Physiological variables for the objective detection of intraoperative nerve block failure in dogs

by

Pierre Etienne Basson

Submitted in partial fulfilment of the requirements for the degree Master of Medicine in Veterinary Science (Anaesthesiology) in the Department of Companion Animal Clinical Studies, Faculty of Veterinary Sciences,

University of Pretoria

Date submitted:

August 2023

Supervisor : Prof Gareth E Zeiler

Co-supervisor : Dr Abdur R Kadwa

Acknowledgements

God for providing me with the opportunity and ability to specialise.

I would like to thank my wife, Laura, for supporting me through this journey and always encouraging me. For her understanding and sacrifice throughout the last few years.

To my supervisors Prof Gareth Zeiler and Dr Abdur Kadwa for their sacrifice and guidance through both the MMed Vet and ECVAA programs. To Dr Justin Grace and Dr Kaegan Boustead in assisting with my training.

To Dr Chris Blignaut for soldering through these last few years of the specialisation with me.

My family Ernrich, Tersia, Anrich and Michelle Basson and Tina De Beer for their support through this.

To all the staff at Valley farm especially Dr Tim Krafft, Dr Dean de kock, Sr. Marilise Vermaak and Sr. Celine Lamprecht.

Declaration of originality

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

UNIVERSITY OF PRETORIA

FACULTY OF VETERINARY SCIENCE

DECLARATION OF ORIGINALITY

This document must be signed and submitted with every essay, report, project, assignment, mini-dissertation, dissertation and/or thesis

Full names of student: Pierre Etienne Basson

Student number: 10152564

Declaration:

1. I understand what plagiarism is and am aware of the University's policy in this regard.
2. I declare that this dissertation (e.g. essay, report, project, assignment, mini-dissertation, dissertation, thesis, etc.) is my own original work. Where other people's work has been used (either from a printed source, Internet or any other source), this has been properly acknowledged and referenced in accordance with departmental requirements.
3. I have not used work previously produced by another student or any other person to hand in as my own.
4. I have not allowed, and will not allow, anyone to copy my work with the intention of passing it off as his or her own work.

Signature of student: .....

Signature of supervisor: .....

Table of contents

Acknowledgements ii
Declaration of originality iii
Table of contents iv
List of tables vi
List of figures vii
List of abbreviations ix
Abstract xi
1.0 Introduction 1
2.0 Literature review 3
2.1 Nociception and pain 3
2.2 The intraoperative detection of nociception 4
2.2.1 Assessment of the ANS 5
2.3 Anaesthetic and analgesic drugs used in our study and their effect on nociception 8
2.3.1 Anaesthetic drugs 8
2.3.2 Analgesic drugs 9
2.4 Locoregional anaesthesia 13
2.4.1 Neurolocation 13
2.4.2 Anatomy and technique 14
2.4.4 Complications of locoregional anaesthesia 16
2.5 Inference and outlines 17
3.0 Materials and methods 18
3.1 Animals and housing 18
3.2 Study design 19
3.3 Sample size 20
3.4 Experimental procedures 20
3.4.1 Procedures of the nerve blocks 23
3.4.2 Intraoperative monitoring 25
3.4.3 Intraoperative analgesia and rescue interventions 26
4.0 Data analysis 27
4.1 Part A 27
4.2 Part B 28
5.0 Results 29
5.1 Part A 29
5.2 Part B 32

6.0 Discussion 36
Conclusion and future research 41
References 42
Appendixes 53
Appendix i 53
Appendix ii 54
Appendix iii 55
Appendix iv 56
Appendix v 57
Declarations 58

List of tables

Table 5.1 Summary of the receiver of operator characteristic (ROC) analysis of guided saline block (GSB; 10 dogs; response to surgical stimulus) and guided bupivacaine block (GBB; 10 dogs; no response to surgical stimulus) undergoing an arthrotomy under surgical anaesthesia [premedicated with 0.01 mg kg^{-1} medetomidine and 0.3 mg kg^{-1} morphine, induced with propofol and maintained on isoflurane (FE´Iso 1.6 %)]. Indicating the maximum potential effectiveness of heart rate (HR), delta HR, respiratory rate (f_R), delta f_R, systolic arterial pressure (SAP), delta SAP, mean arterial pressure (MAP), delta MAP, diastolic arterial pressure (DAP) and delta DAP in detecting a response to surgical stimulus. 31

Table 5.2 Variables that can be used to objectively detect peripheral nerve block failure in dogs undergoing stifle arthrotomy. All dogs were premedicated with 0.01 mg kg^{-1} medetomidine and 0.3 mg kg^{-1} morphine, induced with propofol and maintained on isoflurane (FE´Iso 1.6 %) in oxygen. 35

List of figures

Figure 3.1 Study design indicating the treatment groups during data colection and the relevance of the data collected from the specific sections of the study (Part A & B). 19

Figure 3.2 Order and timing of procedures during data collection. Indicating time segments between events: clinical evaluation, premedication, anaesthetic induction, nerve blocks, recording of the pre-cut physiological variables, the start of surgery for GBB, GSB and BBB treatment groups. Additional block for GSB treatment group post surgery. Time segments for starving (A), patient preparation (B) and recording of data (C). 21

Figure 3.3 Block confidence score used for the guided saline (GSB; 10 dogs), guided bupivacaine (GBB; 10 dogs) and blind bupivacaine (GBB) treatment groups. Scale of 1 (poor) to 4 (high) confidence scoring that the block will be successful for US-NS guided and blind techniques, respectively. The score was performed immediately post block prior to surgery. Modified from Gray et al. (2019). 22

Figure 3.4 US-NS guided psoas compartment (a) and lateral proximal sciatic (b) nerve block being performed on a dog. US images obtained shown on the right. External iliac artery (EIA), seventh lumbar vertebral body (VB) and its transverse process (TP), femoral nerve (FN) and obturator nerve (ON), ischiatic tuberosity (IT), greater trochanter of the femur (GT), and the sciatic nerve (SN) indicated. 25

Figure 5.1 Summary of comparison of subjective (left row) and objective (middle and right rows) assessment of nerve block outcome for blind bupivacaine block (BBB; 10 dogs). Values for Fisher's exact test, McNemar's test and Cohen's kappa coefficient are indicated. The count (number of dogs) undergoing an arthrotomy under surgical

anaesthesia [premedicated with 0.01 mg kg^{-1} medetomidine and 0.3 mg kg^{-1} morphine, induced with propofol and maintained on isoflurane (targeted end-tidal concentration of 1.6%)] is indicated. Black bar: failed blocks; Shaded bar: successful blocks. 33

Figure 5.2 Summary of nerve block outcome (fail or success) for blind bupivacaine block (BBB; 10 dogs). Subjective score (left row) compared to objective scores (middle and right rows) after being stratified into the assigned confidence score (2, 3 and 4) compared using Kendall's tau-b rank correlation coefficient. The count (number of dogs) undergoing an arthrotomy under surgical anaesthesia [premedicated with 0.01 mg kg^{-1} medetomidine and 0.3 mg kg^{-1} morphine, induced with propofol and maintained on isoflurane (targeted end-tidal concentration of 1.6%)] is indicated. Black bar: failed blocks; Shaded bar: successful blocks. 34

List of abbreviations

ANS	-	autonomic nervous system
BBB	-	blind bupivacaine block
BIS	-	bispectral index
CNS	-	central nervous system
DAP	-	diastolic arterial pressure
ECG	-	electrocardiogram
EEG	-	electroencephalography
FE´Iso	-	end-tidal isoflurane concentration
f_R	-	respiratory rate
GBB	-	guided bupivacaine block
GSB	-	guided saline block
HR	-	heart rate
HRV	-	heart rate variability
Hz	-	hertz
kg	-	kilogram
L	-	litre
mA	-	milliampere
MAP	-	mean arterial pressure
mg	-	milligram

mL	-	millilitre
ms	-	millisecond
PI	-	perfusion index
PNS	-	parasympathetic nervous system
SAP	-	systolic arterial pressure
SD	-	standard deviation
SNS	-	sympathetic nervous system
V_T	-	tidal volume

Abstract

Objective To identify physiological variables that can be used to objectively detect intraoperative nociception to indicate peripheral nerve block failure.

Study design A prospective blinded randomized clinical study.

Animals A sample of 14 male (40.8 ± 12 kg; mean ± SD) and 16 female (34.3 ± 11.4 kg) client-owned dogs undergoing a stifle arthrotomy.

Methods Dogs were randomly assigned to one of three treatment groups for psoas compartment and proximal sciatic nerve blocks (0.2 mL kg^{-1} per site): guided bupivacaine (GBB) or saline (GSB) blocks or blind bupivacaine block (BBB). Guided blocks were performed using ultrasound and nerve stimulation. Dogs were premedicated intramuscularly with 0.01 mg kg^{-1} medetomidine and 0.3 mg kg^{-1} morphine. General anaesthesia was induced with propofol (to effect to achieve tracheal intubation) and maintained with isoflurane in oxygen (targeted end-tidal concentration of 1.6%). The assigned investigator, based on randomisation, allotted a confidence score [1 (poor) to 4 (high)] that the block will be successful after administering the assigned nerve block treatment. The blinded investigator allotted a binomial subjective score of the nerve block outcome ("Yes": response to surgical stimulation; "No": no discernible response) at each time point. Receiver of operator characteristic curve analysis was used to compare actual values and change in values of physiological variables between GSB (Yes nociception) and GBB (No nociception) at the time of the arthrotomy. The Youden index and associated criterions for each physiological variable were used as an objective measure. Fishers exact t-test, McNemar's test and Cohens kappa statistical analysis were used to determine association, differences and inter-score reliability, respectively between the objective and subjective scoring for the BBB. The subjective score was compared to objective scores after being stratified into the assigned confidence scores using Kendall's tau-b rank correlation coefficient.

Results The cardiovascular variables had good discriminating ability in distinguishing a nociceptive response ($p < 0.01$). The Youden indexes for MAP and DAP had the best potential effectiveness in detecting a response to surgical stimulus. The highest sensitivity was that of delta MAP (100%). Good agreement was indicated between the subjective and objective scores with delta HR or SAP. The use of delta MAP (> 6 mmHg), delta SAP (> 10 mmHg), delta DAP (> 8 mmHg) had the best ability in indicating peripheral nerve block failure ($p < 0.001$).

Conclusions and clinical relevance The use of delta MAP, delta SAP or delta DAP can be considered as objective measures to detect intraoperative peripheral nerve block failure in anaesthetised dogs undergoing stifle arthrotomy. The determination of criterion values for different populations and conditions will benefit future clinical trials.

Keywords analgesia, intraoperative nociception, nerve block, physiological variables.

Chapter 1

1.0 Introduction

Nociception is the process by which the peripheral and central nervous systems (CNS) encodes a noxious stimulus (Merskey & Bogduk 1994). Pain due to the perception of these encoded signals plays an integral role in postoperative outcome. Concepts such as Enhanced Recovery After Surgery (ERAS) and fast-track surgery, focus on abbreviating the postoperative recovery period. Peripheral nerve blocks play an important role in ERAS (Campoy 2022). There is a correlation between the intensity of the immediate postoperative pain and the development of persistent (maladaptive) pain (Kehlet et al. 2006). Unfortunately, the successful outcome of a peripheral nerve block is not a given (Vettorato et al. 2012, Portela et al. 2013). Therefore, the importance of detecting nerve block failure intraoperatively prior to the recovery period is apparent.

The change in value of physiological variables relating to the autonomic nervous system (ANS) forms the basis of several methods that have been suggested for the assessment of nociception. Nociception results in an increased sympathetic tone (Miller & O'Callaghan 2002) which is mostly due to the intersection of the sympathetic branch of the ANS and nociceptive pathways (Benarroch 2006). Intersections occur between neurons in the dorsal horn and the preganglionic sympathetic nerves via somatosympathetic reflexes in a spinal cord segment. Further intersections occur in higher centres of the CNS such as the amygdala, nucleus of solitary tract, parabrachial nucleus, ventrolateral reticular formation and the hypothalamus (Benarroch 2006). Physiological variables [heart rate (HR), blood pressure, respiratory rate (f_R) and tidal volume (V_T)] are used as subjective assessments to detect nociception in humans (Stomberg et al. 2001). A 20% increase in mean arterial pressure (MAP)

and HR has been used as a criterion to determine peripheral nerve block failure in dogs (Papadopoulos et al. 2022). An increase in MAP is a sensitive indicator of nociception in isoflurane anaesthetised pigs and horses (Haga et al. 2001; Haga & Dolvik 2005). Derivatives of physiological variables have been used to create objective measures for assessing the absence of nociception. The most common of these derivatives is heart rate variability (HRV) which has been assessed in anaesthetised dogs (Bergfeld et al. 2014). Algorithms that incorporate various combinations of HR, blood pressure responses, HRV, pulse beat interval and pulse wave amplitude have also been used to assess nociception in anaesthetised humans (Rossi et al. 2012; Bergmann et al. 2013).

Despite these assessments, there remains no consensus on how to objectively confirm nociception, especially in anaesthetised humans (Ledowski 2019) and animals. There is need for a practical, objective way to detect intraoperative nociception which can indicate peripheral nerve block failure and prompt the veterinarian to administer analgesics before recovering the dog.

Chapter 2

2.0 Literature review

2.1 Nociception and pain

The pain pathway can be divided into four processes namely: transduction, transmission, modulation and perception (Osterweis et al. 1987). Transduction is the process by which a mechanical, chemical or thermal stimulus is converted into an electrical signal (action potential). Transmission is the propagation of an action potential along a nerve fibre. The main afferent fibres involved in action potential propagation along ascending pain pathways are slow conducting C-fibres (small unmyelinated fibres transmitting dull poorly localised pain sensations) and fast conducting A-δ fibres (larger myelinated fibres transmitting sharp, localised pain sensations). During surgery, nociceptors that are found in tissues are activated and transduce a mechanical (or chemical or thermal) stimulus into an action potential. The action potential is propagated along the afferent, first order neuron through the dorsal root and the tract of Lissauer to the dorsal grey horn. In the dorsal grey horn, within the respective Rexed laminae, the first order neuron synapses with a second order neuron. The second order neuron then propagates this action potential further via the ventrolateral [spinothalamic (neospinothalamic and palaeospinothalamic)] pathways to the thalamus and reticular formation. The reticular formation further propagates the action potential to the thalamus via the reticulothalamic pathways. The action potential is then relayed from the thalamus to the cerebral cortex (Osterweis et al. 1987). Modulation is the process by which the intensity of a nociceptive message is altered. The well described pathway of modulation is in the dorsal grey horn where higher centres found in the brain (from the rostral medulla and periaqueductal grey matter) alter the response for the signal (Purves et al. 2001).

The process of perception requires consciousness and this seldom occurs during surgical anaesthesia and therefore adequately anaesthetised patients do not perceive pain. However, while under anaesthesia the processes of transduction, transmission and modulation occurs in response to surgical stimulus. Therefore, under general anaesthesia, noxious stimulus still results in the active propagation of impulses along the pain pathways i.e. nociception. However, the perception and experience of pain can only occur once the patient is awake.

2.2 The intraoperative detection of nociception

Cowen et al. (2015) identified five intraoperative methods for objective assessment of nociception: neuroimaging, assessment of autonomic nervous system changes, biopotential monitoring, biomarker analysis (e.g. stress hormonal assays) and composite algorithms (combinations of variables and modalities). These methods target three physiological systems: ANS, the CNS and spinal reflexes (Martinez-Vazquez & Jensen 2022). Assessment of the CNS relies on neuroimaging (functional near-infrared spectroscopy) and biopotential monitoring [electroencephalography (EEG) and EEG based indices] (Cowen et al. 2015). The use of biopotential monitoring such as bispectral index (BIS), an EEG derived algorithm, has been suggested as a plausible modality for the detection of pain in dogs and cats (Hernandez-Avalos et al. 2019). There is a rationale that inadequate analgesia results in the active propagation of impulses along nociceptive pathways which can be detected in a similar way to which BIS monitoring can be used to determine the adequacy of anaesthetic depth. Assessment of spinal reflexes relies on the detection of clinically invisible nociceptive reflexes during general anaesthesia by means of electromyographic biopotential monitoring (Lichtner et al. 2018). Assessment of the ANS (i.e MAP and HR) was more sensitive in detecting nociception than assessment of the CNS (i.e. EEG) in isoflurane anaesthetised pigs and horses (Haga et al. 2001;

Haga & Dolvik 2005). Furthermore, assessments of the CNS and spinal reflexes are limited by cost, practicality and expertise. Therefore, the literature review is focussed on the ANS.

2.2.1 Assessment of the ANS

The assessment of respiratory and cardiovascular system, which is predominantly regulated by the ANS, is integral to the guidelines for basic anaesthetic monitoring (Committee on Standards and Practice Parameters 2020). There is a complex and dynamic relationship between the parasympathetic (PNS) and sympathetic (SNS) divisions of the ANS. The SNS has a rapid response time of less than one second (Nunan et al. 2010). Due to the intersection of autonomic and nociceptive pathways, surgical stimulus can result in an increased sympathetic tone (Miller & O'Callaghan 2002). Anaesthetic drugs can supress the neuroendocrine response of the SNS in a dose dependent manner during surgery (Roizen et al. 1981). Yamashita et al. (2012) indicated that during the maintenance of anaesthesia with sevoflurane in oxygen, ANS responses were preserved at clinically relevant inhalant anaesthetic concentrations in dogs. Cardiovascular and respiratory variables form the basis for efforts in detecting nociception (Yamashita et al. 2012). The most basic of these variables is detecting changes in HR, respiratory rate, blood pressure and pupillary size.

Measures of HRV and pulse rate variability (PRV) can be used to evaluate the ANS tone (Malik 1996). HRV is based on electrocardiogram (ECG) monitoring and provides real-time measurements by using the R-R interval to determine changes in the ANS tone. Similarly, PRV is based on the peak to peak analysis of arterial pulse wave intervals. HRV and PRV are influenced by several factors, such as species, breed, age, surgical stimulation, co-morbidities drugs and anaesthetic depth (Cowen et al. 2015). Several HRV algorithms have been developed to increase the accuracy of HRV. These algorithms account for non-nociceptive related factors that influence HRV. The HRV algorithms include the cardiorespiratory coherence algorithm

(Brouse et al. 2013), real time Fourier high/low frequency ratios (Jeanne et al. 2009) and analgesia nociception index (Jeanne et al. 2014). The use of these algorithms in animals shows promise, however, the cost of equipment and practicalities of monitoring precludes their use in day-to-day small animal practice.

The cardiovascular depth of analgesic index (CARDEAN) has been used to guide opioid administration and to limit patient movement during surgery in humans (Cividjian et al. 2007; Rossi et al. 2012). The CARDEAN makes use of ECG (R-R interval) and oscillometric blood pressure (systolic arterial pressure) measurements to calculate an index (Cividjian et al. 2007; Rossi et al. 2012). The index is calculated by plotting these variables on a linear scale to create an index score (0-100). An increase in index score indicates nociception and an index score of > 60 is associated with patient movement during surgical stimulus (Cividjian et al. 2007).

Perfusion index (PI) has been used to determine peripheral nerve block success in dogs (Gatson et al. 2016). The PI uses the waveforms of the plethysmograph to analyse pulse interval and amplitude (Bonhomme et al. 2011; Bergmann et al. 2013). The PI generates values (0-100) on a linear scale, where painful stimuli are associated with values greater than 50. The PI has been used for dose titration studies administering remifentanil to humans (Bonhomme et al. 2011; Bergmann et al 2013). Bonhomme et al. (2011) concluded that the performance of PI is comparable to that of HR and MAP when used to detect nociception. The limitations of PI include cost of the equipment and it is used near the surgical site. The PI measurements as with blood pressure monitoring can be affected by external factors such as anaesthetic depth, intravascular volume, HR, drugs administered and also by factors that interfere with pulse-oximetry.

Küls et al. (2017) assessed the use of thermography to determine epidural and femoral-sciatic nerve block success in dogs undergoing orthopaedic surgery. Thermography was not effective in predicting epidural or nerve block success in dogs.

Pupillometry is another method of detecting pain by evaluating the SNS response to noxious stimuli by means of an infrared pupilometer. The SNS (mydriasis) and the PNS (miosis) control pupil size due to their innervation of the respective smooth muscle fibres in the iris in humans and dogs. The reference intervals for pupillometry have been established for healthy, awake, non-painful and drug naïve dogs (Mills et al. 2022). Although the use of pupillometry to detect pain in humans is established, its use in dogs under general anaesthesia requires validation. It is important to note that the use of certain anaesthetic drugs, when administered at therapeutic doses, influence pupil size. Drugs such as morphine and medetomidine cause miosis in dogs (Lee & Wang 1975; Kanda et al. 2015). Drugs such as adrenaline, atropine and ketamine cause mydriasis in dogs (Gross & Pablo 2015).

Although there are technological advances in devices used to assess ANS response to nociception, it is clear that these modalities have limitations. These, often costly devices do not have a clear advantage over using routinely monitored cardiovascular variables such as MAP to indicate nociception (Haga et al. 2001; Haga & Dolvik 2005). Further evaluation of these regularly monitored physiologic variables can potentially lead to the development of an affordable, accessible clinical tool for the objective detection of nociception in anaesthetised dogs.

2.3 Anaesthetic and analgesic drugs used in our study and their effect on nociception

2.3.1 Anaesthetic drugs

Propofol

Propofol is a hypnotic anaesthetic that is commonly use for the intravenous induction of general anaesthesia. In our study propofol was used as an induction drug and not for maintenance of general anaesthesia. Propofol produces its anaesthetic effect via positive allosteric modulation of the $GABA_A$ receptor (Ying & Goldstein 2005). The surgical anaesthesia produced by propofol inhibits the perception of pain. However, propofol has no antinociceptive properties (Wilder- Smith et al. 1995). Propofol has an effect on commonly measured physiologic variables. Postinduction apnoea and hypoventilation are common with propofol use (Bigby et al. 2017). The mechanism of this respiratory depression is by central and peripheral chemoreceptors suppression (Dahan et al. 2003). Furthermore, it also causes a decrease in arterial blood pressure by decreasing systemic vascular resistance. The decrease in blood pressure is often followed by a compensatory increase in HR. The magnitude of these cardiovascular effects is directly proportional to the propofol plasma concentration (Cattai et al. 2018). Propofol has a short duration of action. Dogs induced with 6-8 mg kg^{-1} propofol intravenously (IV) were completely recovered from anaesthesia after 20 minutes and clinical parameters were returned to pre-administration values after 15 to 20 minutes (Short & Bufalari 1999).

Isoflurane

The contemporarily used inhalant anaesthetics isoflurane and sevoflurane inhibit the perception of pain. However, they have no inherent antinociceptive properties (Steffey et al. 2015). Isoflurane decreases arterial blood pressure in a dose dependent manner by decreasing systemic vascular resistance (Grimm et al. 2015). The minimum alveolar concentration (MAC) of

isoflurane in the dog is 1.28% (Steffey & Howland 1977). A MAC multiplier of 1.2-1.4 (FE´Iso of 1.54-1.68%) is required to inhibit movement in response to a noxious stimulus in 95% of anaesthetised humans (De Jong & Eger 1975; Aranake et al. 2013) and this Effective Dose 95% has been recommended for anaesthetised animals.

2.3.2 Analgesic drugs

Analgesic drugs have antinociceptive effects by altering one or more components of the pain pathway (transduction, transmission and modulation). Analgesic drug classes relevant to the current study include alpha$_2$ adrenoceptor agonists (i.e. medetomidine), opioids (i.e. morphine), non-steroidal anti-inflammatory drugs (i.e. meloxicam) and local anaesthetic drugs (i.e. bupivacaine).

Medetomidine

Alpha$_2$ adrenoceptor agonists are commonly included as part of the preanaesthetic drug combination administered to dogs undergoing orthopaedic surgery (Papadopoulos et al. 2022). Alpha$_2$ adrenoceptor agonists have sedative, analgesic and anaesthetic sparing properties which make them a popular choice as part of an anaesthetic drug protocol. The sedative effect site is in the rostroventral lateral medulla and locus coeruleus (Sinclair 2003). The analgesic effect site is along the afferent nociceptive pathways (peripheral nerves and in the dorsal horn) by inhibiting the release and postsynaptic binding of norepinephrine. Medetomidine has cardiovascular effects that can affect the commonly measured cardiovascular variables. Medetomidine causes an increase in systemic vascular resistance, transient hypertension and baroreceptor mediated reflex bradycardia or centrally mediated bradycardia which results in a decrease in cardiac output (Pypendop & Verstegen 1998; Rankin 2015). Initially the arterial

blood pressure increases where after it returns to a value slightly below baseline (Pypendop & Verstegen 1998). Medetomidine (40 ug kg^{-1}) in dogs has a peak effect at 10-20 minutes for sedation and at 5 minutes for changes in cardiovascular variables after IV administration. Furthermore, systolic arterial pressure (SAP) and diastolic arterial pressure (DAP) returns to normal at 30–40 minutes and HR remains low (roughly 50% of baseline values) 120 minutes post administration (Kuusela et al. 2000).

Morphine

Opioids are regularly administered as part of anaesthetic drug protocols for dogs undergoing orthopaedic surgery (Papadopoulos et al. 2022). Opioids are predominantly administered for their analgesic effects. However, in dogs they also produce an anaesthetic sparing effect. Opioid induced analgesia is predominantly through the binding of opioid receptors within the CNS (periaqueductal grey, nucleus reticularis paragigantocellularis and dorsal horn) (Pathan & Williams 2012). Morphine is the prototypical example of an opioid drug. Morphine is a preferential *mu* receptor agonist, with some *kappa* and *delta* receptor agonistic effects (Kukanich & Wiese 2015). Opioids predominantly exert their analgesic effect via the C-fibres and to a lesser effect via the A-δ fibres (Kukanich & Wiese 2015). Therefore, opioids on their own might not provide adequate analgesia for surgical stimulus or inhibit a response to surgical stimulus at recommended doses. Morphine has a poor lipid solubility profile compared to other opioids. When morphine is administered, even IV, initial drug effect can be noted within 5 minutes but peak analgesic effect is only reached 30 minutes after intravenous injection (Kukanich & Wiese 2015). Morphine has clinically relevant adverse effects (emesis, gastrointestinal hypomotility or stasis and immunomodulation) associated with its use that can potentially prolong (i.e. ERAS) the recovery period after surgery. Morphine has direct and indirect effects that can affect the commonly measured physiological variables. Intravenous

injection of morphine can result in histamine release (degranulation of granulocytes) which results in a decrease in systemic vascular resistance and blood pressure with a compensatory increase in HR (Kukanich & Wiese 2015). Opioids are well known to produce a centrally mediated increase in the PNS tone causing bradycardia. Opioids are also known to cause respiratory depression by supressing the pre-Bötzinger complex in the medulla. Morphine has been shown to directly decrease the thermoregulatory set point in the hypothalamus which could influence *f*R (increase in *f*R to decrease body temperature) when clinically relevant doses are administered to dogs (Adler et al. 1988). Dogs seem to acclimatise to the new thermoregulatory set point within 3–40 minutes where after *f*R returns to normal (Adler et al. 1988). Lucas et al. (2001) concluded that in dogs 0.3–0.5 mg kg^{-1} morphine administered IV provided adequate analgesia for moderate to severe pain.

Meloxicam

The surgical damage of cells results in the release of arachidonic acid from their phospholipid membranes via phospholipase A_2. Arachidonic acid is metabolised by cyclo-oxygenase (COX) and lipoxygenase enzymes to prostanoids and leukotrienes, respectively (Papich & Messenger 2015). The prostanoids increase the excitability of the peripheral somatosensory nerves and thereby causes peripheral sensitisation. Meloxicam is an enolic acid derivative that preferentially blocks COX-2 isoform. At high doses its COX selectivity decreases (Papich & Messenger 2015). The nonsteroidal anti-inflammatory drugs indirectly inhibit nociceptive pain through the prevention of peripheral and central sensitisation and the direct algogenic effects of prostanoids that results from inflammatory pain.". These drugs do not have any direct effects on the commonly measured physiological variables (Papich & Messenger 2015).

Bupivacaine

Local anaesthetic drugs inhibit the propagation of action potentials along the nerve fibres by blocking voltage gated sodium channels. Therefore, these drugs provide full analgesia as it completely inhibits action potentials. The voltage gated sodium channels cycle through different conformational states (open, inactivated or closed) and are mainly blocked in the open or inactivated state. When the nerve is stimulated by means of a nerve stimulator (NS) or by surgical stimulation the voltage gated sodium channels are in its open state and allow for quicker access of the local anaesthetic to the binding site (Starmer et al. 1984). The initial surgical stimulus can result in the opening of voltage gated sodium channels allowing the local anaesthetic to access the binding site i.e. use dependent blockade (Starmer et al. 1984). Action potentials have the ability to jump over blocked nodes of Ranvier especially if less than three nodes in series are blocked (Fink 1989). The percentage of impulses propagated is inversely related to the number of nodes or the length of nerve blocked (Raymond et al. 1989). We speculate that the physiological changes in a response to surgical stimulus might be subtle with partial nerve blocks.

Bupivacaine has a longer duration of action compared to other local anaesthetics, which is desired in dogs undergoing orthopaedic surgery. The recommended dosage of bupivacaine in dogs is 2 mg kg^{-1}, which equates to a total volume of 0.4 mL kg^{-1} of a 0.5% solution. Initially, a volume of 0.4 mL kg^{-1} of the 0.5% solution bupivacaine was suggested for a single point approach to the psoas compartment block (Campoy et al. 2008), however, a volume of 0.15 mL kg^{-1} was shown to be sufficient (Vettorato et al. 2013). A volume of 0.05 mL kg^{-1} was shown to provide adequate distribution for blocking the sciatic nerve (Campoy et al. 2008). Speculatively, the higher prescribed volume of 0.2 mL kg^{-1} per site might be better suited for blind techniques. Bupivacaine has a variable onset time, Gray et al. (2019) indicated a median (range) onset time of 24 (3 – 60) minutes and offset time of 360 (240-360) minutes. Therefore,

nerve block outcome should be assessed within 60-240 minutes from administration of the block.

2.4 Locoregional anaesthesia

The first neuraxial (spinal and epidural) and peripheral nerve anaesthetic block techniques were performed in the late 1800s by Corning and Halstead and shortly after described by Quincke (Ball & Westhorpe 2003). Since then, there have been several advancements in describing different techniques used for neuraxial anaesthesia and peripheral nerve blocks. The field of peripheral nerve blocks has grown and developed further in tandem with the advent and increased availability of ultrasound (US) machines and nerve stimulators.

2.4.1 Neurolocation

The use of nerve stimulation (NS) for nerve localisation dates back to 1912 where it was first reported by the German surgeon, Dr Perthes (Goerig & Agarwal 2000). The NS makes use of a teflon coated needle with an exposed tip, so that current is only delivered through the tip. The negative lead of the NS is connected to the needle and the positive lead is connected to an adhesive electrode or a crocodile clip which is attached on the skin. General use recommendations in dogs are to set the pulse frequency, pulse width and initial current to 1 Hz, 0.1 ms and 2.0 mA. The circuit is closed when tissue is penetrated and a high current density is focused around the exposed tip of the insulated needle. At these prescribed NS settings, a motor response of the relevant muscle group is elicited when the exposed tip is in close proximity to the targeted nerve. Once a motor response is detected the current is reduced in a stepwise manner until a current of 0.3-0.5 mA is achieved which can indicate closer proximity of the needle tip to the nerve (Portela et al. 2018). The injection of local anaesthetic drugs via

the insulated needle at this point, should abolish the muscle response, this is called the Raj test (Dugdale 2020).

The US guided nerve block technique relies on visualisation of the nerve and its surrounding structures which aids in guiding a needle as it is advanced to place the tip in close proximity to the nerve. Orebaugh et al. (2007, 2009) indicated that, in humans, the US guided technique was quicker and resulted in less inadvertent vascular punctures compared to NS guidance alone.

2.4.2 Anatomy and technique

In order to perform peripheral nerve blocks, a good understanding of the relevant anatomy is important (Gurney & Leece 2014). Nerves innervating the canine pelvic limb originate from the lumbosacral plexus. The lumbosacral plexus receives its supply from the spinal nerves originating from lumbar spinal segment (L4-S1) and to a variable degree from sacral spinal segment (S2- S3) (Evans & de Lahunta 2013). The lumbosacral plexus can be separated into two entities, the lumbar and sacral plexuses. The iliopsoas muscle is penetrated by the spinal nerve branches of L4-6, which, after emerging from the intervertebral foramina, they interconnect to form the lumbar plexus. The lumbar plexus continues in the psoas compartment where it branches to form the obturator, genitofemoral, lateral femoral cutaneous and femoral nerves (Portela et al. 2013). The psoas compartment is an anatomical compartment formed by the psoas minor, iliopsoas and the quadratus lumborum muscles (Portela et al. 2013).

Although several approaches have been described for the blocking of these nerves within the psoas compartment, this study focused on the lateral pre-iliac approach (Portela et al 2013). Vettorato et al. (2013) determined that the success rate of the sagittal paravertebral approach was not different to that of the lateral pre-iliac approach to blocking the lumbar plexus

in dogs undergoing orthopaedic surgery of the pelvic limb. Graff et al. (2015) and Tayari et al. (2017) independently determined that injections performed at the most caudal aspect of the psoas compartment resulted in regular staining of both the obturator and femoral nerves. Portela et al. (2013) described the pre-iliac block and indicated that injecting a 0.1 mL kg^{-1} of a coloured solution successfully covered both the obturator and femoral nerves. Anatomical differences in the innervation of the canine stifle exist, therefore, to provide adequate locoregional analgesia of the stifle, it is advisable to block the genitofemoral, lateral femoral cutaneous and obturator nerves before they leave the psoas compartment (Campoy et al. 2008; Portela et al. 2008).

The femoral nerve runs ventral and lateral to the obturator nerve in the caudal aspect of the psoas compartment (Graff et al. 2015). These nerves can be visualised by means of US by placing a 10-12 MHz linear probe ventral to the psoas compartment, cranial to the iliac crest (Echeverry et al. 2012; Mahler 2012). The nerves can be seen as hypoechoic, round structures with a hyperechoic border.

The sacral plexus is formed by the lumbosacral trunk (L6-S1) that runs on the medial aspect of the ilium, ventral to the gluteal muscles in close proximity to the gluteal blood vessels. The branches of the sacral plexus, *inter alia*, form the caudal femoral cutaneous, sciatic and cranial and caudal gluteal nerves (Evans & De Lahunta 2013). The sciatic nerve exits the pelvis at the greater ischiatic foramen and supplies the hip (Portela et al. 2010) where it can be blocked by means of the parasacral approach. Furthermore, Campoy et al. (2008) described the lateral proximal approach, where the sciatic nerve is blocked between the ischiatic tuberosity and the greater femoral trochanter. The lateral proximal approach has been used in several clinical studies (Vettorato et al. 2012; Portela et al. 2013; McCally et al. 2015). Visualisation of the sciatic nerve can be performed by US visualisation with a 10–12 MHz linear probe positioned

between the greater femoral trochanter and the ischiatic tuberosity, with the probe perpendicular to the nerve (Costa-Farré et al. 2011).

2.4.4 Complications of locoregional anaesthesia

Complications of locoregional anaesthesia could be drug related or technique related (i.e. mechanical damage). Adverse reactions to local anaesthetics include systemic toxicity (CNS and cardiovascular toxicity) and peripheral nerve injury (Garcia 2015). Although very serious, systemic toxicity can be avoided by adhering to dosage guidelines and aspirating prior to injecting. To avoid IV injection, the US guided technique is superior to the NS technique due to the visualisation of the needle tip in relation to blood vessels (Orebaugh et al. 2009). Regardless of technique used, the final step is to aspirate prior to injection to ensure that the needle tip is not located intravascularly. The principal component of peripheral nerve injury seems to be associated with intrafascicular injection (Hadzic et al. 2004; Phan et al. 2021). Intrafascicular injection results in mechanical, chemical and vascular injury to the nerve bundle, leading to axonal degeneration (Phan et al. 2021). Visualisation of the needle tip placement with US can be used to avoid intraneural injection (Orebaugh et al. 2007; Orebaugh et al. 2009). Hadzic et al. (2004) determined that intraneural injections are associated with pressures of 1293–2327 mmHg at the beginning of injection and that perineural injections are associated with pressures less than 207 mmHg. In addition, intraneural injectate administration associated with low injection pressures (569 mmHg) resulted in motor return within the expected time period (3 hours) post block (Hadzic et al. 2004). Kapur et al. (2007) indicated that intrafascicular rather than intraneural injections lead to nerve injury.

2.5 Inference and outlines

We concluded from the literature review that there is a need for an affordable, simplified way of detecting intraoperative nerve block failure. Can objective measures be discovered in adequately anaesthetised dogs and how would they perform against subjective assessments in the detection of nerve block failure?

The aim was to identify routinely measured physiologic variables that can be used to objectively detect a response to surgical stimulus and therefore indicate intraoperative nerve block failure.

The first objective was to determine the effectiveness of using routinely monitored physiologic variables to detect peripheral nerve block failure. The second objective was to determine cut-off values for these variables that can be used as an objective score to determine peripheral nerve block failure. The third objective was to assess the performance of the identified cut-off values and to compare it against subjective and confidence scoring in the detection of peripheral nerve block failure.

The null hypothesis was that physiologic variables in anaesthetized dogs will be no different between ultrasound-nerve stimulator guided blocks using saline compared to bupivacaine during stifle arthrotomy.

Chapter 3

3.0 Materials and methods

3.1 Animals and housing

A sample of client owned dogs (*Canis lupus familiaris*) that were to undergo a stifle arthrotomy and dynamic cranial cruciate ligament repair were included in the study upon informed owner consent (Appendix i). Inclusion criteria were dogs weighing ≥ 20 kg with unilateral pathology of a stifle joint (cranial cruciate ligament rupture) requiring corrective surgery as well as an arthrotomy and deemed otherwise healthy on clinical examination and routine haematology (blood smear and packed cell volume) and serum biochemistry (total protein, urea and creatinine) assessments (American Society Anaesthesiologists classification score of I or II). Dogs that had radiographic evidence of concurrent osteoarthritic changes in any other joint of the pelvic limbs and girdle or concurrent neurological disease were excluded. Other exclusion criteria were dogs with contraindications for peripheral nerve blocks such as coagulopathy or infection at the nerve block site, hypotension, or unsuccessful arterial cannulation. The study was conducted at Valley Farm Animal Hospital (VFAH), Pretoria, Gauteng, Republic of South Africa. The dogs were housed in hospital cages. During hospitalisation, the dogs were fed kibble twice a day and water was freely available except when being prepared for surgery. The study was approved by the Animal Ethics Committee of the University of Pretoria (V035-17; Appendix ii).

3.2 Study design

A prospective randomised comparative study was planned. The study complied with the Consolidated Standards of Reporting Trials (CONSORT) guidelines (Appendix iii). Population sampling was opportunistic, where after obtaining client consent, dogs were randomly assigned to one of two investigators and one of three treatment groups: guided bupivacaine block (GBB) or guided saline block (GSB) or blind bupivacaine block (BBB). Randomisation was achieved by means of an online balanced block randomisation technique (3 treatments; Sealed Envelope 2021). The other investigator, not assigned a dog, was blinded to the treatment administered by the assigned investigator and was allocated to recording the variables. The study was divided into two parts (Fig. 3.1):

Part A: The aim was to evaluate routinely measured physiological variables to detect if the magnitude of response to surgical stimulation between GSB and GBB treatment groups were different. These physiological variables were used to objectively score locoregional anaesthesia outcome.

Part B: The aim was to assess the robustness of the objective score of locoregional anaesthesia outcome determined in Part A by comparing outcome to subjective and confidence scores. A BBB was performed and objective and subjective scores were assigned and the agreement between them were assessed.

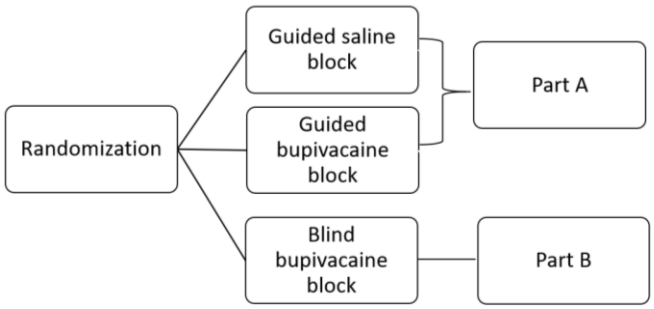


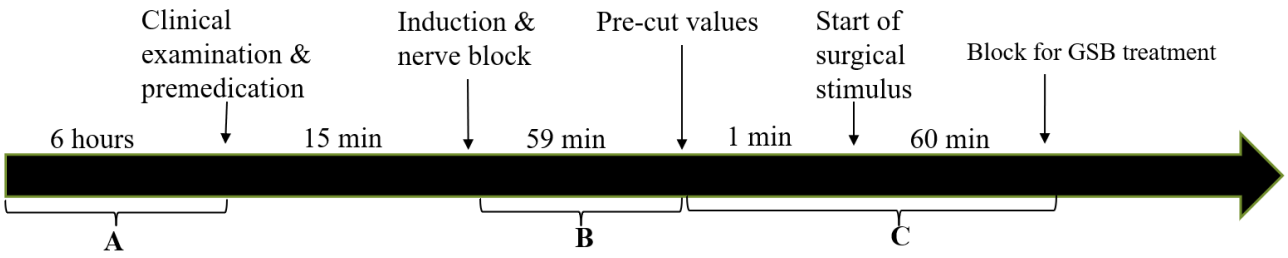
Figure 3.1 Study design indicating the treatment groups during data colection and the relevance of the data collected from the specific sections of the study (Part A & B).

3.3 Sample size

The sample size necessary for comparison of means was calculated at 10 dogs per treatment group (total = 30 dogs). The MAP was used as the variable of interest for the sample size calculation by applying these assumptions: type 1 error of 0.05, type 2 error of 0.20, difference of means of 4 mmHg and standard deviation of 3.

3.4 Experimental procedures

The dogs were fasted of food for 6 hours prior to the administration of the premedication. The order and timing of procedures was standardised (Fig. 3.2). Dogs were premedicated with 0.01 mg kg^{-1} medetomidine (Domitor, 1 mg mL^{-1}; Zoetis; RSA) and 0.3 mg kg^{-1} morphine (Morphine, 10 mg mL^{-1}; Pharma-Q Holdings (Pty) Ltd., RSA) mixed in a single syringe and injected intramuscularly (IM) and left undisturbed in a cage for 15 minutes. An intravenous canula (Jelco 20 G; Smiths medical, Lancashire, United Kingdom) was aseptically placed and secured into one of the cephalic veins. The dogs were induced with 2-4 mg kg^{-1} propofol (Propoven, 1.0%; Fresenius Kabi (Pty) Ltd., RSA) IV until the trachea could be intubated with a cuffed (high volume low pressure) 8.0 – 12 mm internal diameter polyvinyl endotracheal (ET) tube (Ho-lee tube; JC Medical, RSA). The ET tube was connected to a circle breathing circuit for anaesthetic maintenance using isoflurane (Isofor; Safeline Pharmaceuticals (Pty) Ltd., RSA) in oxygen and medical air (FiO_2 of 0.5) with fresh gas flow rate of 2 L $minute^{-1}$. The vaporiser (Isoflurane Sigma Delta; Penlon, UK) was initially set on 2% and titrated to an end-tidal isoflurane concentration (FE´Iso) of 1.6%. The dogs were allowed to breathe spontaneously. Cefazolin (Zefkol, 100 mg mL^{-1}; Litha Pharma (Pty) Ltd., RSA) was administered IV at 20 mg kg^{-1} after induction and repeated every 90 minutes during anaesthesia. The cornea was lubricated (Systane; Alcon laboratories, RSA) and lubrication reapplied as needed.



A: Starving.
B: Shaving and aseptic preparation, arterial cannulisation, FE´Iso titration to 1.6%.
C: Recording of physiological variables.

Figure 3.2 Order and timing of procedures during data collection. Indicating time segments between events: clinical evaluation, premedication, anaesthetic induction, nerve blocks, recording of the pre-cut physiological variables, the start of surgery for GBB, GSB and BBB treatment groups. Additional block for GSB treatment group post surgery. Time segments for starving (A), patient preparation (B) and recording of data (C).

The dog was placed in lateral recumbency with the pelvic limb to be operated on in the non-dependent position. Hair over the block sites was clipped and the skin was aseptically prepared. The blocks were performed as per the randomised treatment by one investigator. The GSB and GBB were both performed using an US-NS guided technique (see later).

The psoas compartment block was performed before the sciatic nerve block. On completion of the blocks, a timer was started to count down 60 minutes. A confidence score of the block (Fig. 3.3) as well as a body condition assessment (American Animal Hospital Association 9 point score) and ease of landmark palpation were recorded. Then the surgical site was shaved and aseptically prepared. A cannula (Jelco, 20 G; Smiths medical, Lancashire, United Kingdom) was aseptically introduced and secured in the dorsal pedal artery of the non-surgical limb. ECG electrode pads were placed on both metacarpal paw pads and the metatarsal paw pad of the non-surgical limb. Once the dog underwent the final surgical preparation, it was moved to the theatre table and placed in dorsal recumbency. The patient was connected to the anaesthetic machine, as described previously, and monitor sensors and leads were attached. The invasive blood pressure transducer (Sembu TR transducers; SSEM Mthembu Medical

(Pty) Ltd, RSA) was zeroed to atmospheric pressure at the level of the sternal manubrium. A fast-flush test was performed to subjectively ensure adequate dampening of invasive blood pressure line. Intravenous fluids using an isotonic crystalloid (lactated Ringer's solution; Fresenius Kabi, RSA) was administered at 5 mL kg^{-1} $hour^{-1}$ for the duration of general anaesthesia. The first surgical incision was planned to be made at 60 minutes post blocks. Furthermore, the GSB treatment group were administered locoregional anaesthesia at the end of the procedure prior to postoperative radiographs and recovery. The postoperative block was an US guided saphenous and lateral proximal sciatic nerve block (Costa-Farré et al. 2011) with 0.1 mL kg bupivacaine per site (Macaine, 5 mg mL^{-1}; Adcock Ingram, RSA).

Guided	
Subjective score	Description
1	Not confident at all, no confidence of needle in correct position
2	Poor confidence, appropriate response with nerve stimulator, but not confirmed with ultrasound
3	Fairly confident, NS response, correct anatomical location but no doughnut sign
4	Very confident, NS response, visualization of doughnut sign

Blind	
Subjective score	Description
1	Not confident at all, can't properly identify anatomical landmarks
2	Poor confidence, unsure of facial plane
3	Fairly confident
4	Very confident, nerve response observed

Figure 3.3 Block confidence score used for the guided saline (GSB; 10 dogs), guided bupivacaine (GBB; 10 dogs) and blind bupivacaine (GBB) treatment groups. Scale of 1 (poor) to 4 (high) confidence scoring that the block will be successful for US-NS guided and blind techniques, respectively. The score was performed immediately post block prior to surgery. Modified from Gray et al. (2019).

3.4.1 Procedures of the nerve blocks

Injectate volume used for all the blocks was standardised at 0.2 mL kg^{-1} per site. Saline (Sodium Chloride 0.9%; Fresenius Kabi, RSA) was administered for the GSB and bupivacaine (Macaine 5 mg mL^{-1}), for the GBB and BBB. Nerve stimulation was performed using a nerve stimulator (Stimpod NMS 450; Xavant technology, RSA). Ultrasound was performed using a portable ultrasound machine (Esaote MyLab-One; Lomean Medical, RSA).

Psoas compartment block (Fig. 3.4a)

- US-NS guided technique (GBB & GSB): The nerve was visualised via ultrasound by placing a 10-12 MHz linear probe ventral to the psoas compartment cranial to the iliac crest. The NS was set at 2.0 mA (pulse width 0.1 ms, 2 Hz) while the tip of a 22 G insulated needle (Stimuplex; B. Braun, RSA) was advanced towards the nerve. The NS amperage was stepwise decreased to 0.5 mA while observing for continued contraction of the quadriceps muscle (extension of the stifle joint) and the injectate was deposited (Mahler et al. 2012; Portela et al. 2013).
- Blind technique (BBB): A 22 G insulated needle was advanced at a 45° angle in a caudomedial direction through the iliopsoas muscle (ventral to the spinal column; L6) into the compartment formed between the iliopsoas and the quadratus lumborum muscles at a point ventral to the sixth lumbar vertebral transverse process and cranial aspect the iliac crest. Once the needle was in place, bupivacaine was deposited.

Lateral proximal sciatic nerve block (Fig. 3.4b)

- The US-NS guided technique (GBB & GSB): The nerve was visualised via ultrasound by placing a 10–12 MHz linear probe on the dorsolateral surface of the proximal pelvic limb, perpendicular to the nerve, between the greater femoral trochanter and the ischiatic tuberosity. The NS was set at 2.0 mA (pulse width 0.1 ms, 2 Hz) while the 22 G insulated needle was advanced. The NS amperage was stepwise decreased to 0.5 mA while observing for continued contraction of the gastrocnemius muscle (extension of the tarsus) and digital flexor or extensor muscles (flexion or extension of the digits) and the relevant solution was deposited (Costa-Farré et al. 2011).
- Blind technique (BBB): A 22 G insulated needle was advanced perpendicular to the skin, at a point between the first and second third section of a line drawn from the greater trochanter of the femur to the ischiatic tuberosity. The needle was advanced until it contacted the pelvic bone where after it was withdrawn for 3 to 5 mm and bupivacaine was deposited.

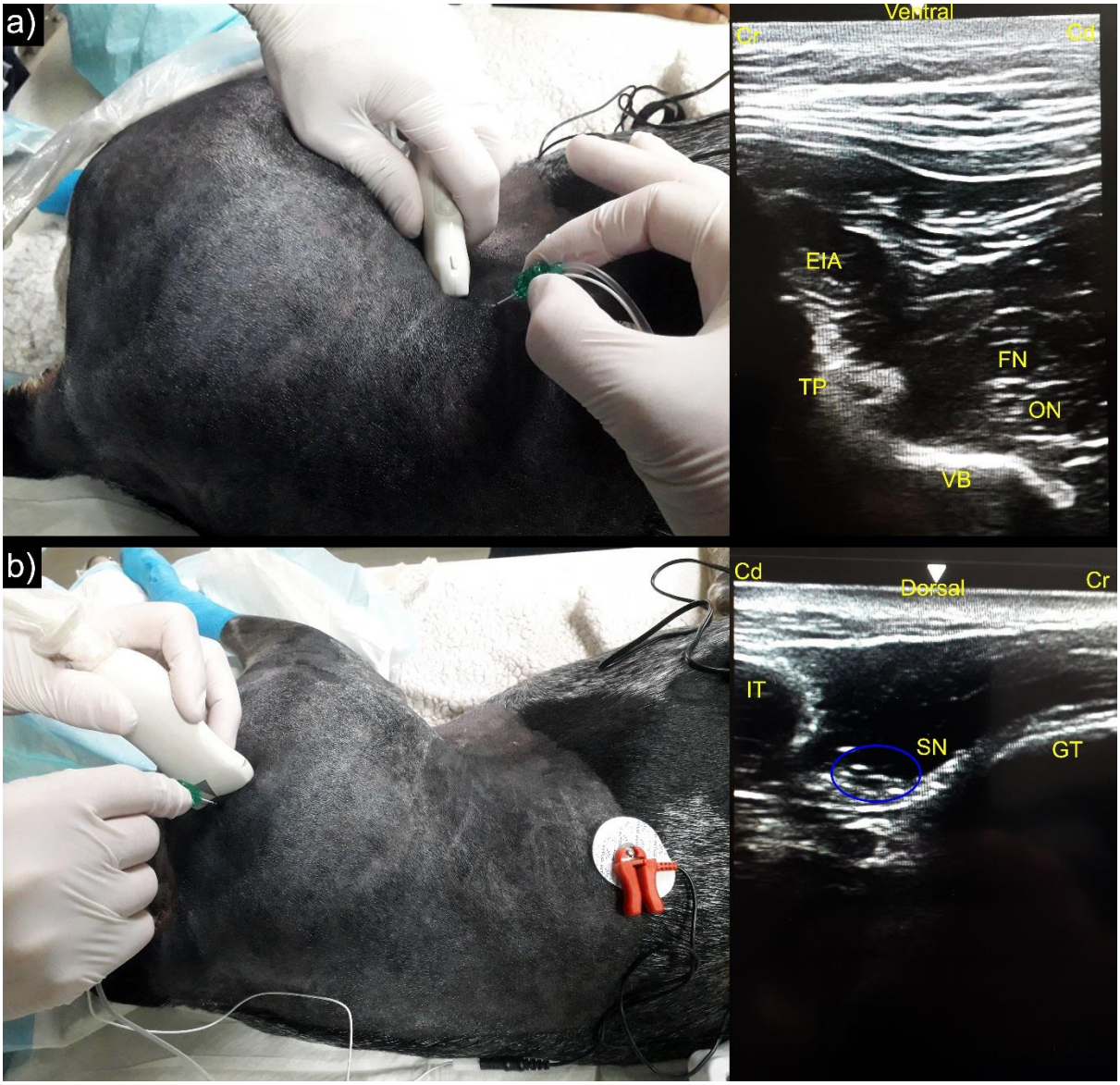


Figure 3.4 US-NS guided psoas compartment (a) and lateral proximal sciatic (b) nerve block being performed on a dog. US images obtained shown on the right. External iliac artery (EIA), seventh lumbar vertebral body (VB) and its transverse process (TP), femoral nerve (FN) and obturator nerve (ON), ischiatic tuberosity (IT), greater trochanter of the femur (GT), and the sciatic nerve (SN) indicated.

3.4.2 Intraoperative monitoring

The physiological variables were monitored with a Primus (Dräger, RSA) anaesthetic workstation and multiparameter physiologic monitor (Infinity Delta XL; Dräger, RSA) by an experienced anaesthetist (blinded investigator). During the surgical procedure, f_R, HR invasive SAP, MAP and DAP, oesophageal temperature (T) and F_E´Iso was monitored continuously but recorded at standardised time points relevant to this study (recorded on the study data collection

sheet; Appendix iv) and at ten-minute intervals (recorded on the VFAH anaesthetic monitoring sheet). The study time points were 1 minute before, at the time of (0 minutes) and 1, 2, 4, 5, 6, 8, 10, 15, 20 and 25 minutes after the skin incision (first surgical event). In addition, event notes were made on the datasheet when the joint was opened and expanded (arthrotomy; second surgical event), using a stifle distractor for at least 2 minutes. A binomial subjective score of the nerve block was assigned by the anaesthetist who was unaware of the treatment group as "Yes" if there was a response to surgical stimulation or "No" if there was not a discernible response at each time point. Criteria for a response to surgical stimulation (Yes) was movement, a lightened plane of anaesthesia (increased jaw tone, palpebral reflex, change in eye position and pupillary size and changes in respiratory rhythm) or an increase (> 25%) in the measured physiological variable values.

3.4.3 Intraoperative analgesia and rescue interventions

All the dogs were administered morphine (0.3 mg kg^{-1}) intraoperatively every two hours and postoperatively every four hours for 24 hours. Meloxicam (Metacam, 5 mg mL^{-1}; Boehringer Ingelheim, RSA) 0.2 mg kg^{-1} was administered subcutaneously (SC) one hour prior to induction, followed by a daily oral dose of 0.1 mg kg^{-1} for 5 days.

In dogs where subjective assessment by the anaesthetist deemed the response to surgical stimulation as severe (tachycardia, tachypnoea, rapidly lightened plane of anaesthesia) rescue analgesic drugs were administered without delay. The rescue protocol consisted of transiently increasing the vaporiser setting to maintain an FE´Iso to 1.8–2.0%, followed by the administration of a single intramuscular dose of 1 mg kg^{-1} ketamine and a constant rate infusion of 0.005 mg kg^{-1} hr^{-1} fentanyl intraoperatively and postoperatively for 12–24 hours.

Chapter 4

4.0 Data analysis

The physiologic variables recorded at the time of the second event (arthrotomy) and up to 2 time points after the event had occurred were examined. The highest values for the majority of variables within that time point were taken as a response to surgical stimulus. If there was no change in the variable values, the value at the time of the event was used. Data distribution was assessed by using descriptive statistics, histograms plots and the Anderson-Darling test for normality.

4.1 Part A

A binomial classification was used where the GSB group was expected to respond (Yes) and the GBB was expected to not respond (No) to surgical stimulation (arthrotomy). This binomial classification was applied to receiver of operator characteristic (ROC) curves comparing the actual value and the change in value of the physiological variables between GSB and GBB at the arthrotomy event. The change in value of a variable (delta value) was calculated by subtracting the value at the event from the value recorded 1 minute before start of surgery (skin incision; first event). The prevalence of the response to surgery was set to 50%. The Youden index and associated criterion for each physiological variable were used as the objective cut-off point between response (indicating block failure) and no response to surgical stimulation. In addition, sensitivity, specificity, positive and negative likelihood ratios, and positive and negative predictive values were calculated from criterion values and coordinates of the ROC curve for each variable.

4.2 Part B

The agreement between the objective score (determined in Part A) and the binomial subjective score of whether there was a response or not to surgical stimulation were assessed. Fisher's exact test was used for determining the association between objective and subjective scores. McNemar's test was used to determine if there are any differences between the objective and subjective scores. Cohen's kappa statistical analysis was performed to assess inter-score reliability. The Kendall tau-b rank correlation coefficient was used to assess the agreement between the binomial subjective and objective score for each of the assigned confidence scores. The variables suggested for the objective detection of response to surgical stimulation (i.e. nerve block failure) were summarised.

Data is presented as mean ± standard deviation. Data analyses were performed using commercially available statistical software (Minitab version 18; Minitab Ltd. LLC; Pensylvania, USA and MedCalc Statistical Software version 20.211; MedCalc Software; Belgium). A Fisher's exact test, McNemar's test and Kendall's tau-b p value of < 0.05 was considered significant. Cohen's kappa coefficient values were assessed as: no (≤ 0), slight (0.01–0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80) and perfect (0.81–1.00) agreement (McHugh 2012).

Chapter 5

5.0 Results

A sample of 14 male (40.8 ± 12 kg body weight; 5 ± 1 out of 9 body condition; 4.4 ± 2.6 years old; five administered BBB, six GBB and three GSB) and 16 female (34.3 ± 11.4 kg body weight; 6 ± 2 out of 9 body condition; 4.6 ± 2.5 years old; five administered BBB, four GBB and seven GSB) dogs were included in the study (data summary table Appendix v). All dogs completed the study and none required rescue analgesic interventions. Anaesthetic times were 116.5 ± 37.4 minutes from premedication to arthrotomy and 71.3 ± 24.4 minutes from block to arthrotomy. All dogs were maintained under general anaesthesia with isoflurane at a FE´Iso of 1.6 ± 0.06%.

5.1 Part A

The results for the ROC analysis indicated that the cardiovascular variables had good discriminating ability in distinguishing a response to surgical stimulus. The use of f_R and delta f_R had a poor ability to distinguish a response to surgical stimulus. The ROC analysis outcomes are indicated in Table 5.1.

In summation of the ROC analysis, evaluation of the Youden index indicated that HR, delta HR, f_R and delta f_R did not meet the empirical benchmark (i.e. J > 0.5) for being used for diagnostic purposes. The other physiological variables had more suitable Youden indexes. The Youden index for delta MAP, SAP, delta SAP and delta DAP were above the empirical benchmark, however, their low Youden indexes were the result of a disproportion between their sensitivity and specificity. The Youden indexes for MAP and DAP had the best potential effectiveness in detecting a reponse to sugical stimulus. The highest specificity was that of SAP. However, the disproportion in sensitivity (60%) and specificity (95%) makes SAP a poor

indicator on its own. The highest sensitivity was that of delta MAP. However, the disproportion in sensitivity (100%) and specificity (60%) makes delta MAP a poor indicator on its own.

Table 5.1 Summary of the receiver of operator characteristic (ROC) analysis of guided saline block (GSB; 10 dogs; response to surgical stimulus) and guided bupivacaine block (GBB; 10 dogs; no response to surgical stimulus) undergoing an arthrotomy under surgical anaesthesia [premedicated with 0.01 mg kg^{-1} medetomidine and 0.3 mg kg^{-1} morphine, induced with propofol and maintained on isoflurane (targeted end-tidal concentration of 1.6%)]. Indicating the maximum potential effectiveness of heart rate (HR), delta HR, respiratory rate (f_R), delta f_R, systolic arterial pressure (SAP), delta SAP, mean arterial pressure (MAP), delta MAP, diastolic arterial pressure (DAP) and delta DAP in detecting a response to surgical stimulus.

Variable	ROC AUC	ROC 95 % CI	*p*	Z	J	J 95% CI	AC	AC 95% CI	Sen (%)	Spe (%)	PPV	NPV	PLR	NLR
HR	0.75	0.59-0.88	0.0023	3.05	0,5	0.22-0.65	> 77	67-83	80	70	72.7	77.8	2.67	0,29
delta HR	0.76	0.6-0.88	0.0007	3.39	0.45	0.2-0.65	>1	-2-10	55	90	84.6	66.7	5.5	0.5
f_R	0.57	0.4-0.72	0.46	0.73	0.2	0.1-0.35	>10	6-13	40	80	66.7	57.1	2	0.75
delta f_R	0.5	0.34-0.66	0.98	0.03	0.15	0.1-0.2	>-1	-4-3	65	20	44.8	36.4	0.81	1.75
SAP	0.82	0.66-0.92	<0.0001	4.77	0.55	0.25-0.65	>127	123-135	60	95	92.3	70.4	12	0.42
delta SAP	0.8	0.64-0.91	0.0001	3.81	0.6	0.35-0.75	>10	2-19	95	65	73.1	92.9	2.71	0.08
MAP	0.89	0.76-0.97	<0.0001	7.77	0.7	0.45-0.85	>80	74-84	90	80	81.8	88.9	4.5	0.13
delta MAP	0.8	0,69-0.94	<0.0001	4.90	0.6	0.3-0.75	>6	1-12	100	60	71,4	100	2.5	0.00
DAP	0.93	0.8-0.99	<0.0001	9.69	0.75	0.42-0.9	>73	68-78	85	90	89.5	85.7	8.5	0.17
delta DAP	0.86	0.72-0.95	<0.0001	5.76	0.65	0.33-0.8	>8	2-21	95	70	76.0	93.3	3.17	0.07

Area under the curve (AUC), confidence interval (CI), significance level (*p*), Z-score (Z), Youden index (J), confidence interval (CI), associated criterion (AC), sensitivity (Sen), specificity (Spe), positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR).

5.2 Part B

The results for the Fisher's exact test, McNemar's test and Cohen's kappa values are indicated in Figure 5.1. Fisher's exact test indicated an association between the subjective scoring and the objective score with delta HR or SAP only. McNemar's test indicated no difference between the performance of subjective scoring and objective scoring with delta HR or SAP in determining a response to surgical stimulation. Cohen's kappa coefficient values indicated: no agreement for delta f_R, slight agreement for HR, delta SAP, delta MAP and f_R, fair agreement for MAP, DAP and delta DAP, moderate agreement for delta HR and substantial agreement for SAP with subjective scoring. The strength and direction of the association (Kendal tau-b rank correlation coefficient) of the subjective score compared to objective scores after being stratified into the assigned confidence scores is shown in Figure 5.2. A summary of the variables and their effectiveness in the objective detection of nerve block failure is presented in Table 5.2.

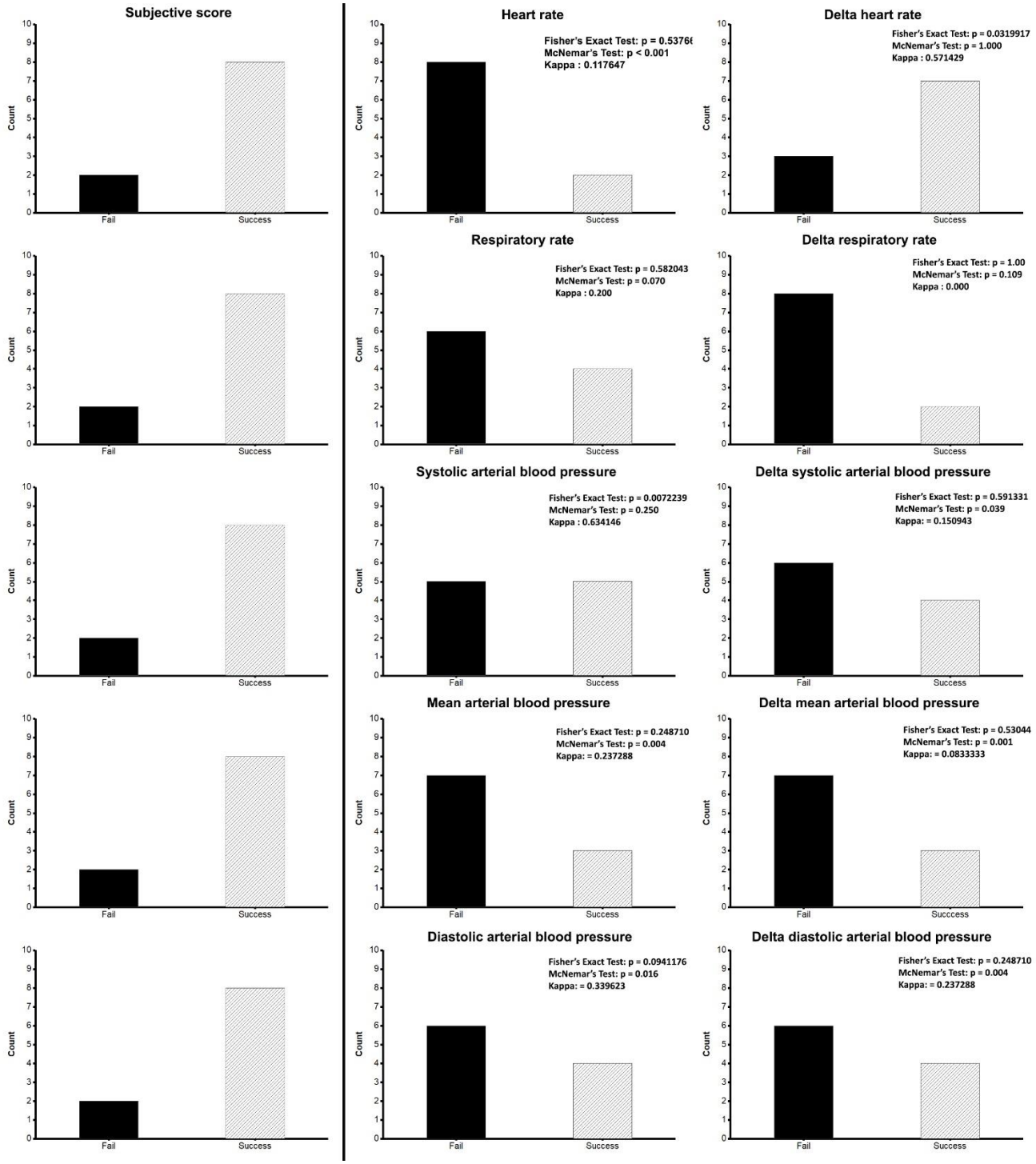


Figure 5.1 Summary of comparison of subjective (left row) and objective (middle and right rows) assessment of nerve block outcome for blind bupivacaine block (BBB; 10 dogs). Values for Fisher's exact test, McNemar's test and Cohen's kappa coefficient are indicated. The count (number of dogs) undergoing an arthrotomy under surgical anaesthesia [premedicated with 0.01 mg kg^{-1} medetomidine and 0.3 mg kg^{-1} morphine, induced with propofol and maintained on isoflurane (targeted end-tidal concentration of 1.6%)] is indicated. Black bar: failed blocks; Shaded bar: successful blocks.

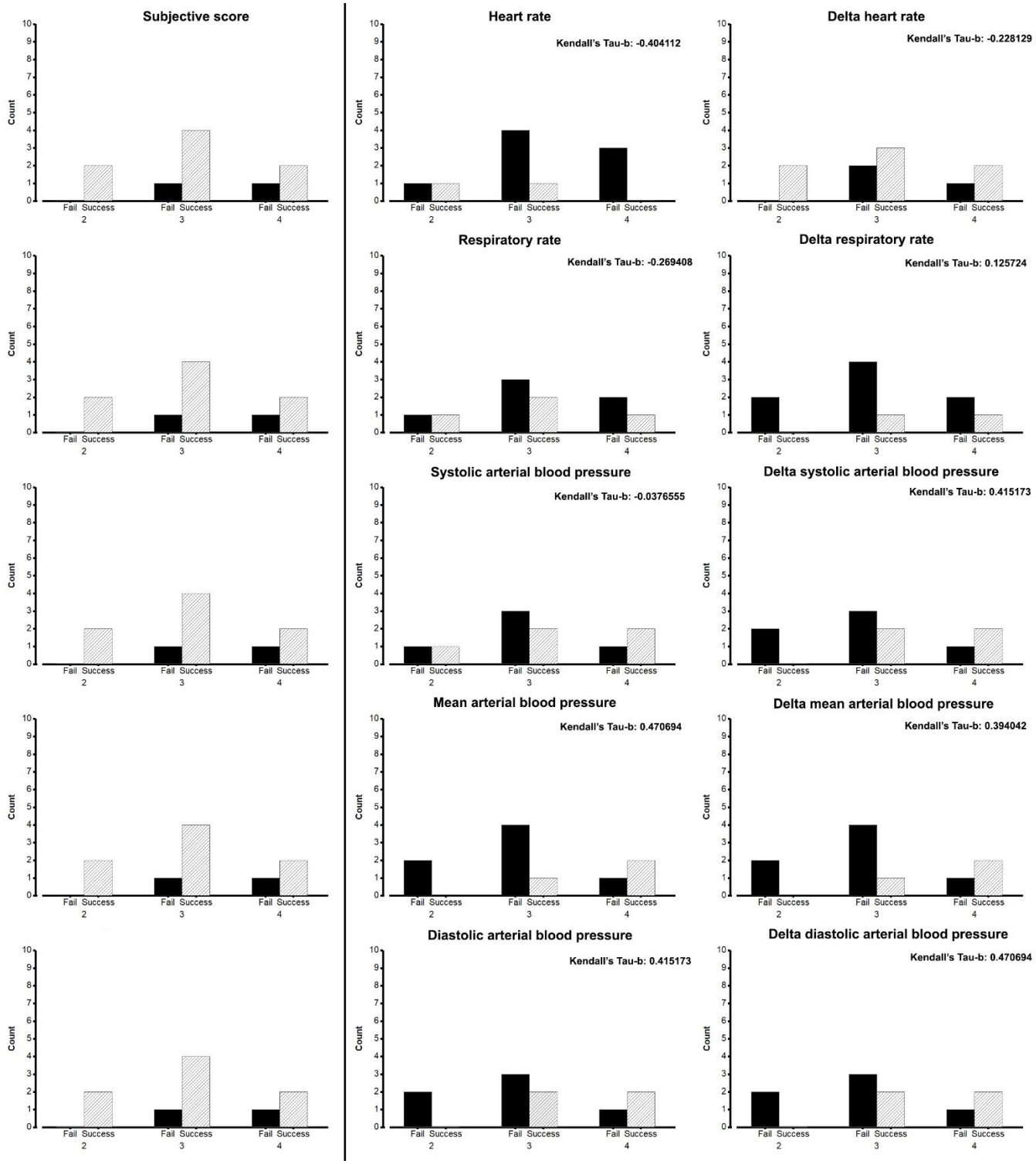


Figure 5.2 Summary of nerve block outcome (fail or success) for blind bupivacaine block (BBB; 10 dogs). Subjective score (left row) compared to objective scores (middle and right rows) after being stratified into the assigned confidence score (2, 3 and 4) compared using Kendall's tau-b rank correlation coefficient. The count (number of dogs) undergoing an arthrotomy under surgical anaesthesia [premedicated with 0.01 mg kg^{-1} medetomidine and 0.3 mg kg^{-1} morphine, induced with propofol and maintained on isoflurane (targeted end-tidal concentration of 1.6%)] is indicated. Black bar: failed blocks; Shaded bar: successful blocks.

Table 5.2 Variables that can be used to objectively detect peripheral nerve block failure in dogs undergoing stifle arthrotomy. All dogs were premedicated with 0.01 mg kg^{-1} medetomidine and 0.3 mg kg^{-1} morphine, induced with propofol and maintained on isoflurane (targeted end-tidal concentration of 1.6%) in oxygen.

	Indicator	Value (mmHg)	Sen (%)	Spe (%)	PLR	NLR	
SAP	Weak	> 127	60	95	12	0.42	
delta SAP	Strong	> 10	95	65	2.71	0.08	*
MAP	Strong	> 80	90	80	4.5	0.13	*
delta MAP	Strong	> 6	100	60	2.5	0.00	**
DAP	Moderate	> 73	85	90	8.5	0.17	
delta DAP	Strong	> 8	95	70	3.17	0.07	*

Suggested for use in the detection of peripheral nerve block failure (*), sensitivity (Sen), specificity (Spe), positive likelihood ratio (PLR), negative likelihood ratio (NLR) for the detection of peripheral nerve block failure.

Chapter 6

6.0 Discussion

Dogs in the GSB treatment group that were surgically stimulated during a surgical plane of anaesthesia demonstrated a detectable response to arthrotomy. The null hypothesis was rejected. The cardiovascular variables, unlike the respiratory variables, showed good diagnostic ability to detect nociception. We propose the use of use of delta MAP (> 6 mmHg) or delta SAP (> 10 mmHg) or delta DAP (> 8 mmHg) as cut-off values for the objective detection of peripheral nerve block failure. With the exception of delta HR and SAP, there was a poor association between objective and subjective scores. There is a poor association between nerve block confidence scoring and the outcome of the block based on subjective and objective scoring.

Nurse anaesthetists, practicing in human medicine, speculated that a degree of overlap exists in the physiologic variables used for the intraoperative assessment of response to surgical stimulation and anaesthetic depth (Stomberg et al. 2001). Cardiovascular variables (HR and blood pressure), f_R, V_T had better association with response to surgical stimulation (i.e. nociception) compared to assessing anaesthetic depth in human patients (Stomberg et al. 2001). Though, an inadequate plane of anaesthesia can potentially make distinguishing nociception from arousal difficult. The current study was not a MAC finding study and all dogs were maintained at a clinically relevant F_E´Iso to simulate what would likely occur in routine non-specialised practice. In human medicine, isoflurane at a concentration of 1.5 times MAC did not prevent a haemodynamic response to noxious stimuli (Inada et al. 1997). Similarly, in our study, where dogs were anaesthetised at 1.25 times MAC, a detectable haemodynamic response to surgical stimulation was observed in the GSB treatment compared to GBB treatment.

Ward et al. (2018) suggested that an overconfidence in the success of peripheral nerve block techniques leads to a biased subjective assessment of the response to surgical stimulus. Furthermore, intraoperative subjective assessment of a response to surgical stimulation in humans is reliant on a change in cardiovascular and respiratory variables (Stomberg et al. 2001). This can explain why our subjective score was similar to the objective scores for delta HR and SAP in the detection of nerve block failure. However, the clinical application of using the cut-off values for HR [(> 77 beats per minute] and delta HR (> 1 beat per minute) to qualify a response to surgical stimulation is impractical. The reason being is that the normal resting HR of most dogs fall within the range of 77 beats per minute or more and a change in 1 beat per minute is too small a fluctuation to arouse any concern. In some of the dogs that were administered the GSB treatment, a decrease in HR was evident. The decrease in HR occurred concurrently with a substantial increase in DAP and was most likely due to an arterial baroreceptor reflex.

The use of the absolute associated criterions values of the cardiovascular variables in this study might not be directly translatable to every dog in every scenario. The cardiovascular effects of isoflurane and medetomidine and the antinociceptive effect of medetomidine and morphine most likely had an unquantifiable influence on the associated criterion values. It is important to consider the effect of the different anaesthetic drug combinations administered and cardiovascular system function of the dog. Therefore, the authors speculate that using delta values are better suited than absolute cardiovascular variable values in detecting a response to surgical stimulation. Furthermore, based on negative predictive values, we speculate that the delta blood pressure variables will have fewer false negatives associated with their use. However, the delta blood pressure variables lack specificity (≤ 70%). Based on the positive predictive values we speculate that the delta blood pressure variables might have a degree of false positives associated with their use. The relevance of false positive nerve block failures in

veterinary science and its effect on ERAS should be considered (Campoy 2022). Unnecessary analgesic interventions (polypharmacy), due to a high proportion of false positives, could result in longer periods of sedation, increased time to ambulation, postoperative nausea and vomiting, increased time to intake of oral solids and fluids and therefore an increased duration of hospital stay. However, under treating can also result in similar outcomes such as delayed time to ambulation, increase hospital stay and unnecessary suffering due to pain. From an animal ethics perspective, and to prevent the development of maladaptive pain physiologic variables and criterion values with a higher sensitivity are required to curtail immediate postoperative pain.

There is a major caveat with regards to the application of the recommended blood pressure variables. Consideration should be given to the availability and accuracy of blood pressure monitoring equipment. We speculate that the use of oscillometric blood pressure measurement is more conventional than invasive blood pressure measurement for orthopaedic surgery in otherwise healthy animals. The degree of agreement required between invasive arterial blood pressure and veterinary specific oscillometric devices is wide. According to the ACVIM consensus statement for dogs and cats (Brown et al. 2007; Acierno et al. 2018) 50% of oscillometric measurements should be within 10 mmHg, or 80% of their measurements within 20 mmHg of invasive pressure readings (SAP, DAP). These ranges are larger than those proposed for our delta blood pressure variables and we therefore hesitate to recommend use of an oscillometric device in detecting of a response to surgical stimulus.

The BBB treatment was expected to have a larger proportion of block failure associated with its use. Therefore, the use of the BBB treatment was used to assess and compare the performance of subjective and objective measures in detecting nerve block failure. The failure rate of blind nerve block techniques in veterinary species is highly variable with a range of 42.9% to 85% (Thomson et al. 2021; Van der Laan et al. 2021; Podsiedlik et al. 2022). The high variability is most likely due to the interplay of several factors such as the complexity of

the block, body condition of the animal (ease of structural palpation) and the operator (skill, familiarity and anatomical knowledge). Therefore, the expected BBB failure rate seems to be in better agreement with objective scoring with the delta blood pressure variables (60% to 70%) than with the subjective scoring (20%). The success rate of NS guided femoral and sciatic nerve blocks in dogs has been shown to be between 76% to 86 % (Vettorato et al. 2012, Portela et al. 2013). Vettorato et al. (2012) used intraoperative fentanyl requirement to qualify nerve block success. Portela et al. (2013) used the downward titration of FE´Iso and the concurrent cardiovascular response (>25% increase of HR or MAP) to determine success rate. Unfortunately, the possibility of nerve block failure within the GBB group cannot be excluded. The investigators routinely perform these blocks on a daily basis and speculate that the success would be similar to those reported above. If seven out of ten were successful then that would aide in the belief that our ROC analysis was accurate enough and the criterions are clinically relevant. However, criterion values should be interpreted with caution.

Serious complications (nerve injury, systemic toxicity) with peripheral nerve blocks are extremely rare. A prospective study of 7000 nerve blocks in human patients indicated that the prevalence of nerve injury and systemic toxicity as 0.0004% and 0.00098%, respectively (Barrington et al. 2009). In the present study, there was an inherent degree of risk associated with all three treatment groups. According to the proposed serious harm and morbidity (SHAM) score by McGuirk et al. (2011) the score for the use of a placebo (GSB) and a blind (BBB) group in the current study was 3 out of 4 (i.e. moderate risk). McGuirk et al. (2011) questioned the use of placebo treatments in peripheral nerve block studies. However, placebo blocks do have significance and are used in peripheral nerve block studies in animals (Warrit et al. 2019; Papadopoulos et al. 2022; Garbin et al. 2023). The BBB treatment had a higher degree of risk for nerve injury and block failure. In an attempt to reduce the incidence of serious complications, a good standard of practice was maintained in all three treatments. Excessive

tissue probing was avoided during needle tip positioning, aspirations were performed prior to injection and the needle tip was repositioned if there was resistance to injection. Upon critical evaluation of this study based on the declaration of Helsinki (DOH), we feel that the inclusion of the saline block did not adversely affect any of the dog's health. The shortfall of this study with regards to the DOH was that dogs administered the GSB treatment did not receive any direct health benefits from the preoperative block (Ashall et al. 2023). The GSB treatment group were administered adequate systemic analgesia throughout the surgery and an additional postoperative nerve block prior to recovery. Therefore, we feel that the experimental procedures were ethical best practice.

The study had some notable limitations, especially because the recommended variables were derived from an otherwise healthy dog population undergoing surgery using a standardised anaesthetic protocol. We speculate that the population of dogs we sampled would be similar in most veterinary practices. Regardless of this speculation, the effect of different: pain states, MAC multiples, anaesthetic drug combinations (antinociceptive and cardiovascular effects) and physiological states (especially cardiovascular function) on the associated criterion values for the cardiovascular variables remains to be determined. We recorded quantitative physiologic values at structured time points. Therefore, observer bias was removed from the data collection and analysis.

Conclusion and future research

The use of delta blood pressure variables should be considered as an objective score to detect a response to surgical stimulation and therefore peripheral nerve block failure. The determination of criterion values for different populations and conditions will benefit future clinical trials in critically evaluating nerve block techniques and patient care by improving ERAS. The objective scoring is a tool with the potential of being translatable to other studies. This study will hopefully set the stage for others to contribute in developing an accessible, easy to use objective tool with clear criterions for the detection of intraoperative peripheral nerve block failure.

References

Acierno MJ, Brown S, Coleman AE et al. (2018) ACVIM consensus statement: guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. J Vet Intern Med 32, 1803-1822.

Adler MW, Geller ED, Rosow CE et al. (1988) The opioid system and temperature regulation. Annu Rev Pharmacol Toxicol 28, 429–449.

Aranake A, Mashour GA, Avidan MS (2013) Minimum alveolar concentration: ongoing relevance and clinical utility. Anaesth 68, 512-522.

Ashall V, Morton D, Clutton E (2023) A Declaration of Helsinki for animals. Vet Anaesth Analg 50, 309-314.

Ball C, Westhorpe R (2003) Local Anaesthesia – early nerve blocks. Anaesth Intens Care 31, 347.

Barrington MJ, Watts SA, Gledhill SR et al. (2009) Preliminary results of the Australasian Regional Anaesthesia Collaboration: a prospective audit of more than 7000 peripheral nerve and plexus blocks for neurologic and other complications. Reg Anesth Pain Med 34, 534-541.

Benarroch EE (2006) Pain-autonomic interactions. Neurolog Sci 27, 130–133.

Bergfeld C, Beyerbach M, Voigt A et al. (2014) Evaluation of heart rate variability for monitoring the depth of anaesthesia in dogs. Tierarztl Prax Ausg Kleintiere/Heimtiere 43.

Bergmann I, Gohner A, Crozier TA et al. (2013) Surgical plethindex-guided remifentanil administration reduces remifentaniland propofol consumption and shortens recovery times in outpatient anaesthesia. Brit J Anaesth 110, 622–628.

Bigby SE, Beths T, Bauquier S, Carter JE (2017) Effect of rate of administration of propofol or alfaxalone on induction dose requirements and occurrence of apnea in dogs. Vet Anaesth Analg 44, 1267-1275.

Bonhomme V, Uutela K, Hans G et al. (2011) Comparison of the surgical Pleth Index with haemodynamic variables to assess nociception-anti nociception balance during general anaesthesia. Brit Anaesth 106, 101–111.

Brouse CJ, Karlen W, Dulmont GA et al. (2013) Monitoring nociception during general anesthesia with cardiorespiratory coherence. J Clin Mon Comp 27, 551–560.

Brown S, Atkins C, Bagley R et al. (2007) Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. J Vet Intern Med 21, 542–558.

Campoy L, Martin-Flores M, Looney AL et al. (2008) Distribution of a lidocaine-methylene blue solution staining in brachial plexus, lumbar plexus and sciatic nerve blocks in the dog. Vet Anaesth Analg 35, 348–354.

Campoy L (2022) Development of Enhanced Recovery After Surgery (ERAS) protocols in veterinary medicine through a one-health approach: the role of anesthesia and locoregional techniques, J Am Vet Med Assoc 260, 1751-1759.

Cattai A, Rabozzi R, Ferasin H et al. (2018) Haemodynamic changes during propofol induction in dogs: new findings and approach of monitoring. BMC Vet Res 14, 282.

Cividjian A, Martinez JY, Combourieu E et al. (2007) Beat-by-beat cardiovascular index to predict unexpected intraoperative movement in anesthetized unparalyzed patients: a retrospective analysis. J Clin Monit 21, 91–101.

Committee on Standards and Practice Parameters, CSPP (2020) Standards for basic anesthetic monitoring. https://www.asahq.org/standards-and-guidelines/standards-for-basic-

anesthetic-monitoring. Last accessed 02 January 2023.

Costa-Farré C, Blanch XS, Cruz JI, Franch J (2011) Ultrasound guidance for the performance of sciatic and saphenous nerve blocks in dogs. Vet J 187, 221-224.

Cowen R, Stasiowska MK, Laycock H, Bantel C (2015) Assessing pain objectively: the use of physiological markers. Anaesth 70, 828-847.

Dahan A, Nieuwenhuijs DJ, Olofsen E (2003) Influence of propofol on the control of breathing. Adv Exp Med Biol 523, 81–92.

De Jong RH, Eger EI (1975) MAC expanded: AD50 and AD95 values of common inhalation anesthetics in man. Anaesth 42, 408–419.

Dugdale AHA (2020) Local Anaesthetic Techniques for the Limbs: Small Animals. In: Veterinary anaesthesia principles to practice (2nd edn). Dugdale AHA, Beaumont G, Bradbrook C, Gurney M (eds). Wiley, UK. pp. 215-235.

Echeverry DF, Laredo FG, Gil F et al. (2012) Ultrasound-guided “two-in-one” femoral and obturator nerve block in the dog: an anatomical study. Vet Anaesth Analg 39, 611–617.

Evans HE, de Lahunta A (2013) Chapter 17: spinal nerves. In: Miller’s anatomy of the dog (4th edn). Evans HE, de Lahuntapp A (eds). Elsevier, Missouri, USA. 611–657.

Fink BR (1989) Mechanisms of differential axial blockade in epidural and subarachnoid anesthesia. Anesth 70, 851–858.

Garbin M, Ruel HL, Watanabe R et al. (2023) Analgesic efficacy of an ultrasound-guided transversus abdominis plane block with bupivacaine in cats: a randomised, prospective, masked, placebo-controlled clinical trial. J Feline Med Surg 25, 1-10.

Garcia ER (2015) Local anesthetics. In: Lumb and Jones (5th edn). Grimm KA, Lamont LA,

Tranquilli WJ, Greene SA, Robertson SA (eds). Wiley, USA. pp. 332–354.

Gatson BJ, Garcia-Pereira FL, James M et al. (2016) Use of a perfusion index to confirm the presence of sciatic nerve blockade in dogs. Vet Anaesth Analg 43, 662-669.

Goerig M, Agarwal K (2000) Georg Perthes—the man behind the technique of nerve-tracer technology. Reg Anesth Pain Med 25, 296-301.

Gurney MA, Leece EA (2014) Analgesia for pelvic limb surgery. A review of peripheral nerve blocks and the extradural technique. Vet Anaesth Analg 41, 445-458.

Graff SM, Wilson DV, Guiot LP et al. (2015) Comparison of three ultrasound guided approaches to the lumbar plexus in dogs: a cadaveric study. Vet Anaesth Analg 42, 394–404.

Gray TR, Dzikiti BT, Zeiler GE (2019) Effects of hyaluronidase on ropivacaine or bupivacaine regional anaesthesia of the canine pelvic limb. Vet Anaesth Analg 46, 214-225.

Grimm KA, Lamont LA, Tranquilli WJ et al. (2015) Inhalation anesthetics. In: Lumb and Jones (5th edn). Grimm KA, Lamont LA, Tranquilli WJ, Greene SA, Robertson SA (eds). Wiley, USA. pp. 297–331.

Hadzic A, Dilberovic F, Shah S et al. (2004) Combination of intraneural injection and high injection pressure leads to fascicular injury and neurologic deficits in dogs. Reg Anesth Pain Med 29, 417–423.

Haga HA, Dolvik N (2005) Electroencephalographic and cardiovascular variables as nociceptive indicators in isoflurane-anesthetized horses. Vet Anaesth Analg 32, 128-35.

Haga HA, Tevik A, Moerch H (2001) Electroencephalographic and cardiovascular indicators of nociception during isoflurane anaesthesia in pigs. Vet Anaesth Analg 28, 126-131.

Hernandez-Avalos I, Mota-Rojas D, Mora-Medina P et al. (2019) Review of different methods used for clinical recognition and assessment of pain in dogs and cats. Int J Vet Sci Med 18, 43-54.

Inada T, Inada K, Kawachi S et al. (1997) Haemodynamic comparison of sevoflurane and isoflurane anaesthesia in surgical patients. Can J Anaesth 44, 140-145.

Jeanne M, Logier R, De Jonckheere J, Tavernier B (2009). Heart rate variability during total intravenous anesthesia: effects of nociception and analgesia. Auton Neursci 147, 91–96.

Jeanne M, Delecroix M, De Jonckheere J et al. (2014) Variations of the analgesia nociception index during propofol anesthesia for total knee replacement. Clin J Pain 30, 1084–1088.

Kanda T, Iguchi A, Yoshioka C et al. (2015) Effects of medetomidine and xylazine on intraocular pressure and pupil size in healthy beagle dogs. Vet Anaesth Analg 42, 623–628.

Kapur E, Vuckovic I, Dilberovic F et al. (2007) Neurologicand histologic outcome after intraneural injections of lidocaine in canine sciatic nerves. Acta Anaesthesiol Scand 51, 101–107.

Kehlet H, Jensen TS, Woolf CJ (2006) Persistent postsurgical pain: risk factors and prevention. The Lancet 367, 1618–1625.

Kukanich B, Lascelles BD, Papich MG (2005) Use of a von Frey device for evaluation of pharmacokinetics and pharmacodynamics of morphine after intravenous administration as an infusion or multiple doses in dogs. Am J Vet Res 66, 1968-7194.

Kukanich B, Wiese A (2015). Opioids. In: Lumb and Jones (5th edn). Grimm KA, Lamont LA, Tranquilli WJ, Greene SA, Robertson SA (eds). Wiley, USA. pp. 207-226.

Küls N, Blissitt KJ, Shaw DJ et al. (2017) Thermography as an early predictive measurement for evaluating epidural and femoral-sciatic block success in dogs. Vet Anaesth Analg 44, 1198-1207.

Kuusela E, Raekallio M, Anttila M et al. (2000) Clinical effects and pharmacokinetics of medetomidine and its enantiomers in dogs. J Vet Pharmcol Ther 23, 15–20.

Ledowski T (2019) Objective monitoring of nociception: a review of current commercial solutions. Br J Anaesth 123, 312–321.

Lee HK, Wang SC (1975) Mechanism of morphine-induced miosis in the dog. J Pharmacol Exp Ther 192, 415–431.

Lichtner G, Auksztulewicz R, Velten,H et al. (2018) Nociceptive activation in spinal cord and brain persists during deep general anaesthesia. Br J Anaesth 121, 291-302.

Lucas AN, Firth AM, Anderson GA et al. (2001) Comparison of the effects of morphine administered by constant- rate intravenous infusion or intermittent intramuscular injection in dogs. J Am Vet Med Assoc 218, 884–891.

Mahler SP (2012) Ultrasound guidance to approach the femoral nerve in the iliopsoas muscle: a preliminary study in the dog. Vet Anaesth Analg 39, 550–554.

Gross ME, Pablo LS (2015). Opthalmic patient. In: Lumb and Jones (5th edn). Grimm KA, Lamont LA, Tranquilli WJ, Greene SA, Robertson SA (eds). Wiley, USA. pp. 963-982.

Malik M (1996) Heart rate variability: standards of measurement, physiological interpretation and clinical use. Circulation 93, 1043–1065.

Martinez-Vazquez P, Jensen EW (2022) Different perspectives for monitoring nociception

during general anesthesia. Korean J Anesthesiol 75, 112-123.

McCally RE, Bukoski A, Branson KR et al. (2015) Comparison of short-term postoperative analgesia by epidural, femoral nerve block, or combination femoral and sciatic nerve block in dogs undergoing tibial plateau leveling osteotomy. Vet Surg 44, 983–987.

McGuirk S, Fahy C, Costi D, Cyna AM (2011) Use of invasive placebos in research on local anaesthetic interventions. Anaesth 66, 84–91.

McHugh ML (2012) Interrater reliability: the kappa statistic. Biochem Med 22, 276-282.

Merskey H, Bogduk N (1994) International Association for the Study of Pain. Part III: Pain terms, a current list with definitions and notes on usage, classification of chronic pain, Second Edition (209-214). https://www.iasp-pain.org/resources/terminology/. Last accessed 06 December 2022.

Miller DB, O'Callaghan JP (2002) Neuroendocrine aspects of the response to stress. Metab 51, 5–10.

Mills EP, Combs-Ramey K, Kwong GPS, Pang DSJ (2022) Development of reference intervals for pupillometry in healthy dogs. Front Vet Sci 26, 1020710.

Nunan D, Sandercock GRH, Brodie DA (2010) A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. Pacing Clin Electrophysiol 33, 1407–1417.

Orebaugh SL, Williams BA, Kentor ML et al. (2007) Ultrasound guidance with nerve stimulation reduces the time necessary for resident peripheral nerve blockade. Reg Anesth Pain Med 32, 448–454.

Orebaugh SL, Williams BA, Vallejo M et al. (2009) Adverse outcomes associated with stimulator-based peripheral nerve blocks with versus without ultrasound visualization.

Reg Anesth Pain Med 34, 251–255.

Osterweis M, Kleinman A, Mechanic D (1987) The Anatomy and Physiology of Pain. In: Pain and Disability. Osterweis M, Kleinman A, Mechanic D (eds). National Academies Press, USA. https://www.ncbi.nlm.nih.gov/books/NBK219252/. Last accessed 08 December 2022.

Papadopoulos G, Duckwitz V, Doherr MG (2022) Femoral and sciatic nerve blockade of the pelvic limb with and without obturator nerve block for tibial plateau levelling osteotomy surgery in dogs. Vet Anaesth Analg 49, 407-416.

Papich M, Messenger K (2015) Non-steroidal anti-Inflammatory drugs. In: Lumb and Jones (5th edn). Grimm KA, Lamont LA, Tranquilli WJ, Greene SA, Robertson SA (eds). Wiley, USA. pp. 227-243.

Pathan H, Williams J (2012) Basic opioid pharmacology: an update. Br J Pain 6, 11-6.

Phan KH, Anderson JG, Bohay DR (2021) Complications associated with peripheral nerve blocks. Orthop Clin North Am 52, 279-290.

Podsiedlik M, Hofmeister EH, Duke-Novakovski T (2022) Comparison of 2 blind approaches to the paravertebral brachial plexus regional block in canine cadavers. Can J Vet Res 86, 20-26.

Portela DA, Melanie P, Briganti A et al. (2008). Nerve stimulator-guided paravertebral lumbar plexus anaesthesia in dogs. Vet Res Commun 32, 307-310.

Portela DA, Otero PE, Tarragona L et al. (2010) Combined paravertebral plexus block and parasacral sciatic block in healthy dogs. Vet Anaesth Analg 37, 531–541.

Portela DA, Otero PE, Briganti A et al. (2013) Femoral nerve block: a novel psoas compartment lateral pre-iliac approach in dogs. Vet Anaesth Analg 40, 194–204.

Portela DA, Verdier N, Otero PE (2018) Regional anesthetic techniques for the pelvic limb and abdominal wall in small animals: A review of the literature and technique description. Vet J 238, 27–40.

Purves D, Augustine GJ, Fitzpatrick D et al. (2001) Neuroscience: The Physiological Basis of Pain Modulation. Available from: https://www.ncbi.nlm.nih.gov/books/NBK10985/. Last accessed July 25 2023.

Pypendop BH, Verstegen JP (1998) Hemodynamic effects of medetomidine in the dog: a dose titration study. Vet Surg. 27, 612-622.

Rankin D (2015) Sedatives and tranquilizers. In: Lumb and Jones (5th edn). Grimm KA, Lamont LA, Tranquilli WJ, Greene SA, Robertson SA (eds). Wiley, USA. pp. 196-206.

Raymond SA, Steffensen SC, Gugino LD, Strichartz GR (1989) The role of length of nerve exposed to local anesthetics in impulse blocking action. Anesth Analg 68, 563–570.

Roizen MF, Horrigan RW, Frazer BM (1981) Anesthetic doses blocking adrenergic (stress) and cardiovascular responses to incision-MAC BAR. Anesthes 54, 390-398.

Rossi M, Cividjian A, Fevre MC et al. (2012) A beat-by-beat, online, cardiovascular index, CARDEAN, to assess circulatory responses to surgery: a randomized clinical trial during spine surgery. J Clinical Mon Comput 26, 441–449.

Sealed Envelope Ltd. 2022. Create a blocked randomisation list. [Online] Available from: https://www.sealedenvelope.com/simple-randomiser/v1/lists. Last accessed Jul 26 2021.

Short CE, Bufalari A (1999) Propofol anesthesia. Vet Clin North Am Small Anim Pract 29, 747-778.

Sinclair MD (2003) A review of the physiological effects of alpha2-agonists related to the clinical use of medetomidine in small animal practice. Can Vet J 44, 885-897.

Starmer CF, Grant AO, Strauss HC (1984) Mechanisms of use-dependent block of sodium channels in excitable membranes by local anesthetics. Biophys J 46, 15–27.

Steffey EP, Howland D (1977) Isoflurane potency in the dog and cat. Am J Vet Res 38, 1833-1836.

Steffey EP, Mama KR, Brosnan RJ (2015) Inhalation anesthetics. In: Lumb and Jones (5th edn). Grimm KA, Lamont LA, Tranquilli WJ, Greene SA, Robertson SA (eds). Wiley, USA. pp. 297-331.

Stomberg MW, Sjöström B, Haljamäe H (2001) Routine intra-operative assessment of pain and/or depth of anaesthesia by nurse anaesthetists in clinical practice. J Clin Nurs 10, 429-436.

Tayari H, Tazioli G, Breghi G et al. (2017) Ultrasound-guided femoral and obturator nerves block in the psoas compartment in dogs: anatomical and randomized clinical study. Vet Anaesth Analg 44, 1216– 1226.

Thomson ACS, Portela DA, Romano M, Otero PE (2021) Evaluation of the effect of ultrasound guidance on the accuracy of intercostal nerve injection: a canine cadaveric study. Vet Anaesth Analg 48, 256-263.

Van der Laan M, Raes E, Oosterlinck M (2021) Cadaveric comparison of the accuracy of ultrasound-guided versus 'blind' perineural injection of the tibial nerve in horses. Vet J 269, 105603.

Vettorato E, Bradbrook C, Gurney M et al. (2012) Peripheral nerve blocks of the pelvic limb in dogs: A retrospective clinical study. Vet Comp Orth Traumat 25, 314–320.

Vettorato E, De Gennaro C, Okushima S et al. (2013) Retrospective comparison of two peripheral lumbosacral plexus blocks in dogs undergoing pelvic limb orthopaedic surgery. J Small Anim Pract 54, 630–637.

Ward S, Guest C, Goodall I, Bantel C (2018) Practice and bias in intraoperative pain management: results of a cross-sectional patient study and a survey of anesthesiologists. J Pain Res 15, 561-570.

Warrit K, Griffenhagen G, Goh C, Boscan P (2019) Comparison of ultrasound-guided lumbar plexus and sciatic nerve blocks with ropivacaine and sham blocks with saline on perianesthetic analgesia and recovery in dogs undergoing tibial plateau leveling osteotomy surgery. Vet Anaesth and Analg 46, 673-681.

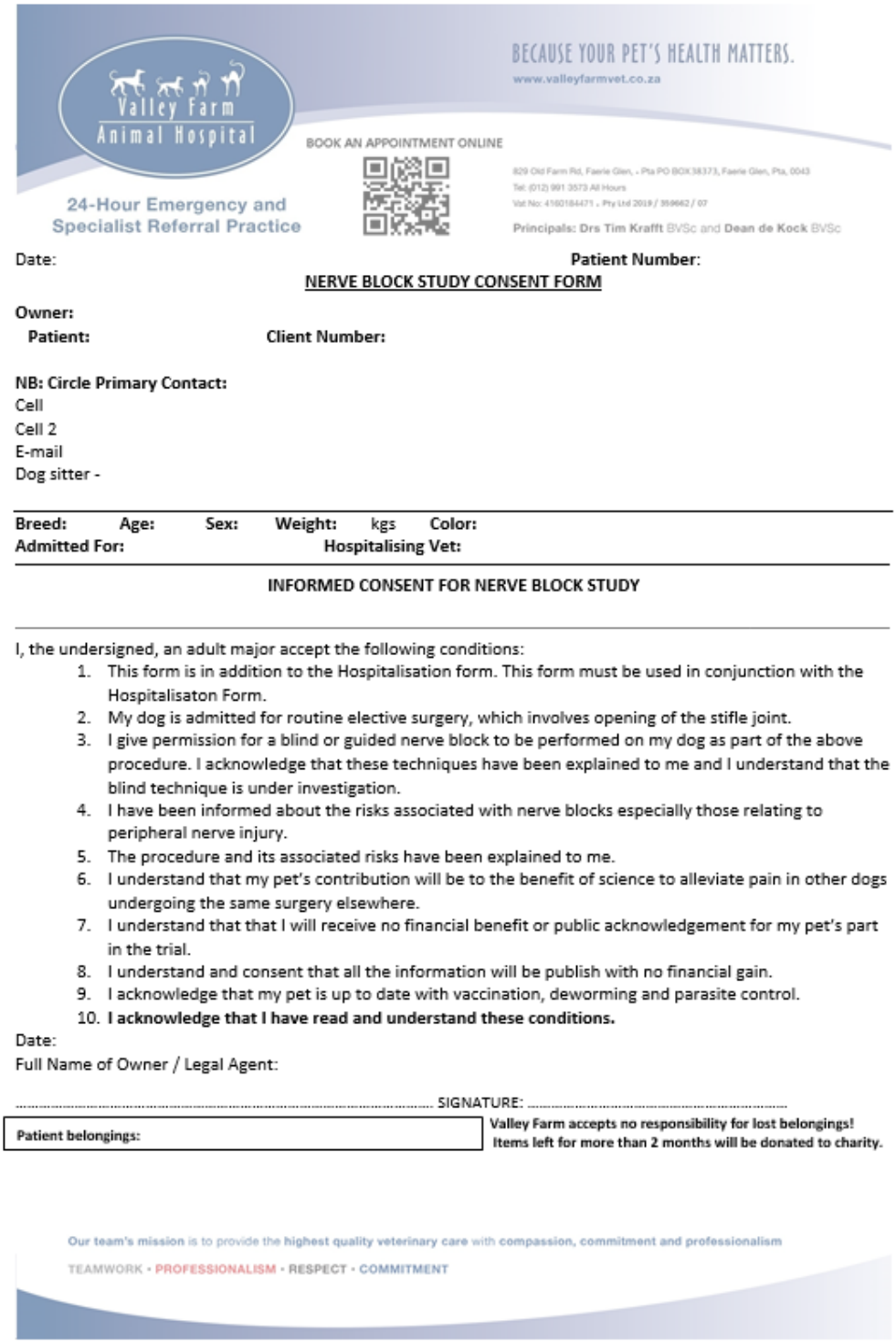
Wilder- Smith OH, Kolletzki M, Wilder- Smith CH (1995) Sedation with intravenous infusions of propofol or thiopentone. Effects on pain perception. Anaesth 50, 218–222.

Yamashita K, Furukawa E, Itami T et al. (2012) Minimum alveolar concentration for blunting adrenergic responses (MAC-BAR) of sevoflurane in dogs. J Vet Med Sci 74, 507-511.

Ying SW, Goldstein PA (2005) Propofol suppresses synaptic responsiveness of somatosensory relay neurons to excitatory input by potentiating GABA(A) receptor chloride channels. Molec Pain 1, 2.

Appendixes

Appendix i



Valley Farm Animal Hospital

BECAUSE YOUR PET'S HEALTH MATTERS.
www.valleyfarmvet.co.za

BOOK AN APPOINTMENT ONLINE

24-Hour Emergency and Specialist Referral Practice

829 Old Farm Rd, Faerie Glen, - Pta PO BOX 38373, Faerie Glen, Pta, 0043
Tel: (012) 991 3573 All Hours
Vat No: 4160184471 . Pty Ltd 2019 / 359662 / 07

Principals: Drs Tim Krafft BVSc and Dean de Kock BVSc

Date: **Patient Number:**

NERVE BLOCK STUDY CONSENT FORM

Owner:
Patient: **Client Number:**

NB: Circle Primary Contact:
Cell
Cell 2
E-mail
Dog sitter -

Breed: **Age:** **Sex:** **Weight:** kgs **Color:**
Admitted For: **Hospitalising Vet:**

INFORMED CONSENT FOR NERVE BLOCK STUDY

I, the undersigned, an adult major accept the following conditions:

1. This form is in addition to the Hospitalisation form. This form must be used in conjunction with the Hospitalisaton Form.
2. My dog is admitted for routine elective surgery, which involves opening of the stifle joint.
3. I give permission for a blind or guided nerve block to be performed on my dog as part of the above procedure. I acknowledge that these techniques have been explained to me and I understand that the blind technique is under investigation.
4. I have been informed about the risks associated with nerve blocks especially those relating to peripheral nerve injury.
5. The procedure and its associated risks have been explained to me.
6. I understand that my pet's contribution will be to the benefit of science to alleviate pain in other dogs undergoing the same surgery elsewhere.
7. I understand that that I will receive no financial benefit or public acknowledgement for my pet's part in the trial.
8. I understand and consent that all the information will be publish with no financial gain.
9. I acknowledge that my pet is up to date with vaccination, deworming and parasite control.
10. **I acknowledge that I have read and understand these conditions.**

Date:
Full Name of Owner / Legal Agent:

..... SIGNATURE: .....

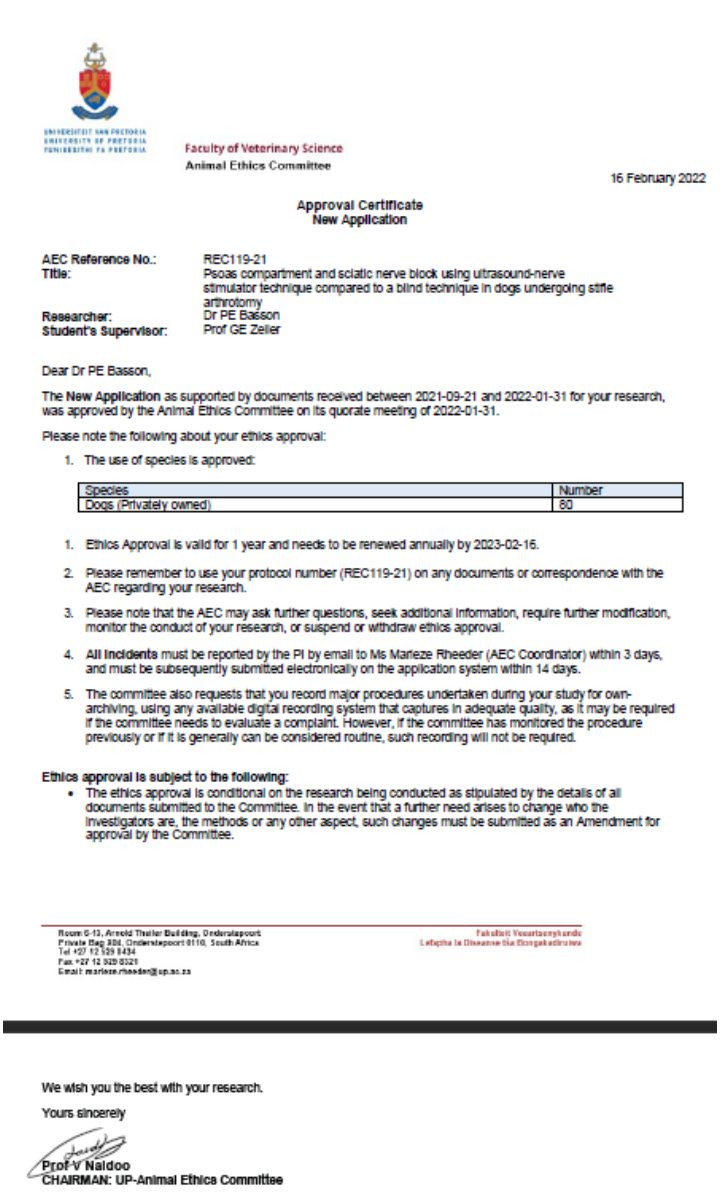
Patient belongings:

Valley Farm accepts no responsibility for lost belongings! Items left for more than 2 months will be donated to charity.

Our team's mission is to provide the highest quality veterinary care with compassion, commitment and professionalism

TEAMWORK • PROFESSIONALISM • RESPECT • COMMITMENT

Appendix ii



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

16 February 2022

Approval Certificate
New Application

AEC Reference No.: REC119-21
Title: Psoas compartment and sciatic nerve block using ultrasound-nerve stimulator technique compared to a blind technique in dogs undergoing stifle arthrotomy
Researcher: Dr PE Basson
Student's Supervisor: Prof GE Zeiler

Dear Dr PE Basson,

The **New Application** as supported by documents received between 2021-09-21 and 2022-01-31 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2022-01-31.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	Number
Dogs (Privately owned)	80

1. Ethics Approval is valid for 1 year and needs to be renewed annually by 2023-02-16.
2. Please remember to use your protocol number (REC119-21) on any documents or correspondence with the AEC regarding your research.
3. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
4. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
5. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

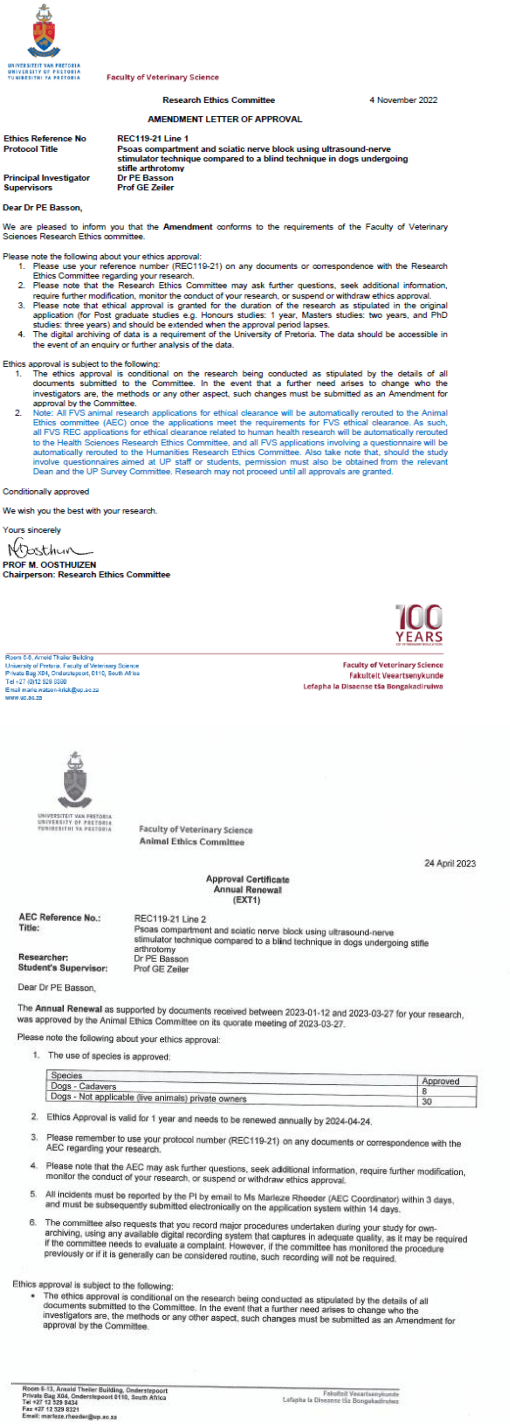
Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Disense tša Bongakadiruiwa

We wish you the best with your research.

Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee



UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science

Research Ethics Committee 4 November 2022

AMENDMENT LETTER OF APPROVAL

Ethics Reference No REC119-21 Line 1
Protocol Title Psoas compartment and sciatic nerve block using ultrasound-nerve stimulator technique compared to a blind technique in dogs undergoing stifle arthrotomy
Principal Investigator Dr PE Basson
Supervisors Prof GE Zeiler

Dear Dr PE Basson,

We are pleased to inform you that the **Amendment** conforms to the requirements of the Faculty of Veterinary Sciences Research Ethics committee.

Please note the following about your ethics approval:

1. Please use your reference number (REC119-21) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application (for Post graduate studies e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. Note: All FVS animal research applications for ethical clearance will be automatically rerouted to the Animal Ethics committee (AEC) once the applications meet the requirements for FVS ethical clearance. As such, all FVS REC applications for ethical clearance related to human health research will be automatically rerouted to the Health Sciences Research Ethics Committee, and all FVS applications involving a questionnaire will be automatically rerouted to the Humanities Research Ethics Committee. Also take note that, should the study involve questionnaires aimed at UP staff or students, permission must also be obtained from the relevant Dean and the UP Survey Committee. Research may not proceed until all approvals are granted.

Conditionally approved

We wish you the best with your research.

Yours sincerely

PROF M. OOSTHUIZEN
Chairperson: Research Ethics Committee

100 YEARS

Room 5-8, Arnold Theiler Building
University of Pretoria, Faculty of Veterinary Science
Private Bag X04, Onderstepoort, 0110, South Africa
Tel +27 (0)12 529 (0)26
Email marie.mateo-kriuk@up.ac.za
www.up.ac.za

Faculty of Veterinary Science
Fakulteit Veeartsenykunde
Lefapha la Disense tša Bongakadiruiwa

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

24 April 2023

Approval Certificate
Annual Renewal
(EXT1)

AEC Reference No.: REC119-21 Line 2
Title: Psoas compartment and sciatic nerve block using ultrasound-nerve stimulator technique compared to a blind technique in dogs undergoing stifle arthrotomy
Researcher: Dr PE Basson
Student's Supervisor: Prof GE Zeiler

Dear Dr PE Basson,

The **Annual Renewal** as supported by documents received between 2023-01-12 and 2023-03-27 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2023-03-27.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	Approved
Dogs - Cadavers	6
Dogs - Not applicable (live animals) private owners	30

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2024-04-24.
3. Please remember to use your protocol number (REC119-21) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. All incidents must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Disense tša Bongakadiruiwa

Appendix iii

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	
Title and abstract			
	1a	Identification as a randomised trial in the title	✗
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	✓
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	✓
	2b	Specific objectives or hypotheses	✓
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	✓
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	✓
Participants	4a	Eligibility criteria for participants	✓
	4b	Settings and locations where the data were collected	✓
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	✓
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	✓
	6b	Any changes to trial outcomes after the trial commenced, with reasons	✓
Sample size	7a	How sample size was determined	✓
	7b	When applicable, explanation of any interim analyses and stopping guidelines	✓
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	✓
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	✓
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	✓
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	✓
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	✓

Appendix iv

ID: _____ Randm: _____ Weight: _____ BC: _____ BLIND / GUIDED

Pre-Anaesthetic assessment:

_____

_____

Performed by: EPB / GEZ Monitored by EPB / GEZ

Pre-med time: _____ Block time: _____ SAL/BUP VOL: (0.2 mL/kg/site) _____mL

Cut time: _____

Joint cut time time: _____ Bone cut time: _____ Block duration_____

Surgical end time: _____ Anaesthetic end time: _____ Pain score time: _____ (____)

	Time point (minutes)													Max DEV %
	T_{-1}	At cut T_0	1	2	3	4	5	6	8	10	15	20	25	
Time														
HR														
RR														
SAP														
DAP														
MAP														
Temp														
Et_{inh}														
Move	Yes/no													
Response	Yes/no													
Fail/succ.														

Guided	
Subjective score	Description
1	Not confident at all, no confidence of needle in correct position
2	Poor confidence, appropriate response with nerve stimulator, but not confirmed with ultrasound
3	Fairly confident, NS response, correct anatomical location but no doughnut sign
4	Very confident, NS response, visualization of doughnut sign

Blind	
Subjective score	Description
1	Not confident at all, can't properly identify anatomical landmarks
2	Poor confidence, unsure of facial plane
3	Fairly confident
4	Very confident, nerve response observed

100% normal func T:_____

Comments: _____

Appendix v

Summary of mean ± standard deviation for delta respiratory rate (fR), delta heartrate (HR), delta systolic arterial pressure (SAP), delta mean arterial pressure (MAP), delta diastolic arterial pressure (DAP) for the treatments guided saline block (GSB; 10 dogs; response to surgical stimulus), guided bupivacaine block (GBB; 10 dogs; no response to surgical stimulus) and blind bupivacaine block (GBB; 10 dogs) in dogs undergoing an arthrotomy under surgical anaesthesia [premedicated with 0.01 mg kg-1 medetomidine and 0.3 mg kg-1 morphine, induced with propofol and maintained on isoflurane (targeted end-tidal concentration of 1.6%)].

	Delta f_R	Delta HR	Delta SAP	Delta MAP	Delta DAP
GBB	0.3 ±1.7	-6 ± 12.2	6.3 ± 13.4	3.8 ± 11.3	3.9 ± 10.8
GSB	0.7 ± 4.1	-1.7 ± 10	16.7 ± 11.9	15.6 ± 9.8	17.2 ± 11.1
BBB	0.6 ± 4.5	-1.4 ± 8.6	13.3 ± 14.9	11.4 ± 12.2	11.4 ± 12.2

Declarations

The authors declare no conflict of interest.

The authors declare that artificial intelligence was not used in this study or during the preparation of the manuscript.

The data set is available upon reasonable request.

PE Basson