Effects of hyaluronidase when administered with ropivacaine or bupivacaine for regional anaesthesia of the pelvic limb in dogs

By

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SIGNATURES

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Abstract

EFFECTS OF HYALURONIDASE WHEN ADMINISTERED WITH ROPIVACAINE AND BUPIVACAINE DURING REGIONAL ANAESTHESIA OF THE PELVIC LIMB

Objective To determine the effect of hyaluronidase on the time to onset and offset of anaesthesia in ropivacaine or bupivacaine femoral-ischiatic nerve blocks.

Study Design Blinded randomised crossover trial.

Animals Eight beagle dogs.

Materials and Methods Each dog underwent four treatments separated into two treatment blocks. All dogs received each treatment once in a balanced randomised order within each block. A three-week washout period between treatments within each block was observed. Initially, the dogs were exposed to the ropivacaine treatment block: RS (ropivacaine 0,5% plus saline 0.9%); RH (ropivacaine 0,5% plus hyaluronidase 100 IU ml^{-1}); followed three weeks later by the bupivacaine treatment block: BS (bupivacaine 0,5% plus saline); BH (bupivacaine 0,5% plus hyaluronidase). The local anaesthetics were administered at 0.1 mL kg^{-1} per site. Hyaluronidase and saline were administered at 0.02 mL kg^{-1} per site. Femoral-ischiatic nerve blocks were performed using a combined ultrasound-guided/electrolocation technique. The mechanical nociceptive threshold was measured using an algometer to ascertain baseline, onset and offset of anaesthesia. Time 0 was deemed to be immediately after completion of both nerve blocks. Mechanical nociceptive threshold was measured at 3-minute intervals for the first 30 minutes (onset period), then at 30-minute intervals up to a maximum of 360 minutes (offset period). Onset and offset of regional anaesthesia were defined as a 25% increase above and as a return to <25% above baseline nociceptive threshold readings, respectively. Nociceptive thresholds were evaluated until offset or until 360 minutes post-block. The times to onset and offset were compared between treatments

within a block using Mann-Whitney U test. Data were analysed using commercially available software and significance interpreted at $p < 0.05$.

Results There were no differences between treatments with regards to onset and offset times of regional anaesthesia. The median (range) onset of anaesthesia for RS and RH was 21 (3 to 60) and 12 (3 to 21) minutes, respectively ($p = 0.141$). The offset was 270 (90 to 360) and 180 (30 to 300) minutes for RS and RH, respectively ($p = 0.361$); while the onset was 24 (3 to 60) and 9 (3 to 27) minutes ($p = 0.394$), and offset was 360 (240 to 360) and 330 (210 to 360) minutes for BS and BH, respectively ($p = 0.456$).

Conclusion and clinical relevance Hyaluronidase had no effect on the onset and offset times of ropivacaine and bupivacaine femoral-ischiatic nerve blocks in dogs compared to saline. The onset and offset times were highly variable in all treatments. Clinically, the high variability of the onset and offset times of the regional anaesthesia of these local anaesthetic drugs mean that clinicians must monitor the patient's response and, if required, provide additional analgesic drugs.

Keywords: ropivacaine, bupivacaine, femoral nerve block, ischiatic nerve block, hyaluronidase

List of abbreviations

%	percent
<	less than
>	greater than
mg	milligram(s)
kg	kilogram(s)
mL	milliliter(s)
mHz	millihertz
ms	millisecond
mA	milliampere
pH	inverse logarithm of the hydrogen ion concentration
pKa	logarithm acid dissociation constant of a drug

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Introduction

Literature review

Local anaesthetic drugs have increasing importance in veterinary clinical practice Quandt & Rawling 1996). One notable function is their use in regional anaesthesia blocks. While several drugs have been used successfully as adjuvants to improve the duration of the block, little has been reported in literature regarding use of adjuvants to hasten onset time of local anaesthetic drugs. Hyaluronidase has been used as an adjuvant drug in ophthalmologic blocks (Rodgers & Craven 2014), subcutaneous infusions with local anaesthetics in humans (Koh et al. 2015) as well as epidural blocks in dogs (DeRossi et al. 2011) with most studies reporting a hastened onset of action. Bupivacaine and ropivacaine are commonly used long-acting local anaesthetic drugs in human medical practice and have gained popularity in veterinary practice (Adami et al. 2012).

Mechanism of action of local anaesthetic drugs

Local anaesthetic drugs inhibit nerve impulse transmission by blocking the inward movement of sodium at the sodium ionophore on the neurolemma (Columb & Hartley 2014, Li et al. 2014). Nerve impulse transmission is blocked as there will, consequently, be neither an action potential generated nor propagation of the impulse along the nerve. Nerve fibres can either be unmyelinated or have various degrees of myelination and the degree of myelination affects how sensitive the nerve will be to the effects of local anaesthetic drugs. Myelination means that the nerve fibres are wrapped in layers of a lipoprotein sheath. There are regular interruptions in the myelin coating called nodes of Ranvier. In myelinated nerves, at least two to three adjacent nodes of Ranvier need to be submerged in local anaesthetic drug to be blocked effectively, or an impulse may skip a blocked node and continue down the nerve fibre (Malamed 2014).

To gain access to the sodium ionophore, a local anaesthetic drug must be in a non-ionised state to cross the neurolemma and enter the neuron. The local anaesthetic drug

must then repolarise to an ionised form before it is able to bind the ionophore from within the cell (Columb & Hartley 2014, Becker & Reed 2012). The time necessary for these events to occur determines the onset of action of the drug. Sensory fibres such as unmyelinated C-fibres and partially myelinated A-delta (Aδ) fibres are the most sensitive to local anaesthetic drugs. The larger, thickly myelinated (A-alfa) (Aα) fibres, which innervate muscle tissue are the last to be blocked as they are the least sensitive. The reverse occurs during recovery from local anaesthesia; voluntary motor function returns before sensation (Becker & Reed 2012). Ropivacaine is less lipid soluble than bupivacaine, which implies that it is less able to penetrate the Aα fibres and is more selective for the smaller diameter pain transmitting Aδ and C fibres (Kuthiala & Chaughary 2011). For this reason; ropivacaine displays a more pronounced motor-sparing effect compared to bupivacaine.

There are several factors which influence the onset of action of the local anaesthetic drugs; the most notable being the proportion of molecules at the neurolemma being in the non-ionised state (Columb & Hartley 2014, Becker & Reed 2012). This is dependent on the acid dissociation constant (pKa) of the drug, the number of molecules reaching the membrane and the tissue pH (Columb & Hartley 2014). Lipid solubility of a local anaesthetic drug determines how quickly it diffuses over the neurolemma. Highly lipid soluble drugs tend to become sequestered in myelin or surrounding adipose tissue, and high lipid solubility tends to delay the onset of action in practice (Becker & Reed 2012).

Onset and offset times for bupivacaine and ropivacaine

One of the methods to classify local anaesthetic drugs is by their time to onset of action (Garcia 2015). These times are grouped into three categories, being fast (or rapid), moderate (or intermediate) or slow (Feldman & Covino 1988; Feldman et al. 1996; Martin-Hansen 2004; Flores 2013; Clarke et al. 2014; Garcia 2015). These categories have been derived by comparing the relative speeds of onset of the various drugs, but no actual times are quoted. What is known is that bupivacaine and ropivacaine have been categorised as being “moderate” or “slow” in onset of action depending on site of administration (Martin-

Flores 2013; Clarke et al. 2014; Garcia 2015). The slow onset of actions for bupivacaine and ropivacaine are when they are administered during peripheral nerve blocks, compared to epidural administration which is classified as being moderate (Feldman et al. 1996; Martin-Flores 2013). However, Clarke et al. (2014) were the exception and do mention that bupivacaine has an expected onset of action of approximately 40 minutes. The textbooks further state that the onset time of ropivacaine is similar to that of bupivacaine. A possible explanation for the vague reports on onset times is due to the different concentration and volumes of the local anaesthetic drugs used, which are thought to alter the onset times. Also, the technique of administration (axial versus peripheral nerve block, for example) and the method of detecting the onset of action (loss of sensation, loss of proprioception, loss of motor function, onset of analgesia) differ among the studies. For example: one study used different doses of bupivacaine for epidural anaesthesia, namely: 0.14 ml kg^{-1} (0.5% bupivacaine solution), 0.22 ml kg^{-1} (0.5% bupivacaine solution) and 0.22 ml kg^{-1} (0.75% bupivacaine solution). In all three treatments the onset time for loss of sensation to the level of the perineum was 3 to 5 minutes (Duke et al. 2000). Another study used a dose of 0.25 ml kg^{-1} (0.5% bupivacaine solution) which resulted in a loss of interdigital sensation in 12 minutes (Cruz et al. 1997). The different onset times in these examples may be due to 1) the loss of sensation being tested at different anatomical regions; or 2) the techniques of determining loss of sensation that were different; and not due to the dosages and volume used in both studies because they were similar in terms of milligrams per kilogram and volume in millilitres per kilogram, respectively. The speed of onset of any form of block is important as prolonged onset times will cause a delay in the treatment of a patient (Koh et al. 2015). Furthermore, a stinging pain sensation is experienced by the patient during injection of the local anaesthetic solution that dissipates once onset of action begins (Jaichandran et al. 2010).

The offset of bupivacaine and ropivacaine are more often quoted in veterinary anaesthesia textbooks, however, there is always a wide range reported. This wide range

could be because of individual variation (in anatomy and pharmacokinetic effects) or different methods of detecting the offset of action (return of motor function, pedal withdrawal reflex or proprioception). The offset times for bupivacaine and ropivacaine are similar and range from 4 to 12 and 5 to 8 hours, respectively (Martin-Flores 2013). An important outcome is that each study must standardise their method of detecting the onset and offset of action to allow fair comparisons to be made. The overall challenge is then comparing the studies outcome to the currently available literature where the methodologies might be different.

Methods used to decrease onset of action time

A number of methods have been investigated to hasten the onset time of local anaesthetic drugs such as increasing the drug formulation concentration, alkalinising the drug solution and adding various adjuvant drugs.

It has been suggested that increasing the concentration of ropivacaine quickens its onset of action when performing regional blocks (El-Sharrawy & Yagiela 2006, Columb & Hartley 2014). However, a recent study comparing equal volumes of 0.5%, 0.75% and 1.0% solutions of ropivacaine reported that increasing the concentration had little effect on the speed of onset in patients undergoing epidural anaesthesia (Brockway et al. 1991).

Alkalinisation of the local anaesthetic solution increases the proportion of non-ionised molecules, which should speed up the onset of action (Robinson et al. 2000). Alkalinising bupivacaine and lidocaine local anaesthetic solutions with sodium bicarbonate produced a block of faster onset compared to bupivacaine and lidocaine alone, in elective caesarean section patients (Fernando & Jones 1991). At present, there has been little further published evidence to support the addition of sodium bicarbonate to a local anaesthetic solution (Brummett & Williams 2011).

Various adjuvant drugs have been added to local anaesthetic drugs in an attempt to quicken the onset of action by increasing the number of molecules reaching the neurolemma

(opioids, alpha2-adrenoceptor agonists, midazolam, adrenaline) (Robinson et al. 2000; Brummett & Williams 2011; Biyani et al. 2014; Trein et al. 2017). Hyaluronidase is an enzyme which has been used to increase the absorption of intramuscular and subcutaneous injected drugs and improve the diffusion of local anaesthetics (Aronson 2016). The enzyme increases tissue permeability and reduces the viscosity of fluids by causing depolarisation of the mucoprotein hyaluronic acid, which is an important component of the “glue” holding cells together (Aronson 2016). Hyaluronic acid also alkalinises the local anaesthetic due to phosphate binders being present in the solution, which also speeds up onset by increasing the proportion of non-ionised molecules reaching the neuron (Palte 2015). Hypersensitivity reactions to hyaluronidase have been reported when used in ophthalmic blocks (Palte 2015).

The use of hyaluronidase as an adjuvant to local anaesthetic drugs has become increasingly popular in ophthalmic blocks, used at a concentration of up to 30 IU ml^{-1} (Rodgers & Craven 2014) and subcutaneous infusions with local anaesthetics (Koh et al. 2015) in humans, with several studies demonstrating a hastened onset of action. A recent study on humans undergoing elective arm surgery found that a mixture of 0.5% ropivacaine and 100 IU ml^{-1} hyaluronidase (total dose not exceeding 3000 IU) had a significantly shorter onset time compared to an equal volume of 0.5% ropivacaine alone (Koh et al. 2015).

In dogs, the onset of epidural anaesthesia with levobupivacaine alone (0.2 ml kg^{-1}; 0.5% levobupivacaine solution) had a longer onset but similar quality of block compared to a levobupivacaine solution at the same dose with 1 ml of hyaluronidase (400 IU ml^{-1}) added (DeRossi et al. 2011). However, the levobupivacaine-hyaluronidase also had a shorter duration of anaesthesia.

Regional nerve block techniques of the canine pelvic limb

Regional anaesthesia using peripheral nerve blocks has become increasingly studied in both human and veterinary anaesthesia (Campoy et al. 2010; Dixon et al. 2010; Costa-Farré et al. 2011; Campoy et al. 2012; Campoy & Mahler 2013; Gurney & Leece 2014). The increased interest is because of the need to improve overall procedure safety by minimising

complications and ensure adequate patient comfort and analgesia following injury or during surgery. The canine pelvic limb can be anaesthetised by administering local anaesthetics into the epidural space or by peripheral infiltration around targeted nerves (Clarke et al. 2014). Complications which have been described in epidural anaesthesia include: hypotension; urine retention; penetration of the dura with CSF leakage; technical failure and local anaesthetic toxicity due to inadvertent intravascular injection.(Jones 2001). Local anaesthetic toxicity can result in neurological complications such as: drowsiness, anxiety, paraesthesia to light and sound, seizures, muscle weakness and coma. Cardiovascular complications of local anaesthetic use include: hypotension, reduced heart contractility and arrhythmias (Cox et al. 2003). Regional anaesthesia nerve blocks are not without risk either. Intravascular injection is always a possibility, and intraneural injection is a potential cause of temporary or permanent nerve injury (Gurney & Leece 2014). However, a review and meta-analysis of available human literature comparing epidural anaesthesia to regional nerve blockade after major knee surgery found that the femoral nerve block provided comparable anaesthesia to the epidural technique, but with a lower likelihood of complications (Fowler et al. 2008).

Additional techniques such as electrical nerve location and ultrasound guidance improve the accuracy of peripheral nerve blocks, although ultrasound guidance requires more technical skill and expensive equipment (Mahler & Adogwa 2008).

Regional anaesthetic blocks of the distal pelvic limb focus on blocking the ischiatic and femoral nerves. The ischiatic nerve can be blocked using a proximal, transgluteal or parasacral approach (Gurney & Leece 2014). The proximal ischiatic nerve block, in which the nerve is targeted between the greater trochanter of the femur and the ischiatic tuberosity, has the highest success rating (77%) of all described approaches to block the ischiatic nerve (Gurney & Leece 2014).

The only sensory branch of the femoral nerve is the saphenous nerve. Described regional anaesthetic approaches to block the femoral nerve include a paravertebral, pre-iliac, suprainguinal and inguinal approach (Gurney & Leece 2014). Since there are no anatomical landmarks that determine the depth that the needle should be inserted for a femoral block, it has been recommended that the block is performed as proximally as possible where the saphenous and femoral nerves run close together, using peripheral nerve stimulation (Mahler & Adogwa 2008).

Methods used to detect regional anaesthesia of canine limbs

Investigators have derived a number of methods to detect the onset and offset of action of local anaesthetic drugs. These methods incorporate techniques to detect sensory and motor function in the region of interest, such as the pelvic limb of dogs. Common practice is to first obtain a baseline score or reading before the local anaesthetic drugs are administered and then perform subsequent measurements at fixed time intervals to detect the change in neural function over time. The scoring of sensory or motor function usually involves a simple descriptive scale whereby the investigator would assign a score according to their unique scoring system (Shilo et al. 2010; Shimada et al. 2017). However, these scoring systems tend to be subjective and are not often used alone to determine the onset and offset of the local anaesthetic block but rather used in combination with an objective measurement too. The objective testing of sensory function can be as simple as applying surgical forceps to pinch the skin at a standardised demarcated anatomical site (Shilo et al. 2010); or as complicated as applying a purpose build algometer device to determine a sensory threshold (Dixon et al. 2010; Lane & Hill 2015). These devices are designed to test either thermal or mechanical nociceptive thresholds (Musk et al. 2014). These devices are designed to pinpoint the onset and offset of regional anaesthesia and have only recently become standard practice for objective measurements (Dixon et al. 2010). Pressure algometry is a technique that determines mechanical nociceptive threshold, which is the point at which an animal will show an evasive behavioural reaction to an increasing pressure stimulus (Lane & Hill 2015).

Literature review outcome

In human medicine, hyaluronidase has shortened the onset of regional anaesthesia of local anaesthetic drugs (Rodgers & Craven 2014; Koh et al. 2015). These favourable results could be relevant in veterinary practice, yet there remains little published data on the effects that hyaluronidase would have on the onset and offset of regional anaesthesia in the pelvic limb of dogs. Clinical practice would likely benefit from an improved onset time and longer duration of action for regional anaesthesia.

Problem statement

Regional anaesthesia is becoming clinically relevant as a means to improve intra- and post-operative analgesia. Despite more accurate peripheral nerve blocking techniques and advancements in technology to study the effects of the local anaesthetic drugs, there remains a paucity in the literature regarding the effects that hyaluronidase has on the onset and offset of ropivacaine or bupivacaine peripheral nerve blocks performed on the pelvic limb.

Aims and objectives

The primary aim of the study was to evaluate the effect that hyaluronidase would have on the times to onset and offset of regional anaesthesia when combined with ropivacaine and bupivacaine in ischiatic and femoral nerve blocks.

This was accomplished by:

- Recording the times to onset and offset of regional anaesthesia when ropivacaine and bupivacaine were combined with hyaluronidase and used in ischiatic and femoral blocks.
- Comparing these times to onset and offset of regional anaesthesia to those of equal volume ropivacaine and bupivacaine solutions mixed with saline used in the same blocks.

The onset and offset of the block were quantitatively calculated using readings measured at regular, scheduled intervals using an algometer.

The beagles were monitored for six hours' post-block to elucidate on the effect that the addition of hyaluronidase may have on the time to offset of the regional anaesthetic block when combined with ropivacaine and bupivacaine in a femoral and ischiatic nerve block.

Hypothesis

H_0: The addition of hyaluronidase to ropivacaine and bupivacaine will not shorten the times to onset or offset of regional anaesthesia compared to the addition of saline in proximal ischiatic and femoral nerve blocks.

H_1: The addition of hyaluronidase to ropivacaine and bupivacaine will shorten the times to onset or offset of regional anaesthesia compared to the addition of saline in proximal ischiatic and femoral nerve blocks.

Materials and methods

Experimental design

A blinded, randomised crossover trial was performed.

Experimental animals

Eight healthy beagle dogs (six males and two females) weighing a mean ± standard deviation (SD) of 15.5 ±1.4 (ranged 13.3 to 18.9) kilograms and aged 2 to 7 years were used for the purposes of this study. The beagles were housed, fed (Vet's Choice, Royal Canin SA, North Riding, South Africa) and cared for by the University of Pretoria Biomedical Research Centre. Environmental enrichment was provided in the form of toys and walks. While the primary investigator was responsible for care of the beagles during the study procedures, a caretaker was responsible for feeding and cleaning pens during the rest periods (washout periods) the beagles were not being worked with.

Before the study began; a full clinical examination was performed on the dogs. This included a physical orthopaedic (pelvic limb joints range of motion, palpation of joints during extension, flexion and rotation) and neurological (gait observation, proprioception and patella reflex testing) examination of the pelvic limbs to detect any pre-existing defects that would influence the results of the study. Venous blood was also drawn to determine haematocrit and total serum protein. As no abnormalities were found, all eight dogs were included in the study.

A minimum sample size of 7 dogs per treatment was required based on a margin of error of 2 minutes when comparing the means of onset and offset times between hyaluronidase and saline when combined with the two local anaesthetic drugs used (estimated 2 standard deviations; 95% confidence level; two-sided).

Study design

Approval for the study was granted by the Animal Ethics Committee of the University of Pretoria (Protocol number V112-16). The study was conducted in two treatment blocks

(ropivacaine and bupivacaine) with a three-week rest period between treatment blocks. The dogs were randomly (online randomisation website http://www.randomization.com) assigned to two treatments within each treatment block in a crossover design, also with a three-week rest (washout) period between the treatments.

Experimental procedures

The experimental procedures were divided into a (a) pilot study and (b) study. The study was divided into a (i) pre-block, (ii) block and (iii) post-block phase.

(a) Pilot study

An initial trial was conducted on 14th and 15th September 2016, before the true data collection period, to allow the primary investigator to test the practicality of the planned experimental procedures. For this purpose, two of the eight dogs were randomly selected. A comprehensive clinical examination was performed and venous blood was drawn to determine haematocrit and total serum protein. As there were no abnormalities detected, the two dogs were taken into the room in which the study took place. Food had been removed from the dog's cages 8 hours before the trial took place, but they did have free access to water.

The dogs were sedated with acepromazine maleate (Neurotranq 1.0%; Virbac; Centurion, South Africa) at a dose of 0.03 mg kg^{-1} intramuscularly. After 30 minutes, a 20 gauge over-the-needle intravenous catheter (Jelco; Smiths Medical International Ltd.; Kent, UK) was aseptically placed in a cephalic vein and secured. The dog was induced into a light plane (relaxed jaw tone, suppressed swallowing reflex) of general anaesthesia, to facilitate endotracheal intubation, using propofol (5.0 mg kg^{-1} to effect; Fresenius Propoven 1.0%; Fresenius Kabi South Africa (Pty) Ltd.; Midrand, South Africa). The intubated dog was then connected to a circle breathing system and maintained on isoflurane (vaporiser set to 1%; Isofor; Safeline Pharmaceuticals (Pty) Ltd.; Johannesburg, South Africa) in oxygen at 40 ml kg^{-1} $minute^{-1}$.

The reason for maintaining the dog on isoflurane was to allow the primary investigator a comfortable time span to familiarise himself with the use of the nerve stimulator (Stimuplex HNS 12; B Braun; Melsungen, Germany) and determine the necessary settings for the ultrasound machine (Noblus; Hitachi Aloka Medical America; Wallingford, CT, USA) equipped with a linear probe (frequency of 5 to 13 mHz) to be used in the study. A balanced isotonic crystalloid fluid (lactated Ringers solution; Fresenius Kabi) was infused at a rate of 5.0 ml kg^{-1} $hour^{-1}$ during general anaesthesia. The temperature, heart and respiratory rate were continuously monitored and recorded every five minutes for the entire period they were under anaesthesia.

Sterile saline (Sabax Sodium Chloride 0.9%; Adcock Ingram Critical Care (PTY) Ltd.; Johannesburg, South Africa) was drawn into a syringe to a volume that each nerve site would receive 0.12 ml kg^{-1} saline on injection. The anatomical sites of the blocks were shaved, scrubbed with surgical soap and sprayed with surgical alcohol (Ethanol). The hands of the primary investigator were scrubbed and sterile gloves were worn.

The femoral block was performed first, with the dog in lateral recumbency and the leg to be blocked in the non-dependent position. An assistant held the leg at an approximate ninety-degree angle to the table to allow the primary investigator a femoral triangle approach (Campoy et al. 2010) to the block. The transducer was placed on the medial aspect of the proximal thigh area. Ethanol was intermittently sprayed onto the skin to enhance the image so that the femoral artery and nerve could be visualised. Once the primary investigator had visualised the nerve, an insulated stimulating needle (21G x 100 mm; Stimuplex A insulated needle; B Braun) was introduced in a medio-lateral direction and monitored on the image, aiming for the nerve.

The nerve stimulator was set to pulse at 1.0 Hz with a pulse width of 0.1 ms. The current was initially set to 1.0 mA until extension of the stifle was elicited. The current was then gradually decreased in a stepwise manner by a co-investigator to 0.4 mA. If a weak stifle extension continued at this current, the calculated dose of saline was injected and the injectate monitored on the ultrasound screen.

The leg was immediately placed in a natural position. Ethanol was sprayed over the lesser ischiatic notch so that the ischiatic nerve block could be performed next. A lateral approach was used (Costa-Farré et al. 2011). The ultrasound probe was then placed over the lesser ischiatic notch and once the ischiatic nerve was visualised, a stimulator needle was introduced, using the settings as described above. The needle was advanced in a dorso-ventral direction to enter the space lateral to the lesser ischiatic notch of the ischial spine, at the level between the proximal part of the greater trochanter of the femur (cranial) and ischiatic tuberosity (caudal). Once a weak flexion the tibio-tarsal joint was observed at a 0.4 mA setting, the calculated dose of saline was injected and monitored on the ultrasound screen.

The dog was then recovered in the procedure room and extubated with return of the swallowing reflex. Once alert and ambulatory, the dog was returned to the yard where the other dogs were kept.

From the pilot study, it became apparent that the initial plan to perform the blocks under conscious sedation using only acepromazine maleate as a pre-medication and propofol as an induction bolus was not in the best interests of the study nor the welfare of the dogs. The time needed to accurately and confidently perform the block was longer than initially anticipated and it was a concern that the dog would begin to rouse before the procedure could be completed. Therefore, it was decided to maintain the dogs on isoflurane throughout the procedure.

It was also noted during the pilot study that late extubation precipitated coughing which may interfere with the measurement of the nociceptive threshold in the early post-block period. Therefore, it was decided that, during the data collection period, the dogs would be extubated as early as possible, before the return of the swallowing reflex, during recovery.

(b) Study

The data collection period took place between the 3rd October 2016 and the 9th December 2016.

(i) Pre-nerve block phase

Before each data collection session, a clinical examination was performed, including an orthopaedic and neurological examination of the pelvic limb. Venous blood was drawn to determine haematocrit and total serum protein. The dogs had been starved for 8 hours prior to presentation in the procedure room but did have free access to water during this fasting period.

Treatments (recording the total doses administered) were prepared as follows:

Treatment block ropivacaine

- Treatment RS: 0.2 ml kg^{-1} ropivacaine HCl (1 mg kg^{-1}; Naropin 0.5%; AstraZeneca UK Ltd.; Luton, UK) plus 0.04 ml kg^{-1} saline (Sabax Sodium Chloride 0.9%)
- Treatment RH: 0.2 ml kg^{-1} ropivacaine HCl (1 mg kg^{-1}; Naropin 0.5%) plus 0.04 ml kg^{-1} hyaluronidase (Hyalase 100 IU ml^{-1}; Kyron Prescriptions; Benrose, South Africa)

Treatment block bupivacaine

- Treatment BS: 0.2 ml kg^{-1} bupivacaine HCl (1 mg kg^{-1}; Marcaine 0.5%; Adcock Ingram Ltd.) plus 0.04 ml kg^{-1} saline (Sabax Sodium Chloride 0.9%), and
- Treatment BH: 0.2 ml kg^{-1} bupivacaine HCl (1 mg kg^{-1}; Marcaine 0.5%) plus 0.04 ml kg^{-1} hyaluronidase (Hyalase 100 IU ml^{-1}).

All drugs were drawn up, 5 minutes prior to administration, to fill a 5 ml syringe (4.2 ml local anaesthetic and 0.8 ml of either saline or hyaluronidase) by a co-investigator who was not involved in the data collection part of the study. All drugs were colourless and transparent and syringes were marked with the dog's name only, enabling blinding of the primary investigator to the treatments. A calculated dose volume of 0.12 ml kg^{-1} was injected per site, based on the dog's body weight.

The room in which the study was performed was equipped as follows:

- Local anaesthetic solutions prepared by someone other than the principle investigator were drawn up as detailed and brought to the room.
- Drugs for premedication and induction of anaesthesia, as well as syringes and needles necessary to administer them.
- A nerve stimulator (Stimuplex HNS 12; B Braun; Melsungen, Germany) fitted with new batteries and a sterilised insulated needle (21G x 100 mm; Stimuplex A insulated needle; B Braun) for each dog.
- An ultrasound machine (Noblus; Hitachi Aloka Medical America; Wallingford, CT, USA) equipped with a linear probe with a frequency of at least 9Hz.
- The prod-pro and leg actuator set (ProdPro; Topcat Metrology; UK), with an air-primed 20 ml syringe.
- Endotracheal tubes
- Anaesthetic machine with a circle breathing system
- Intravenous catheters (Jelco; Smiths Medical International Ltd.; Kent, UK), drip fluid and drip sets.
- Equipment and detergents to clip and surgically prepare the block site.
- Blankets for the dogs' recoveries while still in the procedure room
- Stopwatches to track recording times for each dog
- Crash cart on standby with emergency drugs and an ambubag

On the day of data collection, the dogs were premedicated with acepromazine maleate (0.03 mg kg^{-1}, Neurotranq 1.0%; Virbac; Centurion, South Africa) intramuscularly into the triceps muscle.

A baseline nociceptive threshold was measured 30 minutes later. This measurement was done (each time by the primary investigator) by attaching the actuator of a pneumatically powered mechanical threshold algometer over the lateral aspect of the metatarsus (lateral aspect of metatarsal bone V) of the dog's left *pes* while in right lateral recumbency (Photo 1). The actuator (blunt-tipped 2 mm diameter pin) was advanced, to generate the application force, by gradually pressurising the system by compressing air from an air-primed 20 ml syringe. The system was pressurised until an evasive behavioural response of the dog was elicited, or until a maximum application force of 20 N was reached. Accepted evasive behavioural responses included definite limb withdrawals associated with a rapid head turn towards the probe, biting the probe or vocalisation. The increasing rate of system pressurisation, and thus rate of applied actuator force, was kept as constant (approximately 2.0 N second^{-1}) as possible by monitoring the warning lights built into the device, which indicated if the rate of pressurisation was too fast or slow. Each mechanical nociceptive threshold measurement was taken in duplicate, with a 40 second break between measurements. Duplicate measurements were within 2.0 N of each other and if the behavioural responses were consistent. In the event of duplicate measurements not being within 2.0 N of each other, a third measurement was taken and the two measurements closest in value were accepted, with the remaining measurement being discarded.

Photo 1 Prod Pro actuator attached to the medial aspect of the beagle's metatarsus

A 20 gauge over-the-needle intravenous catheter was aseptically placed in a cephalic vein and secured. The dog was induced into a light plane of general anaesthesia

(medial palpebral reflex and pedal withdrawal reflex obtunded and a relaxed jaw tone) using propofol (5.0 mg kg^{-1} to effect; Fresenius Propoven 1.0%; Fresenius Kabi South Africa (Pty) Ltd.; Midrand, South Africa). The trachea was intubated and connected to a circle breathing system and maintained on isoflurane (vaporiser set to 1%; Isofor; Safeline Pharmaceuticals (Pty) Ltd.; Johannesburg, South Africa) in oxygen at 40 ml kg^{-1} $minute^{-1}$. A balanced isotonic crystalloid fluid (lactated Ringers solution; Fresenius Kabi) was infused at a rate of 5.0 ml kg^{-1} $hour^{-1}$ during general anaesthesia. The temperature, heart and respiratory rate were continuously monitored and recorded every five minutes (example of monitoring sheet used attached in the addenda) for the entire period they were under anaesthesia. The dog was placed in right lateral recumbency and the sites of both peripheral nerve blocks (femoral and ischiatic nerves) of the non-dependent, uppermost pelvic limb (left-hand side) clipped and surgically prepared.

(ii) Nerve block phase

The femoral and ischiatic nerve blocks were performed using a combined ultrasound-guided/electrolocation technique. This technique required the use of an ultrasound machine with a linear probe (frequency of 5 to 13 mHz) and a peripheral nerve stimulator with a new insulated needle for each dog. The primary investigator's hands were surgically scrubbed and sterile gloves were worn.

The femoral nerve block was performed first, using a femoral triangle approach (Campoy et al. 2010). The ultrasound probe was placed in the prepared femoral triangle area (Photo 2). Ethanol was intermittently sprayed onto the site to enhance the quality of generated images. Once the femoral artery and nerve were visualised (Photo 3), a stimulating needle was inserted, aiming for the

Photo 2 Femoral triangle approach to the femoral nerve block

femoral nerve. The nerve stimulator was set to pulse at 1.0 Hz with a pulse width of 0.1 ms. The current was initially set to 1.0 mA until extension of the stifle was elicited. The current was then gradually decreased in a stepwise manner to 0.4 mA. If a weak stifle extension continued at this current, the calculated treatment dose volume (0.12 ml kg^{-1}) was injected (Photo 4).

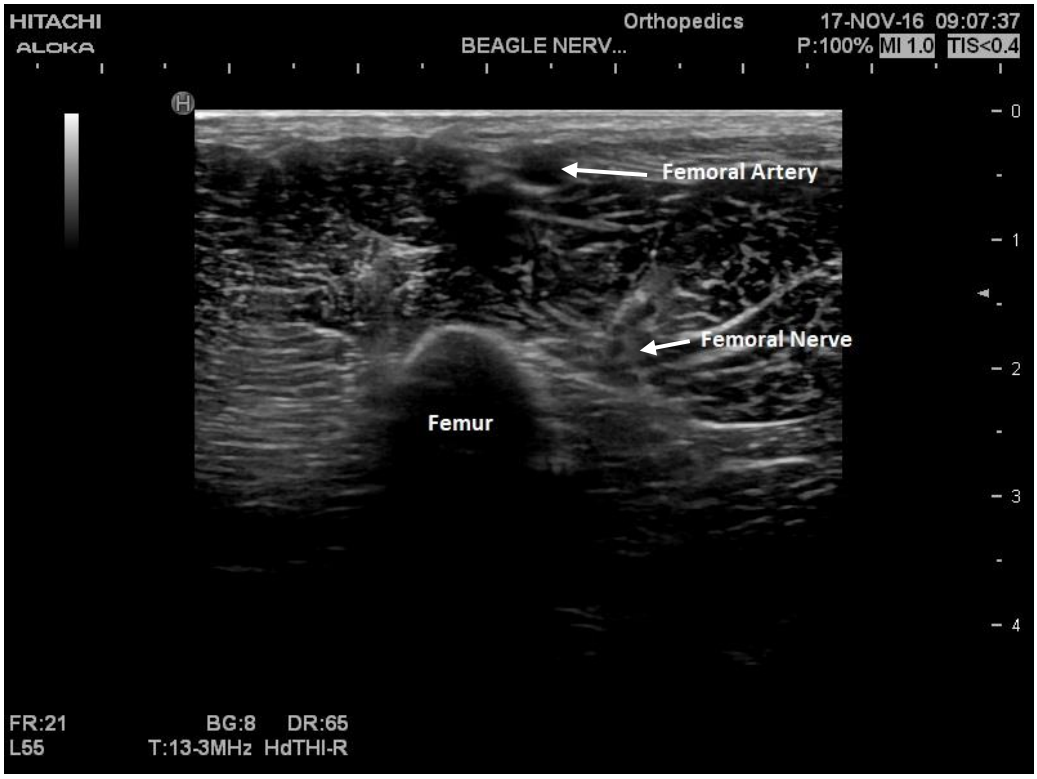

Photo 3 Labelled ultrasound image taken of the femoral nerve before injection of the anaesthetic solution

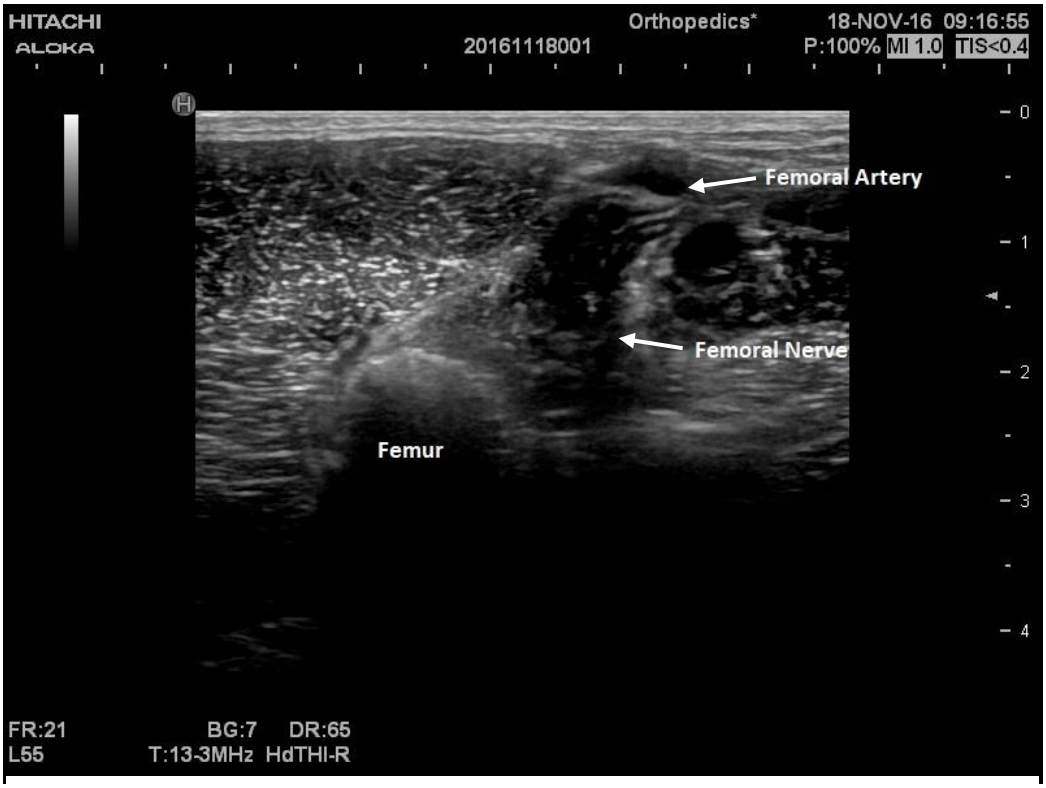

Photo 4 Labelled ultrasound image taken after injection of the anaesthetic solution. Note the anechoic injectate surrounding the femoral nerve (the "doughnut sign")

The ischiatic nerve block was performed next, using a lateral approach (Costa-Farré et al. 2011). The ultrasound probe was placed over the lesser ischiatic notch (Photo 5). Once the ischiatic nerve was visualised (Photo 6), a stimulator needle was introduced, using the settings as described above. The needle was advanced in a dorso-ventral direction to enter the space lateral to the lesser ischiatic notch of the ischial spine, at the level between the proximal part of the greater trochanter of the femur (cranial) and ischiatic tuberosity (caudal). The calculated treatment dose volume (0.12 ml kg^{-1}) was injected after a weak flexion the tibio-tarsal joint was observed at 0.4 mA setting (Photo 7).

Photo 5 Ischiatic block using the lateral approach

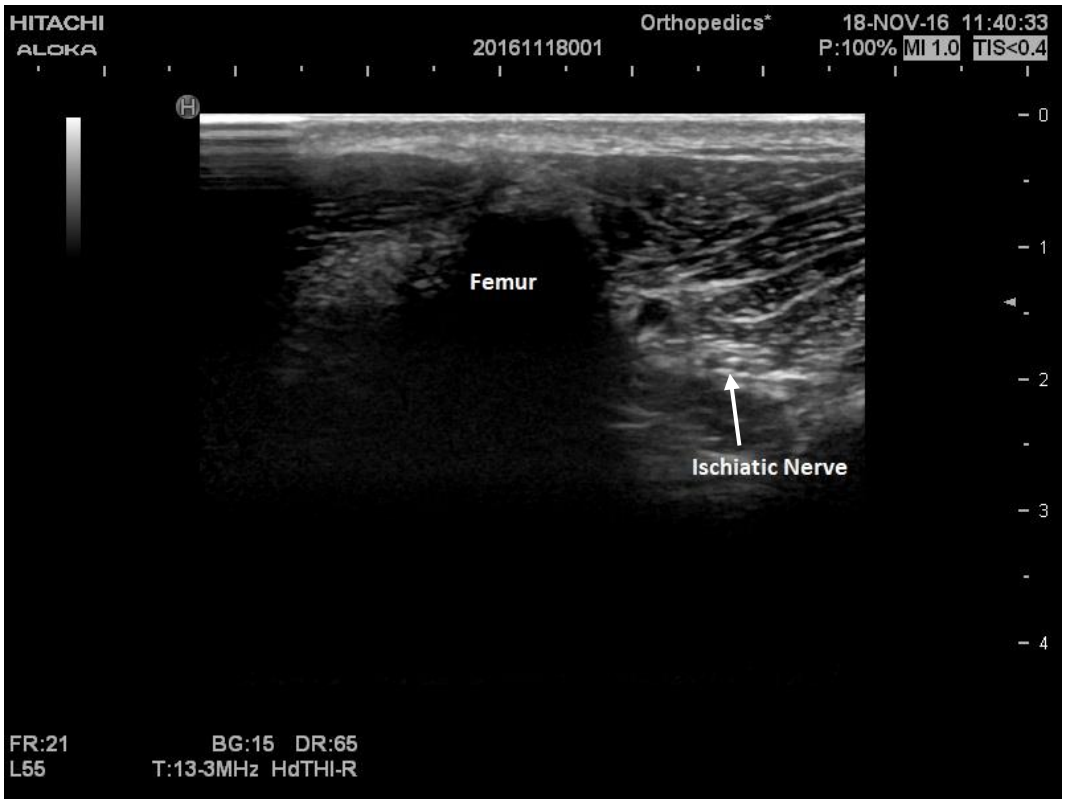

Photo 6 Labelled ultrasound image taken of the ischiatic nerve before injection of the anaesthetic solution

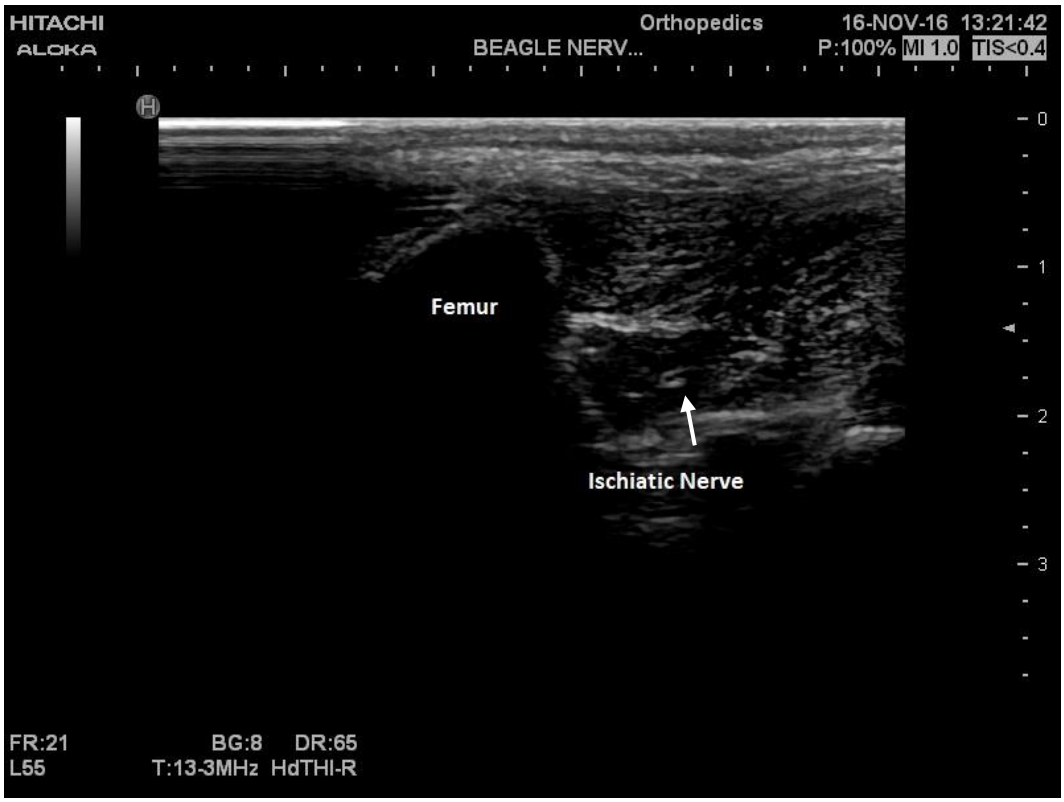

Photo 7 Ultrasound image of the ischiatic nerve taken after injection of the anaesthetic solution. Note the anechoic appearance of the injectate surrounding the nerve (the “doughnut sign”).

In both blocks, the nerve, the stimulating needle and the injectate were monitored on the ultrasound image for the formation of a “doughnut sign”, usually observed as the targeted nerve becomes surrounded by the injectate. The principle investigator assigned the block a subjective score rating the confidence in the block (Table 1). The time taken from induction to completion of the blocks (induction to end block time) and the last measured rectal temperature were also recorded.

Table 1 Subjective scoring system used to grade the confidence in a performed block.

Score	Definition
1	No confidence due to no evidence that the needle was in the correct position
2	Poor confidence. Appropriate response to nerve stimulator but no confirmation of correct placement on ultrasound
3	Fairly confident. Appropriate response to nerve stimulator with deposition of injectate in correct anatomical location as viewed on ultrasound
4	Confident. Appropriate response to nerve stimulator and injectate viewed surrounding the nerve as a “doughnut sign”.

(iii) Post-nerve block phase

Immediately after the blocks were completed, the isoflurane was stopped and the dog was detached from the breathing system. A stopwatch was started, where time T0 was immediately after completion of the ischiatic nerve block. For the first 30 minutes post-block, the dog was recovered on the procedure table (Photo 8). During this time, the mechanical nociceptive threshold was measured every 3 minutes. Each measurement was taken in duplicate, as described in the pre-block phase. The dog's trachea was extubated soon after stopping the inhalation anaesthesia to avoid coughing which could interfere with the interpretation of the dog's reaction to the stimulus.

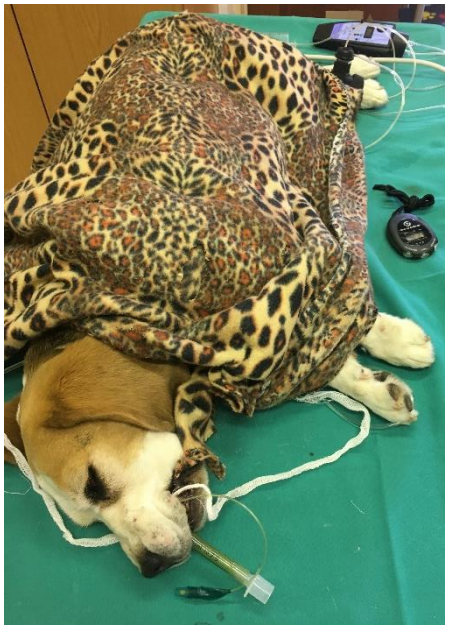

Photo 8 A beagle being recovered on the procedure table for the first 30 minutes post-block

After the first 30-minute recording period, the dog was taken to an outside pen where it could eat, interact and behave normally. The mechanical nociceptive thresholds were then measured every 30 minutes, until two consecutive readings at half hour intervals that were equal to or lower than the initial baseline reading were observed, or for a maximum of 360 minutes (6 hours) post-block. The measurements were taken in the pen with the dog positioned in lateral recumbency with the blocked pelvic limb in the non-dependent, uppermost position.

The quality of the measured mechanical nociceptive threshold data over time and confidence of the block were used to identify block failures (no appreciable drug effect identified by absence of an increase in nociceptive threshold of more than 25% over time). Block failures were also presumed in situations where dogs did not allow baseline measurements (i.e. stoic nature where baseline measurements averaged >15 N) to be taken. Block failures were not included in the statistical analysis.

Data collection

Records were manually collected and managed by the principle investigator. At the end of each day of data collection all completed data collection documents were scanned into a digital format. The principle investigator, supervisor and co-supervisor had access to this data. The original copies of the data sheets were kept by the principle investigator.

Statistical analysis

Duplicate mechanical nociceptive threshold measurements for each time point (baseline and all post-block measurements) were averaged and the average reading was used for further analysis. Data were assessed for normality by evaluating descriptive statistics, plotting of histograms and performing the Anderson-Darling test for normality. Data is reported as median (range), unless otherwise stated. Quantitative data (induction to end block time and last temperature) were compared between treatments within a treatment block using Mann-Whitney U test. The confidence scores assigned after each block (for each dog in each treatment) were compared among treatments using the Friedman test (response: confidence score; Treatment: treatment RS, RH, BS, BH; Block: dog). Block failures were excluded from further analysis. The counts of failed blocks to successful blocks among treatments were tabulated. The mechanical nociceptive threshold data were determined to be non-parametric in distribution and thus were rank-transformed prior to further analysis. The percent change from the baseline measurements was calculated for each post-block measurement (3 to 360 minute readings) within a treatment and was also non-parametric in distribution and thus rank transformed prior to further analysis. Then the mechanical nociceptive threshold and percent change from baseline data was tested for equal variances between treatments using Levene's test and all data had similar variances. The effect of hyluronidase was compared against saline for each local anaesthetics (RS versus RH and BS versus BH) using a general linear mixed model (response: mechanical nociceptive threshold and percent change from baseline; fixed factors: time, treatment; random factors: dog; interaction: time, treatment, treatment x time). The goodness of fit was determined by examining residual plots (histograms, normality plot and residual versus fit

plots). Assessment of the R-squared values (real and adjusted values) were evaluated to determine if the general lineal mixed model was over fitted. Median mechanical nociceptive threshold measurements and change from baseline (%) values over time were plotted on bar charts. The onset of a clinically significant block was set as a 25% increase of mechanical nociceptive threshold from the baseline reading. The offset of the block was defined as a return to a mechanical nociceptive threshold < 25% from baseline. The onset and offset times for each dog receiving each treatment were identified and tabulated (not shown) for comparison. The time to onset and offset of blocks were compared between treatments within a block using Mann-Whitney U test. The duration of the effective block was defined as the time from nociceptive threshold >25% to a return to < 25% from baseline. Data were analysed using commercially available software (MiniTab 17.1; Minitab Inc., Pennsylvania, USA) and results interpreted at the 5% level of significance.

Ethical considerations

- Ethical considerations of this study were aimed at ensuring that the dogs were used in a responsible manner during the project, with no compromise to their welfare. For this reason, no work was conducted on the beagles until approval from the University of Pretoria's research and animal ethics committee had been granted.
- In the event of death, personnel at the UPBRC would have been informed as soon as possible and a post-mortem examination would have been conducted to determine the cause of death. No deaths occurred during the study period.
- In the event of injury, the UPBRC had a contactable veterinarian who would treat the injury encountered.
- After the study period the beagles were rehomed.
- Onderstepoort students and colleagues who helped in the data collection phase of the study were not forced to participate. They assisted by invitation only and did not receive any financial compensation.
- None of the procedures or drugs used in the study were considered harmful to dogs or humans.

Results

All beagles were included into the study based on clinical, neurological and orthopaedic examination findings and therefore they received all treatments. Data from some dogs were excluded from statistical analysis due to failure to obtain a reliable baseline nociceptive threshold reading (n = 4) or failure of the block to demonstrate an increase in nociceptive threshold from baseline (n = 5). Overall, the number of dogs excluded did not differ between the treatments within each treatment block. The body temperature (normothermic for dogs), confidence of the block and time interval from induction of general anaesthesia to the completion of the block did not differ between the treatments within each treatment block (Table 2).

Table 2 Exclusion data, temperature, confidence score and time data for beagle dogs undergoing femoral and ischiatic blocks with ropivacine (R) or bupivacaine (B) with the addition of either hyaluronidase (H) or saline (S).

Block		**Ropivacaine**							**Bupivacaine**						
Treat		**RS**			**RH**				**BS**			**BH**			
Exclusion-inclusion data															
Excluded	NBL	1#			1#				1			1#			
	NB	1			2				1			1			
Included		6			5				6			6			
Total		8			8				8			8			
Clinical and time data															
	Unit	Median	Range		Median	Range		p-v	Median	Range		Median	Range		p-v
			Min	Max		Min	Max			Min	Max		Min	Max	
Temp	°C	36.7	35.8	38.0	37.0	35.6	38.0	1.000	36.4	35.7	36.9	36.5	35.5	37.8	0.495
Confidence	-	4	3	4	3	2	4	0.142	4	3	4	4.0	3	4	0.793
Induction to end block time	Min	17	13	25	22	10	31	0.529	18.5	10	22	18.5	14	24	0.916

Treat: treatment; RS: ropivacaine-saline; RH: ropivacaine-hyaluronidase; BS: bupivacaine-saline; BH: bupivacaine-hyaluronidase; NBL: no baseline reading; NB: no block detected; #: data from the same dog; P-v: p-value; Temp: rectal temperature on completion of block; °C: degree Celsius; Confidence: subjective score of the investigator's confidence in the block; min: minutes. Significance level $\alpha = 0.05$

There were no statistically different observations in both treatment blocks for the "treatment x time" interactions when analysing the onset and offset of action outcome of the

general linear mixed model (Table 3). There were significant findings for the “treatment” interaction for the onset of action for RS vs RH (p = 0.001) and BS vs BH (p < 0.001) and for the offset of action for BS vs BH (p = 0.022). These significant findings indicate that the magnitude of the percent change values was different between the saline and hyaluronidase treatments at each time point. For the “time” interaction the only significant observation was for the onset of action for BS vs BH (p = 0.003). This significant interaction indicates that the change in percentage value over the 3 to 30 minutes time intervals within each treatment were different. Despite no statistical significance, there were observational findings when examining the medians over time which allowed prediction of an onset and offset time (and thus a duration of action) for both treatment blocks, which could assist in a clinical interpretation of the data (Figure 2).

Treatment block: Ropivacaine

The onset of a clinically relevant block for RS was at 21 (3-60) minutes and different to 12 (3-21) minutes for RH (Mann-Whitney U test; p = 0.144; Figure 1a). The offset of the block for RS was at 270 (90-360) minutes and no different to RH which was at 180 (30-300) minutes (Mann-Whitney U test; p = 0.361; Figure 1b). The number of dogs within each treatment remained constant until 90 minutes, which was when the first RS dog’s values returned to baseline. The first dog in the RH treatment to return to baseline occurred at 270 minutes. Shortly thereafter, over the remaining 30-minute monitoring intervals, most of the dogs returned to baseline which suggests that the duration of action for ropivacaine, mixed with either saline or hyaluronidase, does not appear to last longer than 360 minutes. Also, because the number of dogs being monitored decreased rapidly from 240 minutes, the median % change over time became a challenge to interpret from this time point onwards.

This trend is mirrored by the median Newtons measured (Figure 2a, b), with the median Newtons of the RS treatment reaching 20 N at 18 minutes and falling at 300 minutes, compared the RH treatment which reached a median of 20 N at 12 minutes and a sudden temporary drop at 180 minutes.

Treatment block: bupivacaine

The onset of a clinically relevant block for BS was at 24 (3-60) minutes and no different to 9 (3-27) minutes for BH (Mann-Whitney U test; $p = 0.394$; Figure 1c). The offset of the block for BS could not be determined because the % change did not return to <25% above baseline for the entire 360-minute monitoring period. For BH was 330 (210-360) minutes and no different to BS (Mann-Whitney U test; $p = 0.456$; Figure 1d). The number of dogs within each treatment remained constant within each treatment until 210 and 240 minutes, which was when the first BH and BS dog's values returned to baseline, respectively. Half (BS) and two-thirds (BH) of the dogs remained blocked until the end of the monitoring intervals.

The median Newtons measured (Figure 2c, d), the BS treatment reached 20 N at 60 minutes and maintained this threshold for the remaining monitoring period. Whereas, BH, despite having an early onset, demonstrated a progressive increase of force over time and reached 20 N at 90 minutes, but from 270 minutes onwards the median Newtons was variable until the end of the monitoring period.

During the study period, there was one case of injury where a dog developed non-weight bearing lameness of the hind limb that had been blocked several days prior. The dog was prescribed Carprofen (Rimadyl; Zoetis South Africa (Pty) Ltd.; Sandton, South Africa) at a dose of 4,4mg kg^{-1} once daily for 5 days and made an uneventful recovery. The dog remained in the study and showed no more lameness over the rest of the data collection period. We did not determine if the dog suffered transient neuropraxia, myositis or injection site infection.

In summary, hyaluronidase had no effect on the onset and offset times of ropivacaine and bupivacaine femoral-ischiatic nerve blocks in dogs compared to saline. The onset and offset times were highly variable in all treatments. Clinically, the high variability of the onset and offset times of the regional anaesthesia of these local anaesthetic drugs mean that clinicians must monitor the patient's response and, if required, provide additional analgesic drugs.

Figure 1 Bar charts demonstrating the median percentage change from baseline nociceptive threshold values over time for beagle dogs undergoing a femoral and ischiatic nerve block using ropivacaine (R) or bupivacaine (B) mixed with either saline (S) or hyluronidase (H). The number of beagles still enrolled in the calculations is reflected in brackets under treatment and time. The dotted line at 25% indicated the percentage change above baseline that defined the onset and offset of block.

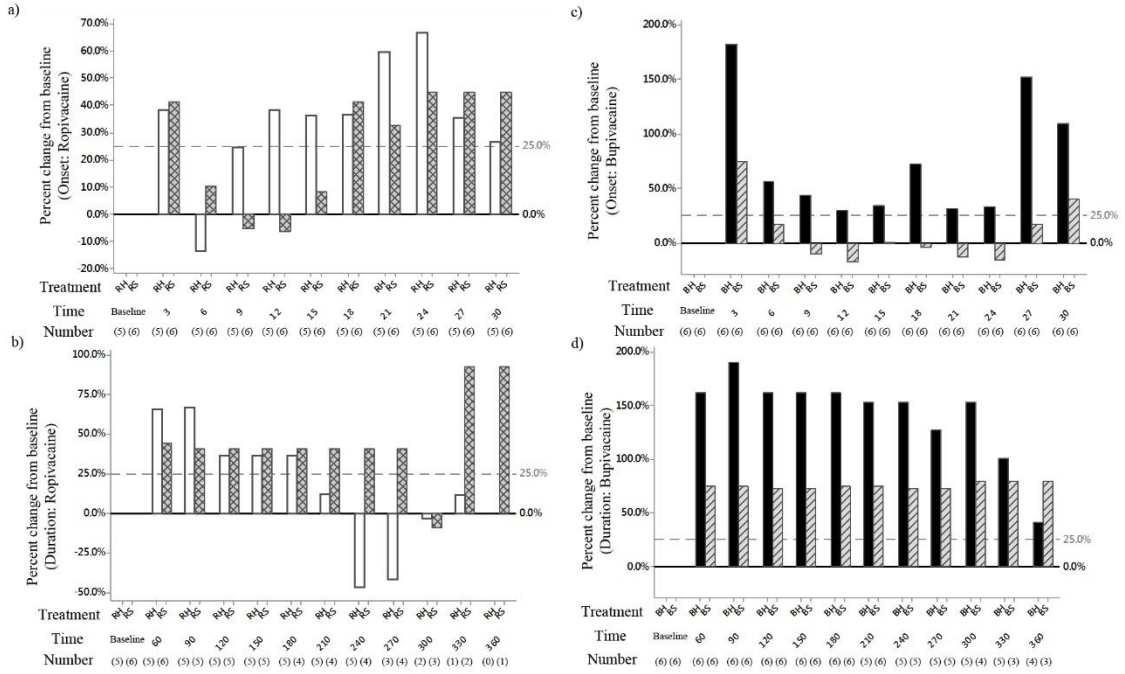

Figure 2 Bar charts demonstrating the median mechanical threshold force (Newtons) over time for beagle dogs undergoing a femoral and ischiatic nerve block using ropivacaine (R) or bupivacaine (B) mixed with either saline (S) or hyaluronidase (H). The number of beagles still enrolled in the calculations is reflected in brackets under treatment and time.

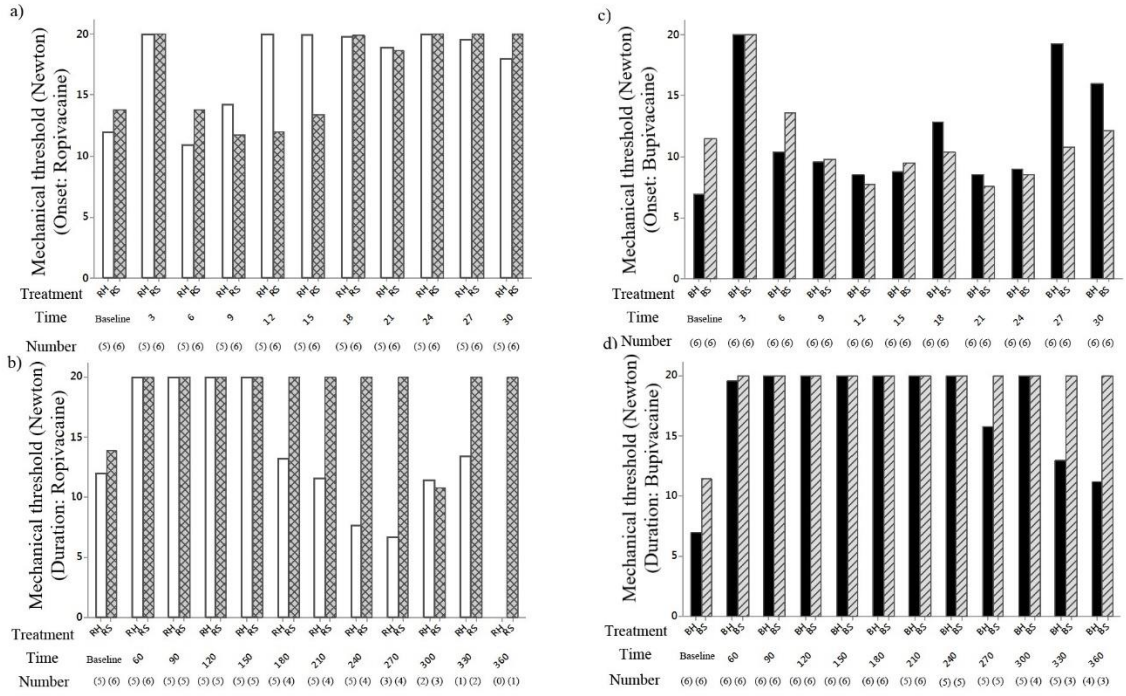

Table 3 Percent change of nociceptive threshold measurements from baseline readings in beagle dogs undergoing a femoral and ischiatic nerve block using ropivacaine (R) or bupivacaine (B) mixed with either saline (S) or hyaluronidase (H). The * denotes no beagles remaining under the effects of the block at that time in the table.

Time (minutes)	**Median**	**Minimum**	**Maximum**		**Median**	**Minimum**	**Maximum**		**p-values**		
					Ropivacaine Treatment Block						
		RS				**RH**			**treat**	**Time**	**treat x time**
			Onset of action (% change from baseline)								
0 (baseline)	0	0	0	Beagles	0	0	0	Beagles			
3	41	-52	96	6	38	34	233	5			
6	10	-50	70	6	-14	-52	233	5			
9	-5	-39	94	6	24	-56	233	5			
12	-6	-43	94	6	38	-16	233	5	0.001	0.659	0.937
15	8	-54	85	6	37	-4	233	5	r^2 =	0.547	
18	41	-4	94	6	37	16	228	5	ar^2 =	0.412	
21	33	-54	92	6	59	31	78	5			
24	45	-15	96	6	67	-66	233	5			
27	45	19	96	6	35	-68	233	5			
30	45	-10	96	6	27	-60	233	5			
			Offset of action (% change from baseline)								
60	45	34	96	6	66	-10	233	5			
90	40	-52	96	5	67	36	233	5			
120	40	-25	96	5	36	-30	233	5			
150	40	-37	96	5	36	-30	233	5			
180	41	-3	96	4	36	-30	92	5	0.895	0.140	0.762
210	42	25	96	4	12	-43	93	5	r^2 =	0.534	
240	42	-6	96	4	-47	-56	100	5	ar^2 =	0.428	
270	42	-51	96	4	-42	-66	62	3			
300	-10	-57	92	3	-3	-31	25	2			
330	92	41	96	2	11	11	11	1			
360	92	92	92	1	*	*	*	0			

Table 3 continued

Time	Median	Minimum	Maximum		Median	Minimum	Maximum		p-values		
					Bupivacaine Treatment Block						
		BS				**BH**					
					Onset of action (% change from baseline)						
0 (baseline)	0	0	0	Beagles	0	0	0	Beagles			
3	75	66	213	6	182	-45	242	6			
6	17	-16	199	6	56	20	187	6			
9	-10	-18	55	6	44	3	153	6			
12	-17	-36	-2	6	30	-19	199	6	<0.001	0.003	0.975
15	0.1	-50	44	6	34	-15	222	6	r^2 =	0.566	
18	-4	-11	70	6	72	-12	242	6	ar^2 =	0.420	
21	-13	-40	15	6	31	-5	153	6			
24	-16	-48	14	6	34	3	123	6			
27	17	-26	79	6	152	-2	231	6			
30	41	-10	86	6	109	-9	231	6			
					Offset of action (% change from baseline)						
60	75	66	213	6	162	-1	231	6			
90	75	66	213	6	190	48	231	6			
120	73	13	213	6	162	107	242	6			
150	73	66	213	6	162	39	242	6			
180	75	66	213	6	162	62	242	6	0.022	0.710	0.976
210	75	66	213	6	153	14	210	5	r^2 =	0.485	
240	73	-0.4	213	5	153	32	210	5	ar^2 =	0.289	
270	73	-14	213	5	127	43	170	5			
300	79	28	213	4	153	-23	210	5			
330	79	58	213	3	101	-21	170	5			
360	79	-21	213	3	42	10	170	4			

RS: ropivacaine-saline; RH: ropivacaine-hyaluronidase; BS: bupivacaine-saline; BH: bupivacaine-hyaluronidase; treat: treatment; Beagles: the number of beagles still blocked at that point of the data collection period; r^2: r-squared value; ar^2: adjusted r-squared value; *: no beagles remaining under the effects of the block at that time. Significance level $\alpha = 0.05$

Discussion

The addition of hyaluronidase to ropivacaine or bupivacaine for femoral-ischiatic nerve blocks in dogs had no effect on the onset and offset times of action. The algometer was an effective objective tool in monitoring the onset and offset of the blocks, however, the data sets were highly variable. Femoral-ischiatic blocks were performed using a combined ultrasound-electrolocation technique and this procedure was considered successful to allow comparisons to be made between the treatments within each treatment block, despite a few failed nerve blocks.

There is scant literature of the combination of hyaluronidase and a local anaesthetic drug administered for nerve blocks in dogs, which makes comparing our findings a challenge. Also, this appears to be the first report of hyaluronidase combined with either ropivacaine or bupivacaine to block the femoral-ischiatic nerves in dogs. Our investigation detected a clinical effect and therefore further investigation into the effects of hyaluronidase has on nerve blockade is warranted.

The lack of statistical significance in our study could be attributed to a number of factors. The sample size calculation assumed that our onset and offset of action time data would have a standard deviation of 2 minutes which was not the case, unlike standard deviations reported in similar studies (DeRossi et al. 2011; Shimada et al. 2017). We observed standard deviations ranging from 11 to 28 minutes. This wide range implies that samples sizes in excess of 119 dogs per treatment are required to detect a statistically significant observation with confidence. Previous studies are equivocal regarding variability in duration of onset and offset times of local anaesthetic blocks in dogs. For example, Shilo et al. (2010) reported that bupivacaine (0.05, 0.1 and 0.2 ml kg^{-1}; 0.5% solution) had a highly variable onset of action which ranged from 20-160 minutes, similar to our study. Whereas, Shimada et al. (2017) report an onset of action of 15 minutes, without variation, for bupivacaine (0.2 ml kg^{-1}, 0.4 ml kg^{-1}; 0.5% solution). The inconsistent findings among these studies could be explained by the different methodologies used to assess the block and criteria used to define the onset and offset of the block. Two methodology factors could

explain the high variability of our data sets: 1) the use of the algometer and, 2) the criteria used to define the onset and offset of action. There are known confounding factors that influence the variability of algometry readings, such as: altering the probe tip diameter, site of application, rate of application and dog position during stimulation (Harris et al. 2015). We standardised our methodology by using one tip diameter (2 mm), used one site of application (lateral aspect of the metatarsus of left pes), applied the stimulation at a fixed rate indicated by the device, and used one dog position during stimulation (right lateral recumbency). Furthermore, Harris et al. (2015) reported that a small (2 mm) diameter probe tip had the least variability of the nociceptive thresholds compared to wider tips. However, our nociceptive threshold readings were still highly variable. Other algometer related causes of the high variability could include 1) the dogs (and primary investigator) learning to anticipate the stimulation and thus reacting at a lower threshold, especially during repeated measures over time (Coleman et al. 2014), and 2) the wrapping of the remote actuator assembly to the metatarsal region may have not been aligned well enough to allow the pin to contact the bone of the metatarsal V during testing in all measurements. The second potential cause of high variability is the arbitrary definition of our onset and offset of action been defined as a 25% increase above and as a return to <25% above baseline nociceptive threshold readings, respectively. Defining the onset and offset of action should be simple and repeatable. For example, Shimada et al. (2017) made use of two categorical scoring system to detect changes in sensory and motor function. Each scoring system had three possible categories and their reported onset and offset times were not variable (Shimada et al. 2017). Unlike, Shilo et al. (2010) who made use of a more elaborate motor block scoring system that included 1) weight bearing when standing and walking, 2) proprioception deficits and 3) leg carrying position. Although the scoring system was simple to apply, it perhaps allowed a more varied outcome. We did not investigate if altering our definition of onset and offset of action would have altered the high variability. Future research in regional anaesthesia requires consensus on how to measure drug effects of the block and how to define the onset and offset of action.

Another limitation to the study was that the dogs were under general anaesthesia for the nerve block procedures. The authors' reasoned that it would have been more important to ensure the most accurate deposition of the treatment solutions around the nerves to ensure the best chance for creating a block. This could only have been achieved while the animals were unconscious and unaware of the procedure. Furthermore, the dogs were awake and conscious within 6 minutes (comments made on the data collection sheets at each time point) and prior to the detection of the increase in applied force, suggesting that the true onset of action was detected when the dogs were awake and conscious. The recovery times were similar to isoflurane-anaesthetised dogs recovering after a magnetic resonance imaging scan (Lozano et al. 2009), and to normothermic dogs premedicated with acepromazine maleate (Kleine et al. 2014). Another limitation was that the ropivacaine treatment block was completed first followed by the bupivacaine treatment block, therefore we cannot rule out the possibility that the investigators got more proficient at completing the study procedures and the dogs perhaps got accustomed to the study over time and the effect this could have on the overall results (Coleman et al. 2014). Furthermore, the interpretation of the linear mixed model outcome, especially for the offset of action comparisons over time, should be interpreted with caution because of the overall modest fit of the data to the model (r^2 and adjusted r^2 values) and the number of dogs decreasing over time.

The combined use of ultrasound guidance and electro-location improved the operator's confidence that the needle tip was close to the nerve. Additionally, the injectate could be visualised on the image, the appearance of which gave an objective indication of block success and reduced fears of accidental intravascular injection. Overall, the failure rate for this study was 28.1% (9/32 blocks). Similarly, a study investigating ultrasound-guided femoral-ischiatic nerve blocks with bupivacaine reported a failure rate of 33% (Shilo et al. 2010). Other investigators have also reported failure rates by indicating that rescue analgesia was required and these rates range from 13% to 50% (Bartel et al. 2016; Congdon et al. 2017; Portela et al. 2013).

The linear mixed model detected a treatment effect only when hyaluronidase was added to ropivacaine and bupivacaine, whereby the magnitude of the percent change from baseline values during the onset of action was different to that of saline. The differences could be because of hyaluronidase increasing the permeability of the tissue, allowing a larger number of molecules to reach the neurolemma in a shorter time which increased the local anaesthetic drug effect (Koh et al. 2015). It could also, in part, be due to the alkalinising effects of hyaluronidase, which would cause a larger proportion of the local anaesthetic to be in the non-ionised state and thus causing a deeper block (Palte 2015). The linear mixed model also detected a difference in the magnitude in percent change from baseline during the offset of action when bupivacaine was combined to hyaluronidase compared to saline. A theory is that wider dispersion through the tissues because of hyaluronidase altering the perineural tissue characteristics reduces the drug's perineural availability by increased absorption into the blood and non-neural tissues, and thus decreased the magnitude of drug effect (Kumar & Macachor 2015).

Conclusion

Hyaluronidase had no effect on the onset and offset times of ropivacaine and bupivacaine femoral-ischiatic nerve blocks in dogs compared to saline. The onset and offset times, determined by the percent change in nociceptive threshold using an algometer, were highly variable in all treatments. Clinically, the high variability of the onset and offset times of the regional anaesthesia of these local anaesthetic drugs mean that clinicians must monitor the patient's response and, if required, provide additional analgesic drugs.

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Addenda

Contents

Anaesthetic monitoring form

Beagle number:_____
Volume of ACP as pre-med:_____
Initial propofol bolus (ml):_____
Anaesthetist:_____ Phone number:_____

Time	Heart Rate	Respiration Rate	Mucous membranes	Temperature	Additional propofol given
5					
10					
15					
20					

IF LOST PLEASE CONTACT
Researcher name: Travis Gray
Researcher cellphone number: 0761949867

Data collection form

Beagle Number: Date: **IF LOST PLEASE CONTACT** Researcher name: Travis Gray Researcher cellphone number: 0761949867		
Time Post Block (Minutes)	Nociceptive Threshold	Comments
Pre-block		
0		
3		
6		
9		
12		
15		
18		
21		
24		
27		
30		
60		
90		
120		
150		
180		
210		
240		
270		
300		
330		
360		

Publications arising from the study

Gray TR, Dzikiti BT, Zeiler GE. Effects of hyaluronidase when administered with ropivacaine or bupivacaine for regional anaesthesia of the canine pelvic limb. *Veterinary Anaesthesia and Analgesia* (In Print) https://doi.org/10.1016/j.vaa.2018.09.041

Animal ethics certificate

UNIVERSITEIT VAN PRETORIA
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Animal Ethics Committee

PROJECT TITLE	**Effects of hyaluronidase on ropivacaine or bupivacaine regional anaesthesia of the pelvic limb**
PROJECT NUMBER	**V112-16**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. T Gray**

STUDENT NUMBER (where applicable)	**UP_28062982**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**Beagles**	
NUMBER OF SAMPLES	**8**	
Approval period to use animals for research/testing purposes		**August 2016 – August 2017**
SUPERVISOR	**Dr. G Zeiler**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 1 September 2016
CHAIRMAN: UP Animal Ethics Committee	Signature