockade (minutes)	30 (15 - 60)	30 (15 - 45)	15 (15 - 60)	15 (15 - 45)
Duration of blockade (minutes)	190 (105 - 235)	400 (160 - 655)*†	332 (190 - 520)	205 (135 - 310)
Onset of complete blockade (minutes)	45 (15 - 105)	45 (15 - 60)	30 (15 - 60)	52 (15 - 105)
Duration of complete blockade (minutes)	75 (45 - 175)	340 (15 - 490)*†	190 (115 - 325)	102 (15 - 280)
Fibular nerve	**DEX0PN**	**DEX1PN**	**DEX2PN**	**DEX1IV**
Onset of blockade (minutes)	30 (15 - 60)	15 (15 - 45)	15 (15 - 30)	15 (15 - 45)
Duration of blockade (minutes)	235 (145 - 325)	415 (160 - 685)*†	347 (235 - 550)*†	197 (175 - 310)
Onset of complete blockade (minutes)	45 (15 - 105)	30 (15 - 45)	22 (15 - 60)	52 (15 - 60)
Duration of complete blockade (minutes)	130 (60 - 190)	355 (90 - 505)*	227 (130 - 400)*	137 (60 -295)
Saphenous nerve	**DEX0PN**	**DEX1PN**	**DEX2PN**	**DEX1IV**
Onset of blockade (minutes)	15 (15 - 45)	15 (15-45)	15 (15 - 15)	15 (15 - 15)

Duration of blockade (minutes)	250 (75 - 430)	445 (370 - 490)*†	400 (160 - 595)†	280 (160 - 310)
Onset of complete blockade (minutes)	45 (15 - 90)	30 (15 - 150)	15 (15 - 30)	45 (15 - 75)
Duration of complete blockade (minutes)	190 (15 - 340)	310 (250 - 415)*†	332 (135 - 550)*†	197 (75 - 265)
Sciatic nerve	**DEX0PN**	**DEX1PN**	**DEX2PN**	**DEX1IV**
Onset of blockade (minutes)	30 (15 - 75)	15 (15 - 45)	15 (15 - 30)	15 (15 - 45)
Duration of blockade (minutes)	145 (105 - 310)	340 (160 - 520)*	340 (190 - 505)*	205 (105 - 310)
Onset of complete blockade (minutes)	60 (15 - 105)	60 (15 - 75)	15 (15 - 30)*	15 (15 - 60)
Duration of complete blockade (minutes)	75 (15 - 135)	190 (75 - 445)*	310 (175 - 460)*	162 (75 - 280)

*different from the DEX0PN group ($P < 0.05$); † different from the DEX1IV group ($P < 0.05$).

Proprioception and Locomotion. No significant differences in the onset times and duration of the proprioceptive and locomotor deficits were observed within and between groups (Table 4).

Table 4. Median (minimum – maximal) onset times and duration of the proprioceptive deficit and abnormal gait (scores 2 and 3) and absent proprioception and complete motor blockade (scores 3) in dogs administered ropivacaine combined with either saline (DEX0PN), 1 µg/kg dexmedetomidine (DEX1PN), or 2 µg/kg dexmedetomidine (DEX2PN) perineurally, or 1 µg/kg dexmedetomidine IV (DEX1IV).

	DEX0PN	**DEX1PN**	**DEX2PN**	**DEX1IV**
Proprioception				
Onset of proprioception deficit (min)	30 (15 - 60)	30 (15 - 60)	22 (15 - 60)	15 (15 - 45)
Duration of proprioception deficit (min)	130 (30 - 160)	250 (60 - 655)	167 (120 - 385)	167 (130 - 280)
Onset of absent proprioception (min)	45 (30 - 60)	45 (15 - 60)	45 (15 - 75)	15 (15 - 60)
Duration of absent proprioception (min)	90 (0 - 130)	175 (0 - 625)	117 (15 - 325)	120 (75 - 250)
Locomotion	**DEX0PN**	**DEX1PN**	**DEX2PN**	**DEX1IV**
Onset of abnormal gait (min)	30 (30 - 30)	45 (15 - 75)	30 (15 - 60)	15 (15 - 60)
Duration of abnormal gait (min)	105 (0 - 190)	265 (15 - 685)	160 (15 - 385)	167 (75 - 280)
Onset of motor block (min)	45 (45 - 60)	45 (15 - 60)	45 (15 - 75)	15 (15 - 90)
Duration of motor block (min)	15 (0 - 130)	175 (0 - 625)	115 (0 - 325)	112 (15 - 220)

Complications. A vessel was punctured during ultrasound-guided saphenous nerve block in one dog. Blood was aspirated in the needle hub before perineural injection. The needle was immediately withdrawn and reoriented to avoid intravascular injection. Three dogs got injured in the interdigital space during the second round of experiments. The injury was not apparent on the day the experiments were carried out. The day after, the paw was red, warm and swollen. The interdigital space was mildly ulcerated in two dogs and moderately ulcerated in one dog. The injury was probably due to excessive weight bearing on dorsal aspect of the digits (paw knuckling) and excessive dragging of the limb. The dogs were medically examined twice a day by a veterinarian and appropriate care were provided. The hairs were clipped and the injury was disinfected with povidone-iodine solution. They were treated with oral carprofen for 5 days and oral amoxicillin-clavulanic acid for 10 days. The third experimental treatment was started after full healing. A soft protective bandage was applied over the digits and a soft mattress was placed in the cage for the rest of the trial in all dogs. The bandage was removed for each assessment and replaced directly afterwards. The paw was carefully examined visually and by palpation for injury at each evaluation time point and three times a day during two days after the experiments.

Discussion

The results of this study indicate that perineural administration of 1 and 2 µg/kg (0.5 and 1 µg/kg per nerve block, respectively) of DEX added to ropivacaine 0.5% significantly prolong sciatic, fibular, tibial and saphenous sensory nerve blocks without increasing the duration of the motor blockade and proprioception deficits in experimental dogs. A systematic review in humans on adjuvants and local anesthesia has revealed that analgesia provided by DEX and ropivacaine can be prolonged by 50 minutes up to 4.5h (Kirksey *et al.* 2015). Our findings are in accordance to the actual data in humans but doses and side effects of DEX and concentrations of ropivacaine varies greatly between trials (Marhofer & Brummett 2016, Abdallah & Brull 2013, Akhondzadeh *et al.* 2018, Fritsch *et al.* 2014, Keplinger *et al.* 2015, Bisui *et al.* 2017, Jung *et al.* 2018). A dose of 0.1 µg/kg of DEX per nerve block combined with bupivacaine for sciatic and femoral nerve block in dogs did not find a significant increase in nerve block duration (Trein *et al.* 2017). The authors suggested a higher dose of DEX is needed and doses of 1µg/kg and 2 µg/kg were evaluated in our study. The choice of the dose for our study has also been guided by practice in human medicine (Kirksey *et al.* 2015). A study compared 1, 1.5 and 2 µg/kg of DEX for interscalene brachial plexus block (Jung *et al.* 2018). The authors suggested that 2 µg/kg might be the optimal dose but that it was associated with an increased risk of hypotension.

Perineural use of DEX has the potential to induce sedation, bradycardia and hypotension already at doses of 1µg/kg in humans (Lin *et al.* 2013, Rancourt *et al.* 2012). There was no decrease in HR when DEX was administered perineurally but it was observed when DEX was administered IV. This might suggest that side effects such as bradycardia might be stronger when DEX is administered IV for locoregional anesthesia in dogs. Sedation scores of dogs in DEX groups were significantly higher in DEX2PN and DEX1IV group up to 30 minutes and extubation time was longer in DEX1IV group. Moderate and deep sedation in human patients was associated with plasma DEX concentrations of 0.2-0.3 ng/mL and 1.9 ng/mL, respectively (Bloor *et al.* 1992, Ebert *et al.* 2000). In the present study, such levels were measured after perineural and intravenous DEX 5, 15 and 30 or even up to 60 minutes after the injections. Plasma level of DEX after perineural brachial plexus block with 150 µg were measured at 0.64 ng/mL 30 minutes after injection and progressively decreased by 0.002 ng/mL per minute

(Fritsch *et al.* 2014). This is similar to the plasma level measured in DEX2PN. The undesired systemic effects of DEX could be avoided or reduced in DEX1PN group. The target for locoregional anesthesia is to keep a low plasma level of DEX. A dose of 1 µg/kg (0.5 µg/kg per nerve block) of DEX seems to be an effective dose to prolong sensory nerve block while minimising systemic side effects. A dose of 2 µg/kg did not provide significant advantages. It is possible that at dose lower than 1 µg/kg per dog (<0.5 µg/kg per nerve block) as evaluated in our study but higher than 0.2 µg/kg per dog (>0.1 µg/kg per nerve block) as evaluated in another study might be effective to prolong sensory sciatic and saphenous nerve block (Trein *et al.* 2017). Bioavailability is lower in DEX1PN group and the perineural rather than the intravenous use of DEX for locoregional anesthesia seems better.

In the present study, DEX administered IV did not significantly prolong peripheral nerve block compared to control group, which is in opposition to findings in humans (Abdallah *et al.* 2016). This difference might be explained by the difference in study design because the duration of analgesia and the 24h cumulative morphine consumption were used as endpoints. The differences in the duration of action on sensory nerve block between perineural and IV administration of DEX might be linked to the mechanism of action by which DEX prolongs locoregional anesthesia. Even though the exact mechanism remains unclear, a recent study has started to provide an answer. Andersen *et al.* have analysed the effect of perineural DEX in volunteers (Andersen *et al.* 2017). They observed that saphenous nerve block was prolonged when DEX was combined with ropivacaine compared with saphenous nerve block performed without DEX in the contralateral limb. They concluded that the perineural mechanism of action of DEX might be peripheral, which is supported by our results. We have observed that sensory nerve blocks were prolonged only when DEX was injected perineurally. Such effect was not correlated to the plasma levels of the drug, which were initially lower and then similar compared with IV DEX. These observations suggest that the main mechanism of action of perineural DEX might be peripheral. The perineural and local mechanism of action of alpha-2 adrenoceptor agonists DEX is thought be related to vasoconstriction, which can delay the absorption of ropivacaine, and inhibit compound action potentials (Yoshitomi *et al.* 2008, Kosugi *et al.* 2010). The perineural action of DEX by blocking the Ih-current to keep the nerve in a hyperpolarised state is an additional

explanation of the peripheral mechanism of action (Brummett *et al.* 2011). A dose-dependent central analgesia produced by DEX seemed unlikely as it did not contribute to increase the duration of the locoregional anesthesia in DEX1IV. A centrally mediated antinociceptive effect of DEX through stimulation of presynaptic alpha-2 adrenoceptors in the central nervous system seems less probable but cannot be excluded. The perineural injection of DEX could be effective through its action at the spinal cord.

Limitations of the study include the small number of animals and some missing data due to the fact that one dog had to be euthanised and that plasma concentrations analysis of DEX of one dog in DEX1IV group could not be repeated. The puncture related vessel damage and self-injuries may have influenced the behavioral results of the dogs. The fact that the investigator was not blinded to the DEX1IV treatment is another main limitation of the study. After randomisation, results regarding nerve block duration were evaluated with unpaired *t*-test. Significant differences were obtained for DEX0PN vs DEX1PN (P = 0.0012) and DEX0PN vs DEX2PN (P = 0.0097) but not for DEX1PN vs DEX2PN (P = 0.0819). It was concluded that 1µg/kg was sufficient to prolong nerve sensory nerve block duration and, therefore, the IV administration of this dose (DEX1IV group) was performed for comparison of the systemic effects. This experimental design was purposefully planned before the experimentations according to the 3R guidelines for animal welfare. A group administered 2 µg/kg IV was not planned to reduce the number of experiments per animal according to the guidelines.

The perineural injection of 1 µg/kg of DEX combined to ropivacaine 0.5% for locoregional anesthesia in dogs seems to balance the benefit of prolonged sensory nerve blocks while minimising side effects. At a dose of 1 µg/kg, the perineural route should be favoured over the IV route to administer DEX for locoregional analgesia. At the tested doses, the plasma level of DEX for locoregional anesthesia were low and did weakly correlate with sensory nerve block duration. These findings and the pharmacokinetic model might guide the route and dose of DEX for locoregional analgesia to be assessed in clinical studies in dogs.

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Footnotes

[a] Diprivan 1%, Astra Zeneca, Belgium

[b] SevoFlo, Abbott Laboratories Ltd, UK

[c] Datex-Ohmeda, Finland

[d] Leader-Flex 19G, Vygon, France

[e] Dexdomitor, Orion, Belgium

[f] Naropin, AstraZeneca, Belgium

[g] Mindray, Schöneiche, Germany

[h] SonoPlex Stim cannula, 21G, 10 cm, Pajunk, Germany

[i] TOF-watch, Organon, Ireland

[j] Guidance for Industry, Bioanalytical Method Validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and research, Center for Veterinary Medicine, May 2001; http://academy.gmp-compliance.org/guidemgr/files/4252FNL.PDF, last accessed 09.08.2020.

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Experimental section

Study 4:

Perineural dexmedetomidine seems to reduce postoperative methadone requirements in dogs after tibial plateau levelling osteotomy: a two-center study

Preliminary Results

Vincent Marolf, Alexandru Tutunaru, Julie Selz, Pierre Picavet, Claudia Spadavecchia, Charlotte Sandersen

Abstract

Objective Evaluate the efficacy of a perineural injection of dexmedetomidine (1µg/kg) combined with ropivacaine to reduce postoperative methadone requirements in dogs after tibial plateau levelling osteotomy (TPLO).

Animals Sixty (30 per institution) client-owned dogs undergoing elective TPLO.

Material and Methods Preoperative ultrasound-guided sciatic and femoral nerve blocks with ropivacaine 0.5% (0.2 mL/kg per nerve block) combined either with dexmedetomidine (0.5 µg/kg per nerve block; group DEX) or with the same volume of saline solution (group CON) was performed in dogs. Postoperative pain was assessed 30 minutes, 2 hours and then every 4 hours for 24 hours with a validated pain scale (4AVet). Rescue methadone (0.2 mg/kg IV) was administered each time a score of ≥ 6 (maximal score 18) was recorded. The total amount and number of doses of methadone and time to first rescue methadone were recorded. The opioids consumption was analysed by Fisher exact tests and time to first methadone by Mann-Whitney U test. The study was performed in parallel at the University clinic of Liege in Belgium and at a private veterinary clinic in Switzerland. Meloxicam (0.15 mg/kg IV) was administered to all dogs at recovery from general anaesthesia.

Results The data of the private clinic were analysed (n = 30). Dogs received a total of 14 and 28 postoperative doses of methadone in groups DEX and CON, respectively (p = 0.0264). Time to first methadone required was not different between groups (p = 0.0901). During the first 24 postoperative hours, analgesia provided by postoperative administration of meloxicam and preoperative nerve blocks was sufficient in 60% and 27% of dogs in group DEX and CON, respectively.

Conclusions and clinical relevance Dexmedetomidine added to ropivacaine for ultrasound-guided sciatic and femoral nerve blocks in dogs seems to reduce the requirements of postoperative methadone in dogs after TPLO.

Keywords Saphenous, pain, opioid, rescue analgesia, fentanyl

Introduction

Cranial cruciate ligament rupture in dogs is one of the most commonly reported orthopaedic diseases in veterinary medicine and usually requires invasive stifle surgery that may include osteotomy and bone plate application in dogs of medium to large size (Hoelzler *et al.* 2005). These surgical procedures are painful and perioperative analgesic plan is mandatory. The adjunct of locoregional anaesthesia such as peripheral nerve blocks clearly improves the pain management and well-being of animals during the perioperative period compared to systemic analgesia (Palomba *et al*. 2020).

Performing locoregional anaesthesia is associated with a certain number of risks. Intraneural fascicular injection of local anaesthetic agents (LAA) can induce detrimental neurologic consequences (Hadzic *et al.* 2004). Infection, bleeding through traumatic vessel puncture, transient tingling or numbness are usually self-limited reported complications. Major complications are rare (Auroy *et al.* 2002). Only one single case of permanent neuropathy has been reported in a review on the risks of peripheral nerve blocks in humans (Brull *et al.* 2007). The benefits of locoregional anaesthesia usually outweigh the risks of complications but a weigh-in of interest must be assessed.

In humans, regional anaesthesia techniques were shown to provide important advantages compared to general anaesthesia and systemic analgesia, including excellent pain control and reduced side effects (Liu & Wu 2007, Liu *et al.* 2005). Continuous improvements in peripheral nerve blocks and the advantages of regional anaesthesia compared with administration of systemic opioids, have strengthened postoperative pain management over recent years (Schnabel *et al.* 2018). However, these early advantages can be short-lived and limited by the relatively brief duration of action of currently available LAA, potentially resulting in early block resolution during the postoperative period (Abdallah *et al.* 2013). The use of perineural dexmedetomidine, a potent alpha-2 agonist, as an adjunct to long-lasting LAA in peripheral nerve blocks has been proven to significantly prolong the duration of sensory nerve block (Study 3). This will contribute to reduce postoperative opioid consumption and improve the

patient's comfort after surgery through reduced pain perception (Abdallah *et al.* 2013, El- Boghadly *et al.* 2017, Vorobeichik *et al.* 2017, Ping *et al.* 2017, Sun *et al.* 2019).

Locoregional anaesthesia techniques of the pelvic limb are commonly used in dogs undergoing invasive stifle surgery. Their efficacy, wide margin of safety and prolonged analgesic effects throughout the perioperative period make them a valuable part of multimodal anaesthetic protocols (Hoelzler *et al.* 2005, Vettorato *et al.* 2012, Caniglia *et al.* 2012). They reduce the need for systemic administration of opioids and so the associated side-effects such as generalized central nervous system depression, dysphoria, prolonged gastric emptying time, vomiting, gastroesophageal regurgitation, urine retention, pruritus and ventilatory depression (Campoy *et al.* 2012). A limited number of studies has investigated the effect of dexmedetomidine perineurally combined with ropivacaine on locoregional anaesthesia in dogs (Trein *et al.* 2017, Bartel *et al.* 2016). Although a longer duration of sensory blockade was observed with perineural dexmedetomidine compared with intramuscular or no administration of dexmedetomidine, the duration was only significantly increased for the tibial nerve. A potential explanation for this finding might be explained by the low dose of dexmedetomidine combined to ropivacaine.

The purpose of our study was to investigate the effect of perineural dexmedetomidine combined to ropivacaine injected preoperatively at the femoral and sciatic nerves of dogs undergoing TPLO. The objective of the study was to evaluate postoperative number of doses of methadone to control pain during the first 24 postoperative hours. Our hypothesis was that dogs which were administered perineural dexmedetomidine combined with ropivacaine would require less rescue methadone to control postoperative pain than dogs which were administered perineural ropivacaine with saline.

Material and Methods

The study has been designed as a prospective, clinical, randomised and blinded trial. Two centres were enrolled for participation: The University of Liège in Belgium and the Veterinary Medical Centre Medi-Vet SA in Switzerland. Ethical committee approval has been obtained by Belgian (Nr. 2138) and Swiss (Nr. 29685, VD3493) authorities. A signed owner informed consent has been collected prior to study participation.

Animals. Thirty dogs per institution were recruited. They were considered eligible for study participation if: a TPLO surgery was planned; if they were 1-15 years old; weighted 10-50 kg; were classified as ASA I or II by the American Society of Anaesthesiologist and if hospitalisation for 24 hours after surgery was possible. Exclusion criteria included: invasive reconstructive knee surgery involving other techniques than TPLO; Body condition score ≥ 8 based on the Nestlé Purina Score; any contraindication for locoregional anaesthesia; any contraindication for the use of ropivacaine, dexmedetomidine or meloxicam; aggressive dogs; skin infection at injection site for sciatic or femoral nerve block; owner refusal; or neuromuscular disorder. At the end of surgery, dogs were classified as small dogs (< 20 kg) or large dogs (> 20kg).

Dogs were randomly assigned to two different groups by drawing a paper from an envelope containing equal number of each group at each institution (30 dogs at each institution). The treatment group (DEX) received an US-guided perineural injection of ropivacaine combined with dexmedetomidine while the control group (CON) received a US-guided perineural injection of ropivacaine combined with saline 0.9% at the sciatic and femoral nerves.

Anaesthesia. Left or right cephalic vein was catheterised and dogs were premedicated with acepromazine 0.01 mg/kg and methadone 0.2 mg/kg administered intravenously (IV). Approximately ten minutes later, anaesthesia was induced with propofol IV titrated to effect until the anaesthetic depth was sufficient to allow orotracheal intubation with an appropriately sized cuffed endotracheal tube. The tube was connected to a circle breathing system. The fresh gas flow of 100% oxygen was set at 1 L/min

and anaesthesia was maintained with an End Tidal (ET) concentration of Isoflurane targeted at 1.3%. Lactated Ringer's solution was administered IV at a rate of 5 mL/kg/hr. A multiparameter monitor including electrocardiogram, pulse oximetry, non-invasive blood pressure, capnography, anaesthetic gas analyser, temperature was used to control vital parameters of the dogs during anaesthesia. Blood pressure was measured by an appropriately sized cuff placed around the forelimb of the dog and measurements were cycled every 3 minutes. Hypotension defined as MAP < 60 mmHg was treated by reduction of the ET of isoflurane by 0.1% every 5 minutes according to clinical anaesthetic depth. If this was insufficient to restore normotension, a bolus of fluids of 10 mL/kg over 20 minutes was administered IV. Finally, dobutamine at 5 µg/kg/min, increased by 2.5 µg/kg/min every 10 minutes was administered if necessary, to restore normotension. Bradycardia was defined as HR < 40 bpm. Bradycardia was treated by administration of IV atropine at 20 µg/kg. Bradycardic and hypotensive events during surgery were recorded. Heart rate, respiratory rate and blood pressure were recorded as baseline value before surgery start. Any increase of 25% in HR or MAP or RR of the recorded baseline value was indicative of nociception and led to the intraoperative administration of fentanyl at 2 µg/kg IV. The number of fentanyl boluses administered during surgery were recorded. Meloxicam 0.15 mg/kg was administered IV shortly after removal of the endotracheal tube.

Surgery. Dogs were operated by three different residency trained surgeons with at least three years of experience as independent surgeon. Two of them are board-certified (Dipl. ECVD). The canine knee joint was instrumentalised with a three-portal method. The arthroscope was inserted laterally to the patellar ligament, the cannula was inserted proximally in the medial joint compartment and the instrument was inserted medially. The surgical procedure for TPLO followed a standard approach (Slocum & Slocum 1993). The type of procedure (arthrotomy *versus* arthroscopy) performed before TPLO was documented. The presence of meniscal tear lesions was recorded as present or absent during arthroscopy/arthrotomy by the operating surgeon.

Locoregional analgesia. A sciatic and femoral ultrasound-guided nerve block was performed preoperatively using a described approach (Campoy *et al.* 2010). The nerve stimulator was used to

confirm right needle placement if necessary. The same experienced anaesthesiologist familiar with US-guidance (Wireless US-probe B024; Konted, China) performed nerve blocks (VM at the Veterinary Medical Center; AT at the University Clinic). A diluted preparation of dexmedetomidine was prepared for dogs of the treatment group. Dexmedetomidine 0.05% (0.1 mL) was diluted with 0.9 mL of NaCl 0.9% in a 1 mL syringe. Care was taken that the preparation was homogenously diluted. Dogs in group DEX received a perineural sciatic injection of 0.2 mL/kg of ropivacaine 0.5% combined with 0.01 mL/kg of diluted dexmedetomidine (equals 0.5 µg/kg of dexmedetomidine). The same volume was injected at the femoral nerve. Dogs in group CON received a perineural sciatic injection of 0.2 mL/kg ropivacaine 0.5% combined with 0.01 mL/kg of NaCl 0.9%. The same volume was injected at the femoral nerve. After perineural injection, the volume of the extension line and needle was flushed with NaCl 0.9%. The heart rate was recorded prior to perform locoregional anaesthesia and 5, 15, 30, 60 and 90 minutes after nerve block.

Postoperative data collection. Thirty minutes (T0.5), two hours (T2), and then every four hours (T4, T8, T12, T16, T20, T24) for 24 hours after recovery, the 4Avet pain score (Holopherne-Doran *et al.* 2010) was used to assess pain. When pain score was ≥ 6 out of 18 (moderate pain), 0.2 mg/kg of methadone was administered IV. The amount of methadone administered were recorded and compared between groups. At the same evaluation time point, a sedation score and a proprioception score were evaluated. The sedation score was evaluated with the following scale (Score 0: fully alert and able to stand and walk; Score 1: alert and able to maintain sternal recumbency; Score 2: drowsy and able to maintain sternal recumbency but unable to stand; Score 3: fast asleep; Campoy *et al.* 2013). The proprioception score consisted on positioning the dorsal part of the digits of the blocked limb on the floor to verify the immediate reposition (knuckling reflex). The following score were attributed (Score 1: absent reposition of the leg; Score 2: retarded, weak or diminished reposition; Score 3: immediate reposition of the leg). Scores of 1 indicated an absent motor nerve block, score of 2 a partial motor nerve block and a score of 3 the presence of a motor nerve block. The veterinarians in charge of collecting the

different scores overnight were previously trained by AT, VM and JS to use the 4Avet pain score until they became familiar with the use of this score if they had not used it previously.

Statistical analysis. An online program (sealedenvelope.com) was used to calculate the number of animals required per experiment at a standard alpha error of 5% and power of 90%. The results of study 3 of sensory sciatic nerve block duration of ropivacaine and perineural dexmedetomidine (321 ± 123 minutes) compared to ropivacaine and saline (171 minutes) was used for calculation. A set up for continuous outcome superiority trial indicated that 15 animals per group were necessary. The experiment was conducted in parallel at two centres to verify if results were reproducible. A statistical analysis was performed using GraphPad Prism Version 5.00 for Windows (GraphPad Software, CA, USA). A Shapiro Wilk test verified distribution of data. Parametric data are presented as mean ± standard deviation (±SD) and non-parametric data as median and interquartile range [IQR]. Demographic data, the duration of surgery, anaesthesia, nerve block to extubation, nerve block to start of arthroscopy/arthrotomy were analysed by unpaired Student *t*-test. Time to first methadone and time to full recovery of proprioception was evaluated by Mann-Whitney U test. A Kaplan-Meier Survival analysis (Log-rank (Mantel-Cox) Test) tested the postoperative survival probability without rescue analgesia (methadone). Fisher exact tests were applied to analyse postoperative number of doses administered. Co-factors, which might have influenced the number of postoperative doses of methadone such as the surgeon, the weight of the dog, the presence or absence of meniscal tear lesions, the type of surgery (arthrotomy *versus* arthroscopy) were analysed by fisher exact and Chi-square tests. Pain scores, sedation scores and proprioception scores were compared with Friedman's test with Dunn's multiple comparison. A p value of ≤ 0.05 was significant.

Results

The results present data of the veterinary centre only because data in Belgium are still being collected. A total of 15 dogs in group DEX and 15 dogs in group CON completed the study. No complications were observed.

Demographic data. Age and weight were normally distributed. There was no significant difference between groups in age (CON: 6.8 ± 2.9; DEX: 6.9 ± 2.9 years) and weight (CON: 25.1 ± 8.5; DEX: 28.0 ± 6.3 kg). Breeds of dogs are presented in table 2.

Surgery. All dogs underwent arthroscopy and TPLO except two dogs which underwent arthrotomy and TPLO. The entire knee joint capsule was not open during surgery except in the two dogs which had arthrotomy. The durations of surgical and anaesthetic procedures are presented in table 1. Co-factors are presented in table 2.

Table 1.

Duration	**Group CON** (minutes)	**Group DEX** (minutes)	*p*-values
Arthroscopy/arthrotomy	27.0 ± 3.2	25.3 ± 2.0	0.6633
TPLO	67.3 ± 3.4	68.6 ± 3.4	0.7827
Anaesthesia (induction to extubation)	173.3 ± 4.7	178.3 ± 3.9	0.4209
Block to extubation	145.3 ± 5.8	138.7 ± 3.5	0.3325
Block to start of arthroscopy/tomy	32.0 ± 1.7	30.0 ± 1.9	0.4435

Duration of surgical and anaesthetic procedures. Dogs received a preoperative ultrasound-guided sciatic and femoral nerve block with ropivacaine 0.5% combined with perineural dexmedetomidine at 1 µg/kg (group DEX) or combined with the equivalent volume of perineural saline solution (group CON). Results are presented as mean ± Standard deviation. TPLO; tibial plateau levelling osteotomy.

Intraoperative data. The percentage of dogs requiring at least one dose of fentanyl (27%) was equal in both groups (4 dogs per group). In group CON, 14% of dogs (n = 2) required fentanyl at 3

occasions, one dog required fentanyl twice and one dog received a single bolus. In group DEX, all four dogs required a single bolus of fentanyl. There was no difference in the total number of doses of fentanyl administered between groups (p = 0.2297). No dogs required atropine. Hypotension was recorded in 20% of dogs (n = 3) in group CON and 7% (n = 1) in group DEX. In group CON, hypotension could be treated by reduction of isoflurane in two dogs and one dog required an additional bolus of isotonic crystalloid. In group DEX, a single isotonic crystalloid bolus restored normotension. No dogs required dobutamine.

Postoperative methadone administration. The total requirements of postoperative doses of methadone were higher in group CON compared to group DEX (table 2; p = 0.0264). The total cumulative fraction of methadone (mg/kg) administered per group is illustrated in figure 1. The number of methadone doses administered during the first 24 postoperative hours were 28 and 14 in the group CON and DEX, respectively (table 2). The highest number of doses of postoperative methadone administered in each group was 6 (n = 2) and 5 (n = 1) in groups CON and DEX, respectively. A total of 60% (9/15) and 27% (4/15) of dogs did not require methadone during the postoperative evaluation period of 24 hours in groups DEX and CON, respectively. The percentage of dogs that required at least one dose of methadone is presented in figure 2. The time to administration of the first postoperative dose of methadone was not different between groups (p = 0.0901). The Kaplan-Meier survival analysis to first dose of postoperative methadone is presented in figure 3 (p = 0.0773).

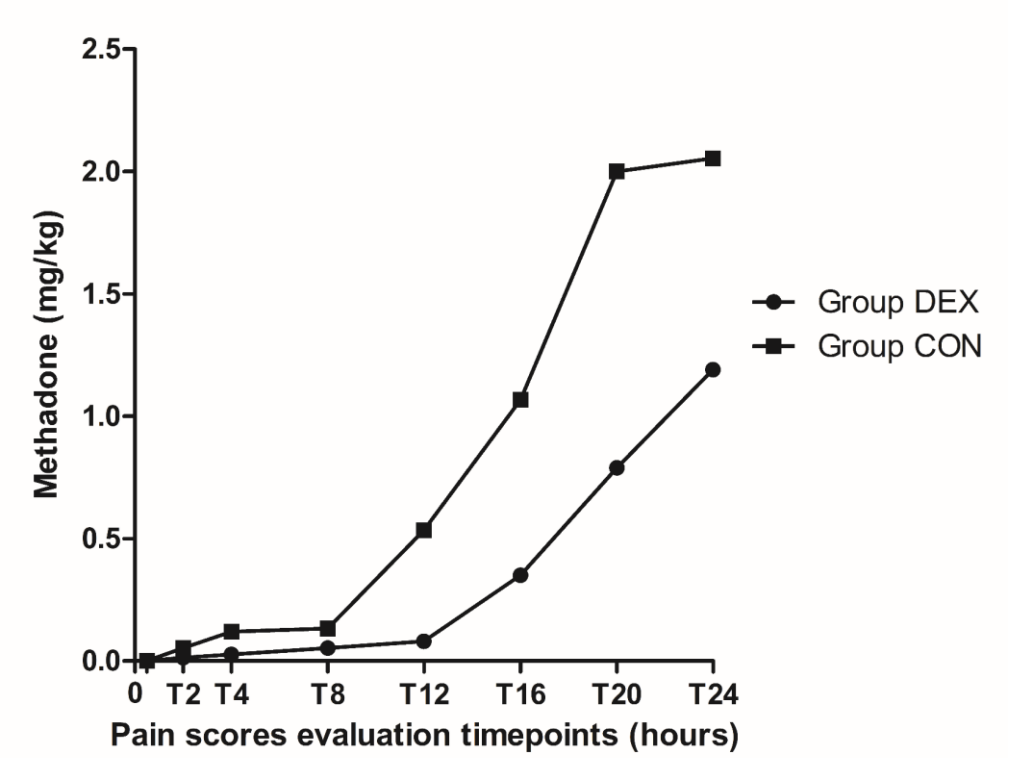
Co-factors. Co-factors such as the surgeon operating, the classification as small or large dog and intraoperative fentanyl requirement might have influenced the number of doses of methadone administered in each dog (table 2). Dogs operated by surgeon 3 required significantly more postoperative doses of methadone. Small dogs required significantly more doses of methadone. In group DEX, dogs which required intraoperative fentanyl never received postoperative methadone (p = 0.02).

Table 2.

Co-factor		**Group CON (n = 15)**	**Group DEX (n = 15)**	***p*-value**
Centre	Private clinic	n = 15 x = 28	n = 15 x = 14	**0.0264**
	University clinic	N/A	N/A	N/A
Weight	Large dogs (> 20kg)	n = 10 x = 16	n = 14 x = 9	**< 0.001**
	Small dogs (< 20 kg)	n = 5 x = 12	n = 1 x = 5	
	p- value	0.2557	**0.0005**	
Meniscal tear	Present	n = 8 x = 18	n = 5 x = 5	0.123
	Absent	n = 10 x = 10	n = 7 x = 9	
	p-value	0.2019	1	
Surgery	Arthroscopy +TPLO	n = 13 x = 23	n = 15 x = 14	0.1669
	Arthrotomy + TPLO	n = 2 x = 5	n = 0 x = 0	
	p-value	0.5251	1	
Surgeon	Surgeon 1	n = 6 x = 5	n = 7 x = 5	**0.003**
	Surgeon 2	n = 6 x = 10	n = 3 x = 0	
	Surgeon 3	n = 3 x = 13	n = 5 x = 9	
	p-value	**0.002**	**0.0171**	**<0.001**
Intraoperative fentanyl	Required at least 1 dose	n = 4 x = 7	n = 4 x = 0	0.126
	No fentanyl required	n = 11 x = 21	n = 11 x = 14	
	p-value	1	**0.02**	
Breed		Mixed breed (n = 5) Labrador (n = 3) Bernese Mountain Dog (n = 2) Australian Shepherd (n = 1) Staffordshire Bull Terrier (n = 1) Beauceron (n = 1) Beagle (n = 1) Airedale Terrier(n = 1)	Labrador (n = 4) German Shepherd (n = 2) Golden retriever (n = 2) Australian Shepherd (n = 1) American Staffordshire Terrier (n = 1) Barbet (n = 1) Boxer (n = 1) English Pointer (n = 1) Dalmatian (n = 1) Spitz (n = 1)	N/A

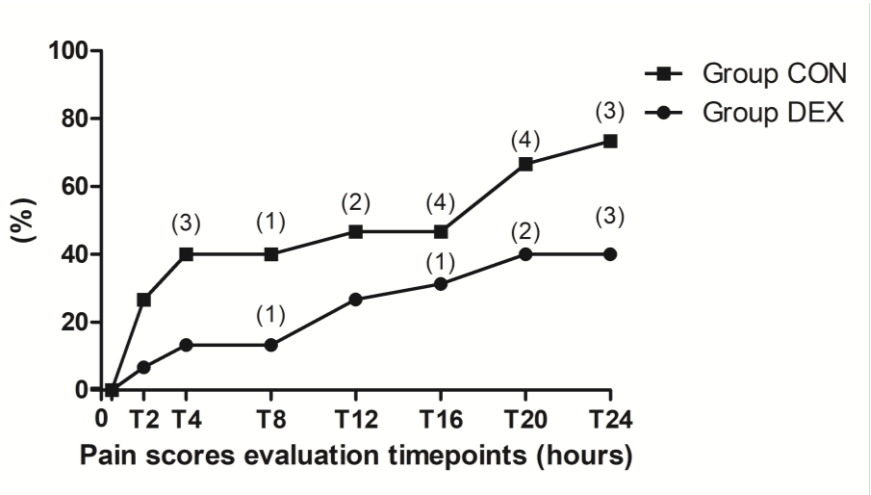
The table shows potential cofounding factors which might have influenced the postoperative methadone requirements of two groups of dogs. Dogs received a preoperative ultrasound-guided sciatic and femoral nerve block with ropivacaine 0.5% combined with perineural dexmedetomidine at 1 µg/kg (group DEX) or combined with the equivalent volume of perineural saline solution (group CON). All co-factors were analysed with fisher exact tests except the co-factor "surgeon" which was analysed by Chi-square test. P-values < 0.05 were considered significant. Significant values are written in bold. N/A, not applicable; n, number of dogs; x = number of postoperative doses of methadone administered

Figure 1.



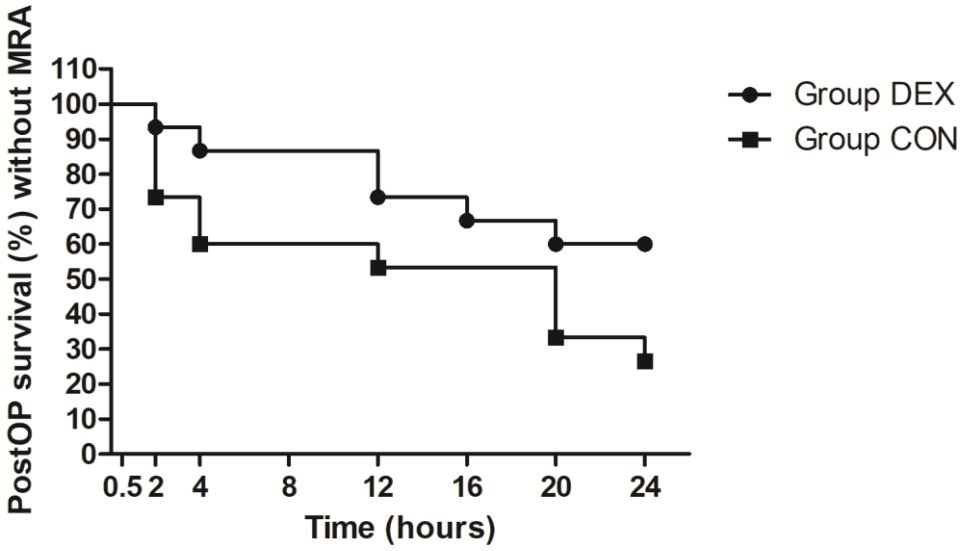
Cumulative postoperative methadone consumption (mg/kg) of dogs after tibial plateau levelling osteotomy. Dogs received a preoperative ultrasound-guided sciatic and femoral nerve block with ropivacaine 0.5% combined with perineural dexmedetomidine at 1 µg/kg (group DEX) or combined with saline solution (group CON). Postoperative pain was assessed by 4Avet pain scores at predetermined timepoints (T0.5 -T24 hours) starting from removal of the endotracheal tube.

Figure 2.



Proportion (%) of dogs which required at least one dose of postoperative rescue analgesia (methadone 0.2 mg/kg IV) after tibial plateau levelling osteotomy. The number in parenthesis is the number of dogs which received ≥ 2 doses of methadone at each evaluation timepoints. Postoperative pain was assessed by 4Avet pain score at predetermined timepoints (T0.5 -T24 hours) starting from removal of the endotracheal tube. Dogs received a preoperative ultrasound-guided sciatic and femoral nerve block with ropivacaine 0.5% combined with (group DEX) or without (group CON) dexmedetomidine at 1 µg/kg injected perineurally.

Figure 3.



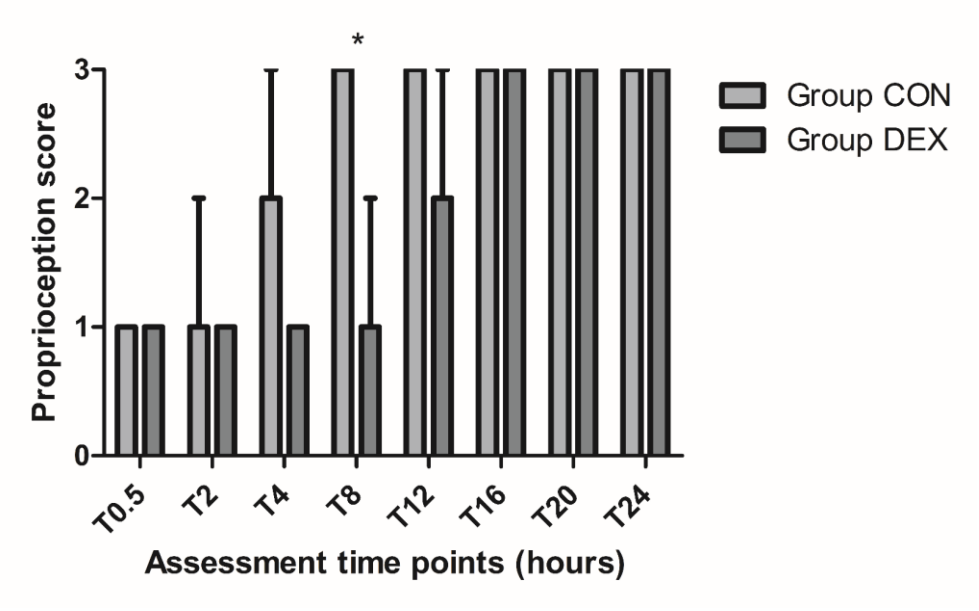
Postoperative (PostOP) survival probability (%) without methadone rescue analgesia (MRA) over time between two groups of dogs after surgery for tibial plateau levelling osteotomy. Dogs received a preoperative ultrasound-guided perineural injection of ropivacaine (0.5%) combined with dexmedetomidine at 1 µg/kg (Group DEX; n = 15) or combined with saline (Group CON; n = 15) at the sciatic and femoral nerve. The table show a Kaplan-Meier Survival Analysis (p = 0.0773; Log-rank Test)

Postoperative pain scores. There was no significant difference in pain scores between the different evaluation time points in each group. Pain scores in group CON were significantly higher at T20 (4[1-7]) and at T16 (4[2-6]) compared to group DEX at T0.5 (1[0-2]), T2 (1[0-2]) and T4 (1[0-2]). Pain scores were significantly higher in group CON at T24 (3[2-6]) compared to group DEX at T2 and T4.

Sedation. Sedation scores were higher in group CON ($p < 0.001$) at T0.5 (1[0-2]) compared to T2 (0[0-0]) and in group DEX ($p < 0.001$) at T0.5 (1[1-2]) compared to T2 (0[0-0]). The sedation scores were not statistically different between group CON and group DEX at all evaluated time points.

Proprioception. All dogs had recovered proprioception (score = 3) at T24. The time to full recovery of proprioception was longer in group DEX than in group CON ($p = 0.0042$). A significant difference between proprioception scores was present at T8 between groups ($p < 0.05$). The time to recovery of proprioception is illustrated in figure 4.

Figure 4



Recovery of proprioception after ultrasound-guided sciatic and femoral nerve assessed at different timepoints after endotracheal tube removal in dogs recovering from tibial plateau levelling osteotomy. Group DEX received 1 µg/kg of dexmedetomidine added to ropivacaine 0.5% for the perineural injection while group CON received an equivalent volume of saline solution instead of dexmedetomidine. A score of 1 indicated absent proprioception, a score of 2 indicated partial or retarded proprioception and a score of 3 indicated recovered proprioception. * Significant differences between groups ($p < 0.05$).

Discussion

Preliminary results of this study suggest that the addition of 1 µg/kg of dexmedetomidine to 0.2 ml/kg of ropivacaine 0.5% for US-guided sciatic and femoral nerve block is beneficial for dogs undergoing TPLO. The postoperative 24 hours cumulated methadone consumption is reduced when dexmedetomidine is added to ropivacaine for peripheral nerve block.

Dexmedetomidine is often used as an adjuvant together with LAA for perineural injection in human patients undergoing orthopaedic surgeries. Several reviews have evaluated the clinical benefits of this drug for peripheral nerve blockade. Doses around 1 µg/kg of DEX will prolong nerve block by approximately 200 minutes (Kirksey *et al.* 2015). Our previous study (study 3) also confirmed that 1 µg/kg might be an effective dose and justify the dose applied in the present study. Other reviews have shown a decreased need of 24 hours cumulated analgesic agents if dexmedetomidine is combined to LAA compared to LAA alone (Vorobeichik *et al.* 2017, Wang *et al.* 2018).

The 24-hour number of postoperative doses of methadone was used as endpoint in this study rather than sensory nerve block duration. The cumulated requirement of postoperative opioids is clinically relevant because the repeated administration of methadone might negatively affect the comfort of dogs (Bini *et al.* 2018). Study 3 revealed that sensory but not motor nerve block was prolonged when dexmedetomidine was added to ropivacaine for peripheral nerve block. In humans, those results are contradictory. Dai *et al.* (2018) reported a longer duration of motor block for supraclavicular brachial plexus block but not for axillary or intermuscular brachial plexus block. Although a trend towards longer motor block was observed in axillary and intermuscular brachial plexus block, it did not reach significant difference. The duration of motor block seems to be dependent on the type of nerve block. In this study, the time to full recovery of proprioception was longer in group DEX. A significant difference in proprioception was observed at T8 between groups. This proves that motor nerve block was longer in group DEX. A prolonged motor nerve block will likely induce a prolonged sensory nerve block. The time to first rescue analgesia was not different between groups. This could also have suggested a block

of longer duration. The reduced cumulated number of doses of methadone required and lower postoperative pain scores in group DEX compared to group CON at specific time points tend to suggest better postoperative analgesia provided by perineural dexmedetomidine. In humans, analgesia is prolonged when dexmedetomidine is added to peripheral nerve blocks (Schnabel *et al.* 2018). Therefore, it remains unclear if prolonged nerve block or a better quality of the nerve block and more solid block explain the decreased the postoperative methadone requirements. A combination of prolonged nerve block, better postoperative analgesia provided by dexmedetomidine and systemic effects of opioids is also possible.

The operating surgeon, the size of the dog (small or large), the breed and the intraoperative fentanyl requirements might have biased the postoperative number of doses of methadone required in each dog. The skills and years of experience vary between surgeons. This might impact on soft tissue trauma and wound size possibly influencing the level of postoperative pain. Common veterinary caseload includes dogs of different weight, size and breeds. Dogs interact differently with humans depending on their breed. Dogs of certain breeds might be shy, stoic, bouncy or happy and interact differently in a postoperative setting. It might influence the final result of the postoperative pain score. Surgery for TPLO is usually performed in larger breed dogs, but the operation might occasionally be indicated for dogs of smaller size. Small dogs required more postoperative doses of methadone than large dogs in our study. Dogs of small size have higher metabolism and higher basal heart rate (Rondo *et al.* 2017). This can lead to faster elimination of drugs from the body. Methadone, dexmedetomidine and LAA might have been metabolised faster in small dogs. There was no difference between intraoperative fentanyl and postoperative methadone requirements between groups but in group DEX, intraoperative fentanyl administration led to decreased postoperative methadone requirements. It seems unlikely that a single dose of 2 µg/kg of intraoperative fentanyl could reduce the postoperative need of methadone. Fentanyl has a short duration of action with a maximum terminal elimination phase of 199 minutes at 10 µg/kg in dogs (Murphy *et al.* 1979). Perineural dexmedetomidine might have influenced the relationship between intraoperative and postoperative need for opioids.

"Rebound pain" is a phenomenon by which the patient perceives severe postoperative pain after peripheral nerve block resolution. It has a negative impact on the patient's comfort and might lead to increased opioid consumption and sleep disturbance (Nobre *et al.* 2019). Rebound pain is not reported in dogs but careful observations of postoperative pain after nerve blocks have weaned off is important. The addition of adjuvants to LAA and the IV injection of anti-inflammatory drugs have been proposed to prevent rebound pain in humans (Dada *et al.* 2019). The injection of perineural dexmedetomidine and IV meloxicam are important multimodal analgesic strategies to prevent the potential underestimated rebound pain phenomenon in dogs.

In humans, many orthopaedic surgeries are performed under locoregional anaesthesia while the patient is awake. When dexmedetomidine is added to LAA for peripheral nerve block, dexmedetomidine might induce sedation (Rancourt *et al.* 2012). After perineural injection of dexmedetomidine, sedation might be deeper in patients receiving a higher dose of the drug (Keplinger *et al.* 2015). In dogs, locoregional anaesthesia is usually combined to general anaesthesia for TPLO surgery. This probably explains why there was no difference in sedation scores between groups in our study.

Some limitations need to be mentioned. The data presented are preliminary results. The statistical analysis of the entire data sets of both centres might reveal different results. A validated French pain scale was applied to evaluate postoperative pain instead of an English pain scale used more frequently in clinical settings such as the Glasgow composite pain scale. Objective scores were recorded by different veterinarians at different institutions. An interobserver variability is not excluded and might have affected validity of data. A two-centre study might be considered an advantage to evaluate the efficacy of perineural dexmedetomidine and generalise its clinical application. However, multiple investigators might have different clinical judgement or different skills in performing US-guided nerve blocks. This might have impacted on the results.

The use of dexmedetomidine as an adjuvant to ropivacaine after femoral and sciatic nerve block, seems to reduce the postoperative consumption of methadone in dogs undergoing TPLO surgery.

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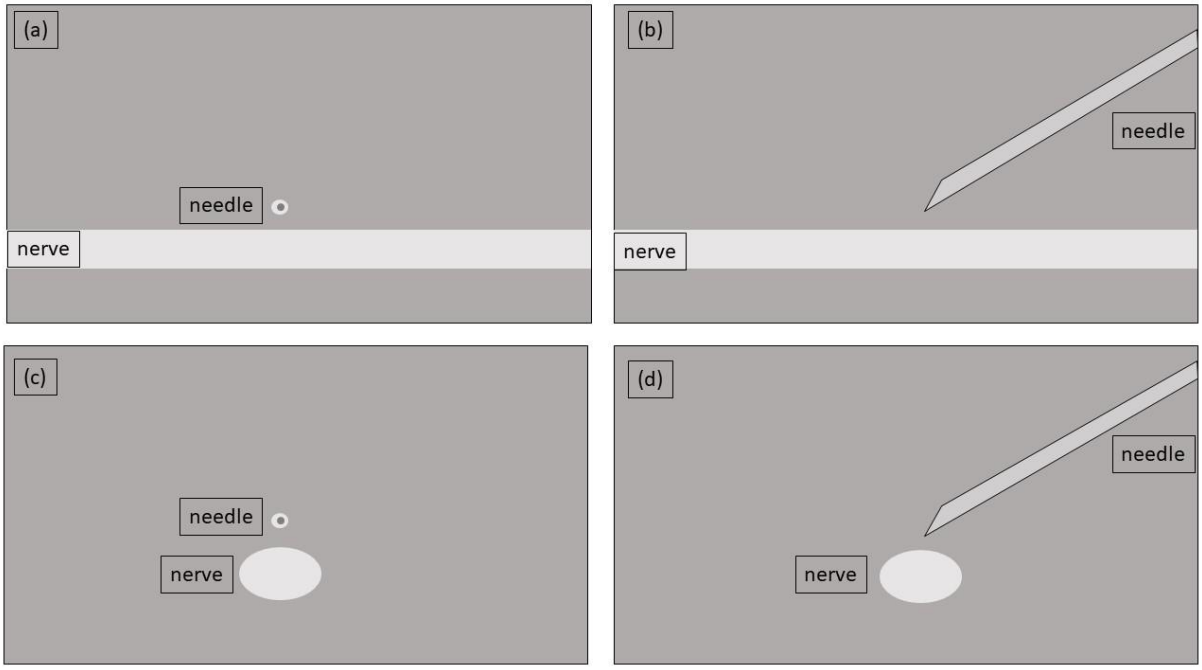
Discussion - Perspectives

Surgical procedures at the pelvic limb in dogs are performed daily in clinical veterinary practices. The procedures vary from hip prothesis, femur head osteotomy, invasive reconstructive stifle surgery, arthrodesis, fracture repair to amputation of the digits or even of the entire pelvic limb. Each surgery is performed on a specific anatomical region of the pelvic limb. As in human medicine, locoregional anaesthesia should be implemented for invasive pelvic limb surgery to provide effective pain relief in dogs. It allows the reduction of isoflurane requirements and side effects associated with high doses of systemic analgesia (Congdon *et al.* 2017, Portela *et al.* 2008, Vettorato & Corletto 2016). Locoregional anaesthesia at the pelvic limb in dogs can be performed by LAA in the epidural space or nearby peripheral nerves. The success rate of epidural anaesthesia varies (Troncy *et al.* 2002, Sarotti *et al.* 2015) and this technique unfortunately induce paralysis of both hindlimbs. Peripheral nerve blocks might be a preferred technique. Ultrasound-guidance allows precise visualisation of the nerves of the pelvic limb and seems superior than the electrical nerve stimulator technique (Haro *et al.* 2016, Akasaka & Shimizu 2017) for peripheral nerve blocks. Some of the US-guided also have a low reported success rate (Shilo *et al.* 2010). This is clinically relevant because dogs might not be able to profit from a specific US-guided approach which might be indicated for a specific surgical procedure. The US-guided parasacral approach to the sciatic nerve in dogs needed modifications to improve its success rate (Shilo *et al.* 2010). Improvements of the US-guided parasacral approach was the goal of study 1. The success rate of the US-guided parasacral approach could be improved by modifications of the ultrasound view of the nerve and by modifying the volume, concentration and type of LAA. An optimised US view was obtained by applying a transverse (short axis) view of the nerve instead of a longitudinal (long axis) view with an in-plane imaging of the needle. This adaptation of the technique yields better results than those obtained in the study by Shilo *et al.* (2010). To discuss the success rate of a US-guided nerve block techniques, it is necessary to understand the different US-guided views in relationship to the nerve and needle as illustrated in the following figure.

Figure: Schematic representation of the nerve and needle view for US-guided nerve block



(a) Longitudinal (long axis) view of the nerve with an out-of-plane needle approach
(b) Longitudinal (long axis) view of the nerve with an in-plane needle approach
(c) Transverse (short axis) view of the nerve with an out-of-plane needle approach
(d) Transverse (short axis) view of the nerve with an in-plane needle approach

In human patients, the insertion of perineural catheters using a longitudinal view requires more time compared to insertions using a transverse view (Mariano *et al.* 2013, Kim *et al.* 2014). Reducing the time needed to perform nerve block will shorten the preparation and procedure time. This advantage is relevant in clinical settings. It is unclear, if the success rate is improved for single nerve blockade with a transverse view compared to the longitudinal view. The transverse out-of-plane approach seems to be easier to use for the inexperienced operator (Huang *et al.* 2018). The transverse view also offers better visualisation of the surrounding structures. Unfortunately, the out-of-plane technique does not allow entire visualisation of the needle which could be inadvertently inserted in the nerve. The transverse in-plane view might be a good alternative to the transverse out-of-plane view for operators in training without underestimating the safety issues of the out-of-plane technique. The transverse view also enables the operator to follow the course of the nerve along its course. In cats, it has been shown that the in-plane technique was more successful than the out-of-plane technique to perform US-guided radial, ulnar, musculocutaneous and median nerve block (Leung *et al.* 2019). The transverse in-plane view offers therefore many advantages and seems safer to avoid nerve damage. The success rate of an US-guided nerve block is highly depending on the training, the skills and experience of the operators (Marhofer *et al.* 2010). Other findings in humans suggests that the operator should use the needle-probe alignment

technique which they are most comfortable with (Fredrickson & Danesh-Clough 2013). Therefore, broadening the range of US-guided nerve block techniques is essential. Study 1 contributed to develop a new approach to the sciatic nerve at the parasacral level which might be preferred by some operators.

The volume, the concentration and the type of LAA might also influence the success rate and the duration of action of an US-guided nerve bloc. In study 1, the volume of LAA was 0.2 mL/kg representing an increase of 0.07 mL/kg of the highest volume used by Shilo *et al.* (2010). The volume of LAA injected plays an important role for the efficiency of the nerve block. This will determine how much and for how long the LAA will surround the target nerve after perineural injection. The sciatic nerve is very large at the parasacral level. This might also explain why a larger volume of LAA is needed to properly diffuse through the entire nerve sheath. The effective dose 50 and effective dose 95 of ropivacaine 0.5% for popliteal sciatic nerve block in humans were 6 mL and 16 mL, respectively (Jeong *et al.* 2015). The effective dose 95 of 16 mL equals a volume of 0.23 mL/kg in a 70 kg person. This volume is very similar to the volume used in study 1 and supports the volume of LAA used in our study. A meta-analysis revealed that increasing the volume of LAA also increased inferior alveolar nerve block success in patient suffering from pulpitis (Milani *et al.* 2018). It remains unclear if a higher volume increases the success of US-guided nerve blocks. An increase in volume of LAA also hastens the nerve block onset time for digital nerve block (Ballo *et al.* 2016). The percentage of persons showing a reduced muscular response of the vastus medialis after LAA injection within the adductor canal was lower after a low injection volume (10 mL) compared to higher volumes (20 mL and 30 mL) (Grevstad *et al.* 2016). A modification of the concentration of the LAA might impact the success rate of the nerve block. It has been proven that the success rate of a sciatic nerve block was higher with low volume high concentration than with high volume low concentration mepivacaine (Taboada Muñiz *et al.* 2008).

In dogs, Portela *et al.* (2010) demonstrated that the duration and intensity of lumbar plexus and sciatic nerve blocks guided by electrical nerve stimulator is more positively affected by the injection of a higher concentration of bupivacaine than by the injection of a higher volume of bupivacaine. The epidural administration of a higher concentration of bupivacaine (0.75% compared to 0.5%) will induce a longer dermatomal block at fixed volumes (Feldmann *et al.* 1996). A concentration of 0.5% of long acting LAA is often used for clinical practice. At fixed concentrations, the author believes that an increase in volume also improves nerve block success rate. A larger volume of LAA might be able to compensate a suboptimal placement of the needle in relationship to the nerve. Experts in US-guided nerve blocks have demonstrated that it is possible to achieve a successful sciatic nerve block with a minimal volume of LAA in human volunteers (Latzke *et al.* 2010). The administration of a higher volume of LAA might be relevant for novice US users to increase their chance for successful nerve block.

The novel use of the LAA levobupivacaine has also been tested in study 1. The use of levobupivacaine for locoregional anaesthesia in dogs remains sparse despite its evidence of lower toxicity compared with bupivacaine (Cerasoli *et al.* 2017, Gomez de Segura *et al.* 2009, Leone *et al.* 2008). Ropivacaine was preferred to levobupivacaine for studies 2, 3 and 4 because this LAA is used more frequently for peripheral nerve blocks in dogs. Additionally, levobupivacaine is nearly twice as expensive compared to ropivacaine. Ropivacaine was used to reduce costs of clinical studies.

These different refinements of the US-guided parasacral approach to the sciatic nerve in dogs contributed to improve the success rate from 67% (Shilo *et al.* 2010) to 93% (Marolf *et al.* 2019).

During US-guided perineural injection, LAA might be observed spreading over the nerve, under the nerve or circumferentially around the nerve. A circumferential spread of LAA around the sciatic nerve is sometimes described as the typical "doughnut sign" and commonly induce a successful nerve block (van Geffen & Gielen 2006, Sites & Antonakakis 2009). However, a circumferential spread of LAA around the nerve cannot always be achieved (Marhofer *et al.* 2014). Trying to obtain a circumferential spread of LAA around the target nerve will increase the amount of needle passes (Szűcs *et al.* 2014) and might increase the risk of iatrogenic nerve damage. The perineural spread of LAA does not appear to influence the success rate of nerve blocks (Marhofer *et al.* 2014, Szűcs *et al.* 2014). There is therefore no clinical advantage to obtain a circumferential spread of LAA around the nerve compared to the spread of LAA above *versus* under the nerve. In this thesis, the needle tip was positioned nearby the nerve and a test dose of 1-2 mL was injected to observe the spread of LAA. If LAA spread above, under or around the target nerve, the total volume of LAA was injected. If the spread was inadequate, the needle was repositioned and another test dose was injected until a correct position of the needle tip and spread of LAA was obtained. In general, the needle tip and spread of LAA were observed over the nerve.

The ultrasound enables the precise localisation of the nerve. Visualisation on the ultrasound image of other anatomical structures adjacent to the nerve will help to localise the nerve. Vessels might be visualised as rounded anechogenic structures surrounded by a hyperechoic rim. The circumflex iliac arteries are useful anatomical landmarks for proximal femoral nerve blocks in humans. The iliac arteries should be considered and special care should be taken to avoid puncture of those vessels as they might lay on the needle pass to perform femoral nerve blocks (Ogami *et al.* 2017).

In dogs, the superficial iliac artery has also been identified as an important landmark for proximal femoral nerve block (Garcia-Pereira *et al.* 2018). This US-guided femoral nerve block approach, or a femoral nerve approach within the psoas compartment (Tayari *et al.* 2017), combined with the modified US-guided parasacral approach described in study 1 might be beneficial for hip joint

surgeries in dogs. According to the author's clinical experience, a single US-guided parasacral sciatic nerve block is not sufficient to block nociception during femur head osteotomy. This observation correlates with the anatomical innervation of the hip joint of the dog. Articular branches to the hip joint rising from the femoral and sciatic nerves were identified in five out of six and in six out of six dog's cadavers, respectively (Huang *et al.* 2013). A combined sacral plexus and psoas compartment nerve blocks has been evaluated for pelvic limb amputation. This nerve block combination provides effective analgesia for this type of surgical procedure (Congdon *et al.* 2017). The sacral plexus nerve block had been performed under electrostimulation guidance. The presence of the ilium and the sacrum over the sacral plexus makes an US-guided approach difficult. We have shown that it was possible to apply a modified parasacral US-guided approach to the sciatic nerve. It is possible that this modified approach combined with an US-guided psoas compartment nerve block (Tayari *et al.* 2017) would be effective to control nociception during pelvic limb amputation in dogs. When performing peripheral nerve blocks, the advantages of the US-guidance over the electrostimulation guidance are, among others, direct visualisation of the nerve and spread of the LAA. Therefore, for pelvic limb amputation in dogs combined US-guided nerve blocks should be considered.

Considering that nerve blocks are usually performed under general anaesthesia or deep sedation, it is challenging to evaluate the success of locoregional anaesthesia in dogs. We differentiate two situations: evaluation of locoregional anaesthesia under experimental or under clinical circumstances.

Under experimental circumstances, nerve blocks evaluation requires different techniques to identify the effects of LAA on the nerves. Success is evaluated directly after performance of the nerve block by the operator as soon as the animal recovered from general anaesthesia or sedation. The nerve block is usually evaluated by motor, proprioceptive and sensory deficits. The evaluation by mechanical nociceptive threshold with the use of an algometer after sciatic and femoral nerve block has also been applied (Gray *et al.* 2019). Sensory and motor blocks can be evaluated by clamp pressure or pinprick applied at different dermatomes and ability to walk (Portela *et al.* 2010, Trein *et al.* 2017). The use of a perfusion index has been proposed as an effective method to evaluate sciatic nerve block success (Gatson *et al.* 2016). The perineural injection of bupivacaine around the sciatic nerve induces vasodilation and increases the perfusion index. The lack of an increase in perfusion index can therefore indicate partial or complete block failure. Sensory deficits by pinprick testing and motor blockade by control of locomotion and proprioceptive deficits were used in study 1 and study 3.

Under clinical circumstances, the nerve block success is usually evaluated while the animal is under general anaesthesia or shortly after recovery once surgery has been completed. Occasionally, locoregional anaesthesia can be combined with procedural sedation in dogs (Campoy *et al.* 2012b). The perioperative opioids requirements needed to control nociception and pain is the most common method used to evaluate the efficacy of locoregional anaesthesia in clinical settings in dogs. Perioperative

opioids requirements are very relevant in clinical settings. Despite being effective to control pain and nociception (Quirion 1984), opioids can induce systemic side effects. Dogs can encounter vomiting, nausea, dysorexia or respiratory depression after opioid administration (Evans 1992, Wilson *et al.* 2005, Bini *et al.* 2018). Locoregional anaesthesia has the potential to reduce the perioperative consumption of opioids in dogs. Studies showed that dogs receiving preoperative locoregional anaesthesia require less opioids to control nociception compared to dogs without locoregional (Boscan *et al.* 2016, Warrit *et al.* 2019b). Better recovery scores at the end of general anaesthesia were also reported in dogs receiving locoregional anaesthesia (Boscan *et al.* 2016). One of the goals of study 2 was to determine which locoregional anaesthesia technique was most likely to reduce the number of doses of opioids required to control pain. We could prove that epidural anaesthesia and combined US-guided sciatic and femoral nerve block reduced the total perioperative opioid requirements compared to single femoral or sciatic nerve block in dogs undergoing TPLO. Other studies have proven the efficacy of combined sciatic and femoral nerve blocks in dogs undergoing TPLO (Caniglia *et al.* 2012; Mc Cally *et al.* 2015) but those studies used the electrical nerve stimulator. This technique is less reliable compared to the US-guided technique for peripheral nerve blocks (Haro *et al.* 2016). Additionally, we demonstrated that combined US-guided sciatic and femoral nerve block reduced early postoperative opioid requirements compared to epidural anaesthesia and single US-guided nerve blocks. This might encourage clinicians to favour the US-guided technique rather than the epidural anaesthesia technique. Arnholz *et al.* (2017) did not prove superiority of US-guided nerve blocks compared to the epidural anaesthesia technique for dogs undergoing pelvic limb surgery. The differences observed between study 2 and the study by Arnholz *et al.* (2017) might be related to the different US-guided approaches to the sciatic and femoral nerves. We used the approaches described by Campoy *et al.* (2010) while Arnholz *et al.* (2017) used the US-guided approaches described by Echeverry *et al.* (2010, 2012a). Finally, study 2 was designed as a pilot study to evaluate the precise perioperative opioid requirements of dogs after US-guided nerve blocks in dogs. The results provide nowadays sufficient data for power calculations of larger randomised controlled trials about US-guided sciatic and femoral nerve blocks in dogs. Pain after surgical orthopaedic procedures might lead to prolonged hospitalisation to administer stronger pain medications. Despite adequate intraoperative analgesia provided by spinal or epidural or peripheral sciatic and femoral nerve block during TPLO, long term chronic pain is not excluded. A total of 41% of dogs showed chronic signs of pain six month after surgery (Pownall *et al.* 2020). This proportion might even be higher if locoregional anaesthesia had not been provided for surgery. The evaluation of perioperative opioid consumption and perioperative pain appear to be adequate outcomes to evaluate the efficacy of peripheral nerve blocks.

In human medicine, the evaluation of nerve blocks in volunteers mimics the situation in experimental dogs. The quantitative effects of nerve blocks include the sensory and motor components. The sensory component of nerve blocks is often evaluated by pinprick, by cold or heat stimulation or by

alcohol swab sensation. The motor component of nerve blocks can be evaluated by active resistive force testing, the ability of thumb adduction, maximal knee extension or maximal hip adduction (Rothe *et al.* 2011, Keplinger *et al.* 2015, Andersen *et al.* 2017, Nielsen *et al.* 2020). In clinical settings, the qualitative effects of nerve blocks are assessed by the duration of analgesia. The cumulative morphine consumption, pain scores evaluation on movement or at rest, patient satisfaction, time to first opioid requirement, 24- or 48-hours total opioids consumption are often applied to evaluate the qualitative component of nerve blocks (Abdallah *et al.* 2016, Baeriswyl *et al.* 2017, Bjørn *et al.* 2017). Qualitative and quantitative evaluations are regularly combined in studies in human medicine. Trying to reduce perioperative opioids requirements is relevant in human medicine because increased opioids consumption might lead to drug induced dependence. Methods used for nerve block evaluation in this thesis seem adequate in comparison to methods used in human medicine. However, the assessment of pinprick and motor scores in studies 2 and 4 might have provided interesting additional information such as the precise duration of sensory and motor nerve block. This could have explained whether the decreased postoperative opioid requirements were related to a longer duration of the nerve blocks or a more solid nerve block.

Evaluating pain in animals is challenging. The inability to communicate verbally complicates the quantification of pain in dogs. Different methods were developed for this purpose. A scoring system is generally completed to determine the level of analgesia required in dogs, with a differentiation between subjective scales and objective scales (Hernandez-Avalos *et al.* 2019). Preventive scoring system, simple descriptive scale, numerical rating scale or visual analogue scale (VAS) are examples of subjective scales. Subjective scales have the advantage of being easy and simple to apply but have a high inter-observer variability (Holton *et al.* 1998). These scales are inadequate when three or more veterinarians simultaneously evaluate pain in dogs after surgery (Holton *et al.* 1998). In study 2, pain was evaluated by a single person and data collected with the use of VAS could be analysed. In study 4, it was logistically impossible for the same veterinarian to apply a VAS during 24 hours and no subjective scales were used. Objective pain scales are multidimensional and evaluate different aspects of pain such as palpation of the surgical site, observation of the dog or cardiovascular parameters. A final score determines the degree of pain at a certain timepoint. The Glasgow Composite Measuring Pain Scale, the University of Melbourne Pain Scale or the Colorado State Acute Pain Scale are examples of objective pain scales (Reid *et al.* 2018). Those pain scales are valid, reliable and useful. Unfortunately, they are only available in English. The 4AVet pain scale is a validated pain scale in French (Holopherne-Doran *et al.* 2010). Veterinarians evaluating postoperative pain in Study 4 were all working in a French speaking practice or university. Under these circumstances, the 4AVet pain scale seemed a judicious choice for postoperative pain evaluation after TPLO in dogs.

The interest about US-guided locoregional anaesthesia in dogs has considerably increased during the last years. This is illustrated by the increasing number of publications available (Portela *et al.* 2018). Peripheral nerve blocks are very effective to control intraoperative nociception and early postoperative pain. Unfortunately, the duration of action of LAA for peripheral nerve blocks is short. This is a clinical issue for dogs recovering from orthopaedic surgeries. It might lead to postoperative pain and increased administrations of opioids. We therefore planned to find a solution to extend the duration of action of LAA in dogs. Adjuvants added to amid-type LAA might contribute to increase the duration of sensory nerve blocks. The ideal adjuvant should not be neurotoxic, should not induce side effects (local or generalised), should shorten the onset time of the nerve block, minimise the systemic absorption of LAA and improve the nerve block intensity and duration (Swain *et al.* 2017). Dexmedetomidine represents a unique adjuvant and meets some criteria of the ideal adjuvant for locoregional anaesthesia. An increasing number of publications is available in human medicine about dexmedetomidine combined to LAA for perineural injections. Dexmedetomidine is safe and does not induce neurotoxicity (Knight *et al.* 2015, Brummett *et al.* 2008). A study performed in rats has shown that intraneural fascicular injection could even have anti-inflammatories and protective neural properties (Kim *et al.* 2018). This might be of particular interest in case of inadvertent intraneural injection. The onset time of nerve block is shorter when dexmedetomidine is added to LAA compared to the control group without dexmedetomidine for supraclavicular brachial plexus block in humans (Bharti *et al.* 2015). Dexmedetomidine does induce mild side effects such as transient hypotension or bradycardia in humans (Lin *et al.* 2013, Rancourt *et al.* 2012, Vorobeichik *et al.* 2017). Systematic reviews and meta-analysis concluded that perineural dexmedetomidine enhances motor and sensory nerve block and, most importantly, increases the degree of analgesia (El-Boghdadly *et al.* 2017, Knight *et al.* 2015, Vorobeichik *et al.* 2017). Surprisingly, very few studies have evaluated the analgesic efficacy of dexmedetomidine for locoregional anaesthesia in dogs. Only two studies on this topic are available. An experimental study in dogs has tested the efficacy of ropivacaine 0.75% for sciatic and femoral nerve blocks combined with or without dexmedetomidine at 0.2 µg/kg injected either perineurally or intramuscularly (Trein *et al.* 2017). Dexmedetomidine did not significantly influence nerve block characteristics between groups. Despite the absence of a significant difference, a trend towards a longer tibial sensory nerve bloc was observed in the perineural dexmedetomidine group. The authors concluded that the dose of dexmedetomidine might have been insufficient. The separated block between the two branches of the sciatic nerve, the tibial and common peroneal nerves, might be due to an extraparaneural injection of LAA. To avoid a differential block, a subparaneural injection might be necessary (Micieli *et al.* 2020). A clinical study also evaluated the efficacy of perineural dexmedetomidine for dogs undergoing stifle arthroplasty. The analgesic efficacy of sciatic and femoral nerve block with bupivacaine 0.5% combined with dexmedetomidine 0.2 µg/kg was compared to the analgesic efficacy of epidural bupivacaine 0.5% combined with buprenorphine 4 µg/kg (Bartel *et al.* 2016). Both

techniques provided sufficient analgesia for up to 24 hours in two third of dogs. Dexmedetomidine will provide postoperative analgesia when administered IV as a continuous infusion rate (Valtolina *et al*. 2009), but the potential of perineural dexmedetomidine needs to be further investigated.

Study 3 and was planned to determine an adequate dose and route of dexmedetomidine used as adjuvants for locoregional anaesthesia in dogs. The plasma level of dexmedetomidine was also determined to identify the systemic effects of dexmedetomidine on peripheral nerve blocks and the mechanism of action of dexmedetomidine for locoregional anaesthesia. We concluded that the perineural injection of dexmedetomidine at 1 µg/kg together with ropivacaine 0.5% was effective to prolong sensory sciatic and saphenous block compared to nerve blocks without dexmedetomidine. At this dose, side effects such as transient sedation were minimal. Dexmedetomidine injected IV and the plasma level of dexmedetomidine apparently do not influence the duration of sensory nerve blocks. The dose of perineural dexmedetomidine used in study 3 is similar to the reported doses used for locoregional analgesia in humans (Marhofer & Brummett 2016, Andersen *et al.* 2017, Jung *et al.* 2018). The clinical efficacy of dexmedetomidine combined to LAA has been proven in humans. It reduces postoperative pain and postoperative opioid consumption and enhance patient's satisfaction (Vorobeichik *et al.* 2017). Clinical studies to evaluate the postoperative analgesic efficacy of perineural dexmedetomidine are needed in dogs. This was the purpose of study 4.

Based on the results of study 3, study 4 was designed as a clinical study to evaluate the clinical efficacy of 1 µg/kg of perineural dexmedetomidine. Dogs undergoing elective TPLO surgery were randomised to receive a perineural US-guided sciatic and femoral nerve bloc with ropivacaine 0.5% combined with or without dexmedetomidine. Results have shown that the total number of postoperative doses of methadone were lower in the group receiving perineural dexmedetomidine and ropivacaine compared to the group receiving perineural ropivacaine without dexmedetomidine. However, some cofounding factors such as the operating surgeon, the breed and/or weight of the dog might have influenced the results. The reduced administrations of postoperative doses of methadone observed in the group of dogs receiving perineural dexmedetomidine is in accordance to clinical studies in human patients. Postoperative analgesic drugs consumption is reduced in groups receiving perineural dexmedetomidine compared to control groups (Fritsch *et al.* 2014, Bengisun *et al.* 2014, Bharti *et al.* 2015). However, a review about the use of dexmedetomidine for locoregional anaesthesia rated the evidence for this finding as low (Vorobeichik *et al.* 2017). In dogs, approximately 66% of dogs did not require hydromorphone rescue analgesia after preoperative sciatic and femoral nerve bloc with bupivacaine and dexmedetomidine for stifle arthroplasty (Bartel *et al.* 2016). Perineural dexmedetomidine has the potential to reduce postoperative opioids consumption and should be considered as part of the analgesic regimen for dogs undergoing invasive knee surgery.

The exact mechanism of action of dexmedetomidine for locoregional anaesthesia remains unclear. Dexmedetomidine might act through systemic or peripheral mechanisms depending if the drug is administered IV or perineurally, respectively. In humans, Abdallah *et al.* (2016) showed that IV dexmedetomidine provides postoperative analgesia after interscalene brachial plexus block. Systemic dexmedetomidine administration prolongs ulnar nerve block but to a lesser extent that perineural dexmedetomidine (Andersen *et al.* 2019). This suggests that dexmedetomidine has at least a partial central mechanism of action. In dogs, we could not show prolonged sensory nerve blocks after IV injection of dexmedetomidine in study 3. Trein *et al.* (2017) also failed to demonstrate any effects on locoregional anaesthesia after a single dose of intramuscular dexmedetomidine. The continuous rate infusion of dexmedetomidine at 1µg/kg/hour combined to spinal anaesthesia provided by a hyperbaric solution of bupivacaine in dogs undergoing pelvic limb surgery delayed the time to first intraoperative fentanyl administration compared to spinal anaesthesia alone (Sarotti *et al.* 2019). The IV injection of dexmedetomidine as a continuous infusion rate will induce a steady state. The constant plasmatic level of the drug in the body might explain better control of intraoperative nociception. In study 3, dexmedetomidine was administered as a single IV bolus injection. The central analgesic mechanism of action of dexmedetomidine might principally be explained by the action of the drug at spinal and supraspinal levels (Murrell & Hellebrekers 2005). After systemic administration of dexmedetomidine, the central release of substance P and glutamate decreases and interneurons remain in a hyperpolarised state which induce an attenuation of pain signals transmission (Weerink *et al.* 2017). Dexmedetomidine seems to stimulates presynaptic alpha-2 adrenoceptors in the central nervous system, especially in the locus coeruleus (Guo *et al.* 1991). The effects of centrally mediated dexmedetomidine induced analgesia might also be dose-dependent. Plasma level of dexmedetomidine up to 1.23 ng/mL did not induce analgesia in healthy volunteers despite an apparent degree of sedation (Angst *et al.* 2004). This concentration is above the highest plasmatic level measured in study 3 and might explain the lack of efficacy of IV dexmedetomidine.

The main mechanism of action of perineural dexmedetomidine seems to be peripheral. Andersen *et al.* (2017) attempted to prove the peripheral mechanism of action of perineural dexmedetomidine based on an experience in healthy volunteers. They observed that the saphenous nerve block was prolonged when dexmedetomidine was combined with ropivacaine compared to saphenous nerve block performed without dexmedetomidine in the contralateral limb. They concluded that the perineural mechanism of action of dexmedetomidine might be peripheral. This observation is also supported by our results of study 3. Different hypothesis might explain the peripheral mechanism of action of perineural dexmedetomidine. Vasoconstriction, hyperpolarisation-activated (Ih) currents or anti-inflammatories properties of dexmedetomidine might be responsible for some of the analgesic effects of perineural dexmedetomidine. Alpha-2 agonists such as dexmedetomidine induce vasoconstriction through reversible binding of the alpha-2A subtypes adrenoceptors located in vessels (Yabuki *et al.*

2014, Yoshitomi *et al.* 2018). This is thought to slow systemic reabsorption of LAA. Consequently, LAA remain perineurally active for a longer period of time and will prolong nerve block. A mechanism of action independent to adrenoceptor is also reported (Kosugi *et al.* 2010). The modulation of Ih-currents via activated cyclic nucleotide-gated cation channels is possible. Dexmedetomidine will inhibit hyperpolarisation-activated cation currents by keeping the nerve in a hyperpolarised state. Thus, preventing further firing of action potential by neurons (Kosugi *et al.* 2010, Brummett *et al.* 2011). Sensory C-fibres seem more sensitive to this mechanism than motoric A-fibres (Weerink *et al.* 2017). Finally, perineural dexmedetomidine might have anti-inflammatories properties. The inflammatory response appears to be attenuated via nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) receptors after perineural injection of dexmedetomidine (Huang *et al.* 2014). An intraneural fascicular injection of dexmedetomidine at 0.5 µg/kg in rats induces anti-inflammatories and protective neural properties (Kim *et al.* 2018). It seems that the peripheral mechanism of action of dexmedetomidine prevails over the centrally mediated effects. The complex mechanism of action of perineural dexmedetomidine is likely multifactorial and a synergistic action with locoregional anaesthesia seems evident.

The initial goal was to design study 4 as a multicentre study. Prospective single-centre randomised controlled trials (RCT) are carefully designed investigations. They can contribute to change clinical practice and improve patient care but methodological problems can occur and centre specific bias might affect the validity of the study (Youssef *et al.* 2008). Results from single-centre RCT might not always be applicable to another centre despite similar clinical settings. The effect of treatments of single centre RCT might be larger than multicentre RCT and results should be interpreted with caution (Dechartres *et al.* 2011, Unverzagt *et al.* 2013). Multi-centre studies have the potential to increase the level of scientific evidence and reduce potential confounder bias (Evans 2013). Their results are usually reproducible but studies can be challenging to conduct (Irving & Curley 2008). They are more complex to perform than single-centre studies and coordination is essential.

An explanatory video of study 4 was projected at the international congress of the Association of Veterinary Anaesthetists in March 2018 in Grenada to recruit clinics to take part to this project. Unfortunately, no veterinary hospital has expressed interest. It might be related to the time-consuming nature of the study, to the inability to motivate a responsible person to conduct the study or to the restricted number of institutions with experience in performing US-guided sciatic and femoral nerve blocks. After discussion, it was decided to perform the study in parallel at two different centres applying the same study design.

The financial impact of US-guidance for locoregional anaesthesia needs further discussion. Costs of the purchase of a performant ultrasound machine have considerably decreased over years. Small

portable or wireless devices are available on the market. This type of equipment is sufficient to perform US-guided nerve blocks. Most hospitals are nowadays equipped with US machines and offer the possibility to use these devices for locoregional anaesthesia. In human medicine, high success rate of nerve block and improvement of anaesthesia-related workflow generate considerable cost savings (Marhofer *et al.* 2010). The use of the US greatly contributes to optimise these conditions. The costs related differences between US-guided interscalene brachial plexus nerve block and general anaesthesia patients undergoing arthroscopic shoulder surgery were compared (Gonano *et al.* 2009). The emergence time and time spent in the post-anaesthetic care unit were shorter and the costs were 19.6% lower in the locoregional anaesthesia group. Sandhu *et al.* (2004) provided a calculation comparing the costs of infraclavicular brachial plexus nerve block guided by ultrasound or electrical nerve stimulator. The costs are lower for US-guided nerve block because it requires less time to perform and the onset time of nerve block is shorter. The US-guidance reduces the procedural time (8.06 ± 1.92 minutes) compared to the electrical nerve stimulator (13.60 ± 4.56 minutes) for femoral nerve block (Forouzan *et al.* 2017). Consequently, the patient spends less time in the operating room which greatly contributes to reduce medical expenses. This seems particularly true in case of catheter placement for continuous nerve blockade (Ehlers *et al.* 2012, Sandhu *et al.* 2004). A model of costs analysis was more balanced regarding this statement (Liu & John 2010). In an ambulatory model setting, the cost of US-guided nerve block is cheaper compared to electrostimulation guidance but in a hospitalisation model setting, the benefit of the ultrasound over the electrical nerve stimulator is masked by the costs for hospitalisation and general anaesthesia (Liu & John 2010).

In dogs, the financial impact is also relevant. One study evaluated the costs of nerve block for dogs undergoing TPLO between sham block with saline or with ropivacaine (Warrit *et al.* 2019). The purchase of equipment (ultrasound and electrical nerve stimulator) increases anaesthesia related costs but might reduce costs related to pain management and treatment of complications. Peripheral nerve blocks offer better analgesia and have an economical benefit compared to systemic analgesia provided by fentanyl (Palomba *et al.* 2020). No studies are comparing the anaesthesia related economic impact between US-guided nerve block and epidural or spinal anaesthesia. Epidural anaesthesia does not require a particular equipment and the simplicity of the procedure makes it a valuable technique for locoregional anaesthesia in dogs. The procedural failure rate of 32% after epidural anaesthesia (Sarotti *et al.* 2015) might induce additional costs through prolonged postoperative hospitalisation or pain treatment. Personal observations have shown that pet owners might dislike the hairless area over the lumbosacral area of their dog required for epidural anaesthesia. Hair re-growth is delayed in 12.2% of dogs over the epidural injection site (Kalchofner Guerrero *et al.* 2014). The authors believe that informing pet owners of the advantages of US-guided nerve block over epidural anaesthesia might lead pet owner to favour the US-guided peripheral nerve block analgesia despite potential higher costs of the technique.

Limitations of the research articles of this thesis have already been discussed individually in the related section of each study. Additional limitations are as follows.

The efficacy of the modified parasacral approach of study 1 has not been evaluated in clinical cases. The difficulty was to recruit specific cases for surgical procedures at the level of the hip joint. Many times, hip joint surgical repair follows traumatic events in dogs. This involves additional lesions around the hip joint such as pelvic fractures for example. Injuries at additional anatomical body regions would be an exclusion criterion, therefore limiting the number of clinical cases available for a study. The veterinary centres involved did not routinely perform total hip replacement surgery. This procedure could not be used as standard surgery to evaluate the efficacy of the modified parasacral approach. The modified parascral approach of study 1 could have been applied in study 2 for dogs undergoing TPLO. We did not use this approach because a proximal approach to the sciatic nerve is not necessary for surgery performed at the knee in dogs. Additionally, the presence of the gluteal vessels nearby the sciatic nerve increases the risk of iatrogenic puncture with the parasacral approach compared to a mid-femoral approach where the sciatic nerve runs under the *biceps femoris* muscle without the proximity of vessels.

A complete qualitative and quantitative assessment of nerve blocks components were not performed in studies 2 and 4. Pinprick testing and detailed motor scores might have provided a better overview of the clinical outcome of US-guided nerve blocks.

In study 3, it was originally planned to perform a US-guided femoral nerve block instead of a saphenous the block. The right position of the needle tip close to the femoral nerve should have been confirmed with the electrical nerve stimulator. Unfortunately, we could not elicit a motor response to confirm the position of the needle tip but an adequate US-image of the nerve was present. We believe that the lack of motor response was related to the needle tip located nearby the saphenous nerve. The saphenous nerve does not elicit a motor response because it is mainly composed of sensory fibres. In study 3, blood was aspirated in the needle hub in 1 dog due to puncture of a vessel. It is possible that this complication was due to the increased amount of needle passes while trying to elicit a motor response. To avoid further puncture of vessels and to decrease the amount of needle passes, the electrical nerve stimulator was not used anymore and the saphenous nerve block was performed in all dogs in study 3.

Perspectives. Many US-guided techniques are described for locoregional anaesthesia in dogs. The combined US-guided sciatic and femoral nerve blocks are valuable for clinical practice in dogs and have been highlighted in this thesis. The use of perineural dexmedetomidine as adjuvant to prolong sensory nerve blocks and reduce postoperative requirements in dogs has also been proven in this thesis. Research should now focus on additional possibilities to prolong the beneficial effects of locoregional anaesthesia.

Perineural catheters might be an effective tool to allow repeated or continuous injections of LAA to prolong nerve blocks. Perineural catheters are regularly placed during the perioperative period in human patients to allow repeated or continuous LAA injections (Afsari & McCartney 2010). Their use improves postoperative pain management in human patients after orthopaedic surgery (Bugada *et al.* 2017). Perineural catheters can be placed under US-guidance using different US views of the nerve and needle (Ilfeld *et al.* 2010, Mariano *et al.* 2013). This allows real-time visualisation of catheter insertion and verification of correct placement. Perineural catheters at the hind limb in humans are commonly placed at the sciatic or femoral nerve. Continuous infusion of ropivacaine at the femoral nerve in patients undergoing total knee arthroplasty will benefit from improved rehabilitation, shorter hospital stays and satisfactory analgesia (De Ruyter *et al.* 2006). Continuous infusion of ropivacaine at the sciatic nerve also improves patients' comfort at home by reducing opioid need and sleep disturbances and decreasing pain after orthopaedic surgery (Ilfeld *et al.* 2002).

In veterinary medicine, the use of perineural catheters is rare. In horses, perineural catheters have been placed at the palmar nerves and infusion of bupivacaine induced sensory nerve block at the distal limb (Zarucco *et al.* 2010). Experimentally induced signs of forelimb pain are reduced by LAA continuous infusion at the lateral and medial palmar nerves (Watts *et al.* 2011). Very recently, an US-guided technique was developed for continuous blockade of ulnar and median nerves in horses (Souto *et al.* 2020). In dogs, preliminary results of perineural catheters placement at the sciatic nerve suggest a minimal dislocation rate and repeated perineural injections were feasible in > 90% of cases (Marolf *et al.* 2015). Monticelli *et al.* (2016) have investigated the possibility of an US-guided catheter placement at the lumbar plexus in dogs' cadavers. They reported good success of the technique but the injection of dye could spread into the abdomen or epidural space. Clinical studies are needed to further evaluate this technique. An interesting report describes the US-guided placement of an epidural catheter at the brachial plexus to prevent postoperative pain by repeated injection of LAA in a dog after invasive front limb surgery (Vettorato & Taeymans 2017). This is a clinical application of the potential benefits of perineural catheter. Perineural catheter might find more clinical application in dogs in the near future. Considering the challenge of avoiding complications related to perineural catheters in canine patients, future studies could focus on efforts to avoid dislocation and catheter related infections. Both of these issues are already described in human medicine (Marhofer *et al.* 2013, Nicolotti *et al.* 2016)

Liposomal bupivacaine might allow prolongation of peripheral nerve blocks. This new LAA formulation composed of liposomal bupivacaine is available for veterinary medicine. The liposomal formulation will induce sustained-releases of bupivacaine at the site of injection for up to 72 hours after a single injection dose. The product has been licensed for surgical wound infiltration in dogs undergoing cranial cruciate ligament rupture in the United States (Lascelles *et al.* 2016). Liposomal bupivacaine appears to have a superior safety profile compared to hydrochloride bupivacaine after IV, epidural,

intrathecal or intra-arterial administration in dogs (Joshi *et al.* 2015). The question whether liposomal bupivacaine would be suitable for perineural injection remains. The website of the manufacturer (https://nocita.aratana.com/, last accessed 06.09.2020) reports approval by the Food and Drug Administration in the United States for peripheral nerve block for cats undergoing onychectomy. The injection of liposomal bupivacaine at the digital nerves seems to provide superior postoperative analgesia up to 72 hours compared to the injection of saline (https://animaldrugsatfda.fda.gov/adafda/app/search/public/document/downloadFoi/3952, last accessed 06.09.2020). The injection of liposomal bupivacaine around the brachial plexus has been tested in dogs and rabbits. Results suggest that the injection and sustained-release was safe at doses 5-fold higher than recommended clinical indication, except for mild granulomatous inflammation observed around the nerve (Richard *et al.* 2012). It is possible that liposomal bupivacaine solution might find clinical application for peripheral nerve injection to prolong duration of nerve blockade. However, prolonged motor nerve block (up to 72 hours) is dangerous and might lead to self-trauma in dogs. The dogs would need to be monitored closely and protective measures such as the application of protective bandages are necessary to prevent injuries.

The US-guided technology for peripheral nerve blockade has considerably evolved during the last decades. Recent advances in human medicine include three- or four-dimensional imaging, improvement of the visibility of needles and needle tips and robotic assistance using special software programs (Sen *et al.* 2019). The three-dimensional imaging can show a nerve and spread of LAA around the nerve on multiplanar or multiorthogonal views (Foxal *et al.* 2007, Gebhard *et al.* 2015). The four-dimensional imaging technology include the real-time component during US-guided nerve block (Gebhard *et al.* 2015). These imaging techniques might increase in popularity as the technology becomes more easily available. Micro air bubbles injection of 0.1-0.2 ml in the needle are described to increase the visibility of the needle by producing hyperechoic rounded structures within the shaft of the needle (Liu & Mei 2018). Micro air bubbles do not only increase needle visibility but also seem to enhance nerve blockade (Cullion *et al.* 2018). This has been evaluated for sciatic nerve blockade in rats with the application of high-frequency, low-intensity ultrasound. Motor and sensory sciatic nerve blocks were prolonged when high-frequency, low-intensity ultrasound was combined to microbubbles but not when both techniques were used alone. Very recently, the same research group demonstrated that insonation through high frequency ultrasound but not the injection of microbubbles enhanced sciatic nerve blockade induced by tetrodotoxin in rats (Cullion *et al.* 2020). Insonation had no effect on sciatic nerve block induced by bupivacaine. The first report about robotic performance of US-guided nerve block evaluated a sciatic nerve block approach in human patients. The principle of robotic assistance was based on a software control system equipped with a joystick and a robotic arm (Hemmerling *et al.* 2013). The nerve success rate was “defined as the introduction of the needle into the nerve sheath” and was

achieved in all patients. The procedural time was short (3 to 4 minutes). Robotic assistance hastens the learning curve of the physician and reduces inter-operator variability performing nerve blocks (Morse *et al.* 2014).

Dexmedetomidine or dexamethasone combined to LAA, repeated or continuous LAA injections through perineural catheter or liposomal bupivacaine seems today the most adequate possibilities to prolong peripheral nerve block effectively (Orebaugh & Dewasurendra 2020). Regional anaesthesia under US-guidance has already been classified as "gold standard" by some authors (Griffin & Nicholls 2010, Hopkins 2007). Ultrasound-guided locoregional anaesthesia coupled with the possibilities mentioned by Orebaugh & Dewasurendra (2020) might today be considered best practice. This thesis might have triggered new insights for research in veterinary medicine, with the hope that the canine population undergoing pelvic limb surgery might soon benefit from these medical advances.

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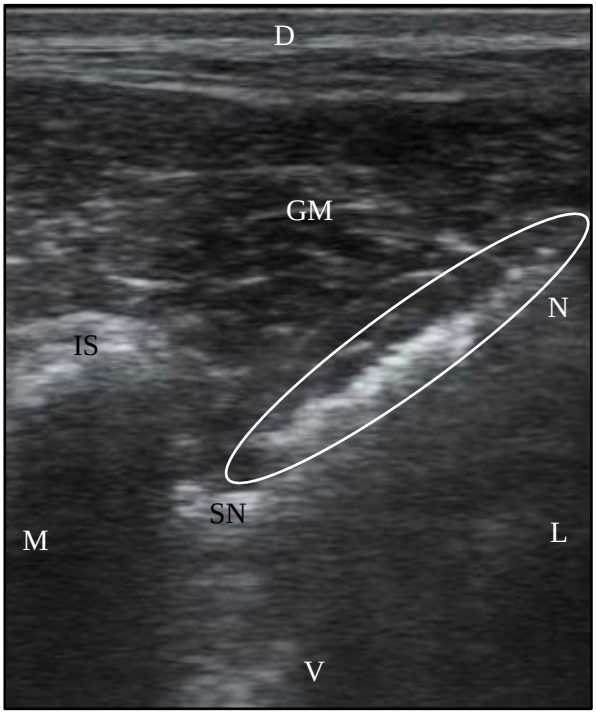
Appendix

Additional figure to study 1



US-image illustrating an in-plane needle view during a modified parasacral approach to the sciatic nerve in dogs. IS, isciatic spine; SN, sciatic nerve; N, needle (circled structure); GM, gluteal musculature; D, dorsal ; V, ventral ; M, medial ; L, lateral.

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