Comparison of Rostral Spread of Lumbosacral Epidural Volume Calculated by Body Weight or Length of the Vertebral Column in Dogs

by

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ABSTRACT

COMPARISON OF ROSTRAL SPREAD OF LUMBOSACRAL EPIDURAL VOLUME CALCULATED BY BODY WEIGHT OR LENGTH OF THE VERTEBRAL COLUMN IN DOGS

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This thesis evaluated in dogs the rostral spread of a lumbosacral epidural volume calculated by body weight or by length of the vertebral column. In phase I of this study, cadaver dogs (4.6–52 kg; 39-99 cm in length) were paired and matched within < 10% for body weight, length of the vertebral column from the sacrococcygeal space to the occipital crest, and body condition score. Within each pair, one dog was randomly allocated to receive an epidural volume by weight and the other dog by length of the vertebral column, using a mixture of dye and contrast medium. Within 5 minutes, the rostral spread of the contrast medium was assessed by computerized tomography (CT), and within one-hour the rostral spread of the dye was assessed by anatomical dissection. In phase II, research Beagle dogs (7.5–10.2 kg; 46–56 cm in length) were used in a randomized crossover design to receive a 1:1 epidural volume of contrast medium and bupivacaine 0.5% based on body weight or length under isoflurane anesthesia, with at least 5 days between doses, and assessed for rostral spread of the contrast medium by CT within 5 minutes of injection. Cardiorespiratory function assessments started immediately after injection, and sensory and motor block from the bupivacaine at 30 minutes post injection and while dogs were conscious.

Phase I and phase II demonstrated a larger volume of injectate using length than for body weight. In cadaver dogs of all sizes this difference was not significant and resulted in a similar number of vertebrae reached by the indicator (dye or contrast medium) between the two methods.
In live dogs and their small size, the larger volume was significantly different and resulted in a significantly greater number of vertebrae reached by the indicators. In phase I, the number of vertebrae reached by dye was significantly greater than for contrast medium. In phase II, the resultant concentration of bupivacaine (0.25\%) resulted in sensory and motor blockade of short duration. Cardiorespiratory function was within normal limits and similar for both dosing methods.
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# TABLE OF CONTENTS

Abstract .................................................................................................................................................. ii

Acknowledgements................................................................................................................................ iv

Table of Contents ................................................................................................................................... v

Declaration of Work Performed ........................................................................................................... viii

List of Figures ........................................................................................................................................ ix

List of Tables .......................................................................................................................................... xiv

List of Abbreviations .......................................................................................................................... xvi

1. General Literature Review ............................................................................................................ 1

   1.1. Introduction .................................................................................................................................... 2

   1.2. Epidural Space Anatomy .......................................................................................................... 4

       1.2.1. Vertebral Canal .................................................................................................................. 4

       1.2.2. Epidural Space .................................................................................................................. 5

       1.2.3. Spinal Cord ....................................................................................................................... 6

           1.2.3.1 Spinal Cord Membranes ............................................................................................. 7

           1.2.3.2 Spinal Cord Anatomy ................................................................................................. 9

       1.2.4. Cerebrospinal Fluid .......................................................................................................... 12

   1.3. Epidural Drugs .......................................................................................................................... 14

       1.3.1. Distribution of Drugs in the Epidural Space ...................................................................... 14

       1.3.2. Local Anesthetics .............................................................................................................. 16

           1.3.2.1. Physiology of the Sodium Channel .............................................................................. 16

           1.3.2.2. Mechanism of Action of Local Anesthetics in the Epidural
Space........................................................................................................18

1.3.2.3. Effects.............................................................................................20

1.3.3. Other Drugs........................................................................................21

1.4. Epidural Injection.....................................................................................23

1.4.1. Lumbosacral Technique.....................................................................23

1.4.2. Spinal Needle.......................................................................................27

1.4.3. Epidural Catheter..................................................................................27

1.5. Epidural Dose and Volume.......................................................................28

1.5.1. Epidural Dose Based on Weight.........................................................31

1.5.2. Epidural Dose Based on Length of the Vertebral Column...............33

1.5.3. Comparison Between Techniques......................................................35

1.5.4. Potential Complications of Epidurals..................................................36

1.5.4.1. Underdosing...................................................................................37

1.5.4.2. Overdosing.....................................................................................37

1.5.4.3. Epidural and Spinal Anesthesia.......................................................38

1.6. Rationale, Hypothesis and Objectives....................................................39

1.7. References................................................................................................42

CHAPTER 2:

2. Comparison of Rostral Spread of Lumbosacral Epidural Volume Calculated by Body
Weight or Length of the Vertebral Column in Dogs.....................................55

2.1. Summary................................................................................................56

2.2. Introduction..............................................................................................59
DECLARATION OF WORK PERFORMED

I declare that the work reported in this thesis was performed by myself and the contributing authors as stated.

The authors’ contribution is as follows:

**Flavio Augusto Vieira Freitag:** Acquisition and preparation of cadaver dogs, epidural injection and dissection of cadaver dogs, data collection, anesthetic and cardiorespiratory monitoring, nociceptive assessments in live dogs, data collection, and thesis preparation. **Alexander Valverde:** Student advisor, study design and funding application, experimental study and dissection of cadaver dogs, assistance with nociceptive assessments, data collection, statistical analysis, and thesis revision. **Monica Jensen:** Advisory committee member, study design, CT imaging interpretation, data collection, and thesis revision. **Andrea Sanchez Lazaro:** Advisory committee member, study design, and thesis revision. **Diego Gomez-Nieto:** Advisory committee member, study design, and thesis revision. **Craig Bailey:** Advisory committee member, study design, and thesis revision.
LIST OF FIGURES

Figure 2.1: Diagram used for number of vertebrae reached by the contrast or dye in dog cadavers and live animals after lumbosacral epidural injection. A maximum of 27 vertebrae was possible (seven lumbar + 13 thoracic + 7 cervical)……………………………………91

Figure 2.2: Volume of lumbosacral epidural injectate in 22 dog cadavers matched by < 10% difference in both weight (BW) and length of the vertebral column (LE), and same body condition score, where one cadaver received an epidural injection of 0.2 mL kg\(^{-1}\) for BW and the paired cadaver received 0.05 mL cm\(^{-1}\) (< 50 cm), 0.07 mL cm\(^{-1}\) (50 to < 70 cm), 0.08 mL cm\(^{-1}\) (70 to < 80 cm), or 0.11 mL cm\(^{-1}\) (≥ 80 cm mL cm\(^{-1}\)) for LE, of a 5:1 volume mixture of iopamide 30% and yellow tissue marking dye…………………………………………………………92

Figure 2.3: Number of vertebrae reached by a 5:1 volume mixture of iopamide 30% (A) and yellow tissue marking dye (B) in 22 cadavers matched by < 10% difference in body weight (BW) and length of the vertebral column (LE), and same body condition score, where one cadaver received a lumbosacral epidural injection of 0.2 mL kg\(^{-1}\) for BW and the paired cadaver received 0.05 mL cm\(^{-1}\) (< 50 cm), 0.07 mL cm\(^{-1}\) (50 to < 70 cm), 0.08 mL cm\(^{-1}\) (70 to < 80 cm), or 0.11 mL cm\(^{-1}\) (≥ 80 cm mL cm\(^{-1}\)) for LE, of the mixture………………………………………………………………………………93

Figure 2.4: Rostral spread of dye in pair #2 of dogs from the small size category (< 10 kg) that received a lumbosacral epidural injection of 0.2 mL kg\(^{-1}\) for BW or 0.05 mL cm\(^{-1}\) (< 50 cm) for LE of a 5:1 volume mixture of iopamide 30% and yellow tissue marking dye. Dog BW spread 19 vertebrae (T\(_2\)) and dog LE spread 26 vertebrae (C\(_2\))………………………………………………………………………………94
Figure 2.5: Rostral spread of dye in pair #3 of dogs from the medium size category (10 to < 25 kg) that received a lumbosacral epidural injection of 0.2 mL kg$^{-1}$ for BW or 0.08 mL cm$^{-1}$ (70 to < 80 cm) for LE of a 5:1 volume mixture of iopamide 30% and yellow tissue marking dye. Dog BW spread 23 vertebrae ($C_5$) and dog LE spread 26 vertebrae ($C_2$)………………………………………………95

Figure 2.6: Rostral spread of dye in pair #3 of cadaver dogs from the large size category (25 to < 45 kg) that received a lumbosacral epidural injection of 0.2 mL kg$^{-1}$ for BW or 0.11 mL cm$^{-1}$ ($\geq$ 80 cm mL cm$^{-1}$) for LE of a 5:1 volume mixture of iopamide 30% and yellow tissue marking dye. Dog BW spread 24 vertebrae ($C_4$) and dog LE spread 27 vertebrae ($C_1$)………………………………………………………………………………………………………………………………96

Figure 2.7: Rostral spread of dye in the pair of cadaver dogs from the giant size category ($\geq$ 45 kg) that received a lumbosacral epidural injection of 0.2 mL kg$^{-1}$ for BW or 0.11 mL cm$^{-1}$ ($\geq$ 80 cm mL cm$^{-1}$) for LE of a 5:1 volume mixture of iopamide 30% and yellow tissue marking dye. Dog BW spread 27 vertebrae ($C_1$) and dog LE spread 27 vertebrae ($C_1$)………………………………………………………………………………………………………………………………97

Figure 2.8: Correlation between distance (cm) (A) or number of vertebrae (B) reached by a volume of injectate of a 5:1 mixture of iopamide 30% and yellow tissue marking dye (B) in 22 cadavers matched by < 10% difference in body weight (BW) and length of the vertebral column (LE), and same body condition score, where one cadaver received a lumbosacral epidural injection of 0.2 mL kg$^{-1}$ for BW and the paired cadaver received 0.05 mL cm$^{-1}$ (< 50 cm), 0.07 mL cm$^{-1}$ (50 to < 70 cm), 0.08 mL cm$^{-1}$ (70 to < 80 cm), or 0.11 mL cm$^{-1}$ ($\geq$ 80 cm mL cm$^{-1}$) for LE, of the mixture………………………………………………………………………………………………………………………………98

Figure 2.9: Comparison of number of vertebrae reached by a 5:1 volume mixture of iopamide 30% and yellow tissue marking dye in 22 cadavers matched by < 10% difference in body weight (BW)
and length of the vertebral column (LE), and same body condition score, where one cadaver received a lumbosacral epidural injection of 0.2 mL kg\(^{-1}\) for BW and the paired cadaver received 0.05 mL cm\(^{-1}\) (< 50 cm), 0.07 mL cm\(^{-1}\) (50 to < 70 cm), 0.08 mL cm\(^{-1}\) (70 to < 80 cm), or 0.11 mL cm\(^{-1}\) (≥ 80 cm mL cm\(^{-1}\)) for LE, of the mixture. A) Data without normality and B) Data transformed to achieve normality…

Figure 2.10: Correlation between number of vertebrae or distance (cm) reached by a volume of injectate of a 5:1 mixture of iopamide 30% and yellow tissue marking dye (B) in 22 cadavers matched by < 10% difference in body weight (BW) and length of the vertebral column (LE), and same body condition score, where one cadaver received a lumbosacral epidural injection of 0.2 mL kg\(^{-1}\) for BW and the paired cadaver received 0.05 mL cm\(^{-1}\) (< 50 cm), 0.07 mL cm\(^{-1}\) (50 to < 70 cm), 0.08 mL cm\(^{-1}\) (70 to < 80 cm), or 0.11 mL cm\(^{-1}\) (≥ 80 cm mL cm\(^{-1}\)) for LE, of the mixture…

Figure 2.11: (a) Heart rate (HR), (b) respiratory rate (\(f_R\)), (c) systolic arterial pressure (SAP), (d) diastolic arterial pressure (DAP), \(\epsilon\) mean arterial pressure (MAP), (f) rectal temperature (TEMP), (g) end-tidal CO\(_2\) (PE\(^{\prime}\)CO\(_2\)), and (h) end-tidal isoflurane concentration (FE\(^{\prime}\)Iso) in six dogs initially under general anesthesia (GA) for placement of an epidural lumbosacral catheter and injection of a 1:1 volume mixture of iopamide 30% and bupivacaine 0.5%, and after anesthesia (RE). The injection consisted of 0.2 mL kg\(^{-1}\) for body weight (BW) on one occasion, or 0.05 mL cm\(^{-1}\) (< 50 cm) or 0.07 mL cm\(^{-1}\) (50 to < 70 cm) for length of the vertebral column (LE) on a different occasion in a randomized crossover fashion. Data are presented as mean ± standard deviation…

Figure 2.12: Comparison of number of vertebrae reached by a 1:1 mixture of iopamide 30% and bupivacaine 0.5% in six dogs administered a lumbosacral epidural injection of 0.2 mL kg\(^{-1}\) for
body weight (BW) and 0.05 mL cm\(^{-1}\) (< 50 cm) or 0.07 mL cm\(^{-1}\) (50 to < 70 cm) for length of the vertebral column (LE) in a randomized crossover fashion, of the mixture.
LIST OF TABLES

Table 2.1: Demographic data of 22 dog cadavers matched by < 10% difference in both weight (BW) and length of the vertebral column (LE), and same body condition score (BCS), where one cadaver received an epidural injection of 0.2 mL kg\(^{-1}\) for BW and the paired cadaver received 0.05 mL cm\(^{-1}\) (< 50 cm), 0.07 mL cm\(^{-1}\) (50 to < 70 cm), 0.08 mL cm\(^{-1}\) (70 to < 80 cm), or 0.11 mL cm\(^{-1}\) (≥ 80 cm mL cm\(^{-1}\)) for LE, of a 5:1 volume mixture of iopamide 30% and yellow tissue marking dye, respectively.

Table 2.2: Rostral spread measured by number of vertebrae and distance (cm) from the lumbosacral space (LS) of a LS epidural dose calculated by body weight (BW; 0.2 mL kg\(^{-1}\)) or by length of the vertebral column (LE; 0.05 mL cm\(^{-1}\) [< 50 cm], 0.07 mL cm\(^{-1}\) [50 to < 70 cm], 0.08 mL cm\(^{-1}\) [70 to < 80 cm], 0.11 mL cm\(^{-1}\) [≥ 80 cm mL cm\(^{-1}\)]) using a 5:1 volume mixture of iopamide 30% and yellow tissue marking dye, respectively, in dog cadavers matched according to < 10% difference in BW and LE, and same body condition score. Spread measured by CT imaging (iopamide) and necropsy of the spinal cord (dye).

Table 2.3: Demographic data of female Beagle dogs that received a lumbosacral epidural injection of 0.2 mL kg\(^{-1}\) for body weight (BW) and 0.05 mL cm\(^{-1}\) (< 50 cm) or 0.07 mL cm\(^{-1}\) (50 to < 70 cm) for the length of the vertebral column (LE) in a randomized crossover fashion of an equal volume mixture of iopamidol 30% and bupivacaine 0.5%.

Table 2.4: Time to presence of a positive response to noxious stimulation, consisting of clamping with a sponge forceps or a hemostat or electrical stimulation, of different dermatomes starting from caudal to cranial in six female Beagle dogs that received a lumbosacral epidural injection of 0.2 mL kg\(^{-1}\) for body weight (BW) and 0.05 mL cm\(^{-1}\) (< 50 cm) or 0.07 mL cm\(^{-1}\) (50 to < 70 cm) for
the length of the vertebral column (LE) in a randomized crossover fashion of an equal volume mixture of iopamide 30% and bupivacaine 0.5%. AN1 = base of the tail, AN2 = pelvic limb paw, AN3 = area surrounding the iliac crest, AN4 = inguinal area near the femoral nerve, AN5 = area surrounding the transverse process of L3 and flank, AN6 = paramedian area next to the umbilicus, AN7 = along the thirteenth rib, AN8 = paramedian area to the xyphoid, AN9 = along the ninth rib, AN10 = along the second rib, and AN11 = thoracic limb paw.
LIST OF ABBREVIATIONS

AN1 – Analgesia assessment at the base of the tail
AN2 – Analgesia assessment at the pelvic limb paw
AN3 – Analgesia assessment at the area surrounding the iliac crest
AN4 – Analgesia assessment at the inguinal area near the femoral nerve
AN5 – Analgesia assessment at the area close to the transverse process of L₃ and flank
AN6 – Analgesia assessment at the paramedian area next to the umbilicus
AN7 – Analgesia assessment along the thirteenth rib
AN8 – Analgesia assessment at the paramedian area to the xyphoid
AN9 – Analgesia assessment along the ninth rib
AN10 – Analgesia assessment along the second rib
AN 11 – Analgesia assessment at the thoracic limb paw
BCS – Body condition score
BW – Body weight
CNS – Central nervous system
CSF – Cerebrospinal fluid
CT – Computerized tomography
DAP – Diastolic arterial pressure
DRG – Dorsal root ganglia
Fe’Iso – End-tidal isoflurane concentration
$f_r$ – Respiratory rate
HR – Heart rate
L7-S; LS – Lumbosacral space

LE – Vertebral column length

MAC – Minimum alveolar concentration

MAP – Mean arterial pressure

mV – Millivolts

Navs – Sodium voltage gated channels

NMDA – N-methyl-D-aspartate

PaCO₂ – Arterial partial pressure of carbon dioxide

Pₑ'CO₂– End-tidal CO₂

SAP – Systolic arterial pressure

SD – Standard deviation

SQ – Subcutaneous

TEMP – Rectal temperature

VL – Volume based on vertebral column length

VW – Volume based on dose by weight
CHAPTER I

GENERAL LITERATURE REVIEW
1.1 INTRODUCTION

The epidural technique is one of the most used regional techniques in clinical practice in small animals to provide anesthesia and/or analgesia mainly of the pelvic limb, pelvis, and abdomen. Local anesthetics, opioids, and alpha-2 agonists are the most common drugs administered by this route, by means of a spinal needle or epidural catheter, introduced at the lumbosacral space (L₇-S; LS). Depending on the anesthetic drug(s) and dose/volume of administration used by the epidural route, the thorax and fore limb can also be affected.

The final volume of the epidural injectate in small animals is often based on the local anesthetic drug to be used and the desired area of interest (dermatome) to be affected. The most common and simple method to calculate the volume of injectate is based on the body weight of the animal (mL kg⁻¹). A different method consists of calculating the injectate based on the length of the vertebral canal, from the occipital crest to the first coccygeal vertebrae in centimeters (mL cm⁻¹).

These two methods have not been compared clinically to elucidate differences, reliability, and efficiency; therefore, there is no knowledge if both methods result in good agreement. When both methods have been compared theoretically, there are differences between the volumes of injectate that are more profound in dogs with weights of less than 25 kg, with larger volumes resulting from using the length calculation method.

The objectives of this research are, first to compare the two methods, dose by weight and dose by length, using first, dog cadavers of a broad range of body weights (from small breeds to giant breeds) to review the vertebral canal anatomy and rostral spread of dye and contrast medium after epidural injection at the lumbosacral space (L₇-S). Second, using live small dogs (< 10 kg) to verify the cadaveric findings of rostral spread, using a local anesthetic (bupivacaine) mixed with
contrast medium, to assess sensory blockade and some cardiovascular parameters (heart rate, blood pressure) using a prospective paired and randomized trial.
The first description of spinal anesthesia occurred in 1885 using experimental dogs (Corning 1885) and by 1899 Bier described a series of human clinical cases in which spinal anesthesia with cocaine was used to operate on cases of osteomyelitis, bone tuberculosis, and fractures, of the pelvic limb (Bier 1899). In 1935, Brook reviewed the use of the epidural technique, using the lumbosacral approach, in several domestic species (dog, horse, cow) (Brook 1935) and its use promoted in dogs two decades later (Joshua 1956).

1.2 EPIDURAL SPACE ANATOMY

1.2.1 Vertebral Canal

The vertebral or spinal canal in dogs is composed by seven cervical, thirteen thoracic, seven lumbar, three sacral and a variable number of coccygeal vertebrae. Therefore, each vertebral segment, represent a different percentage from the total length, with the cervical, thoracic, lumbar and sacrum representing, 27%, 37%, 29%, and 7% respectively (Fletcher & Kitchell 1966; Evans & Miller 1993). The vertebral canal is the sum of vertebral foramina from all vertebrae, extending from the foramen magnum to approximately the sixth coccygeal vertebra. The vertebral canal is limited by the dorsal longitudinal ligament at its ventral aspect, which is attached to the intervertebral disks and the vertebrae. Intervertebral pedicles and foramina form the lateral walls of the canal, and the vertebral laminae and ligamentum flavum (or yellow) are the dorsal limits of the vertebral canal (Evans & de Lahunta 2013). The shape of the vertebral canal changes along the vertebral column; in the first three cervical vertebrae it is almost circular and at the fourth vertebra it enlarges and acquires a slight oval shape. The oval shape and enlargement extend through the second thoracic vertebra and then returns to a circular shape with uniform diameter up to the
eleventh thoracic vertebra. The height of the canal keeps constant at the end of thoracic vertebrae to the lumbar area, but because of the increase in width it becomes oval shape (Evans & de Lahunta 2013).

The components inside the vertebral canal, include the epidural space, meninges, cerebrospinal fluid, and the spinal cord.

1.2.2 Epidural Space

The epidural space or *cavum epidurale* is the annular space, inside the vertebral canal, that surrounds the spinal cord, cerebrospinal fluid (CSF), and meninges. The dorsal, ventral, and lateral limits of the epidural space are the same as the vertebral canal, limited at it innermost by the *dura mater* (Evans & de Lahunta 2013). The epidural space separates the dura mater from the periosteum, except in the ventral aspect of the first two cervical vertebrae. The epidural space contains fat tissue and on the floor of the vertebral canal the internal ventral vertebral venous plexus (Evans & de Lahunta 2013).

The epidural fat tissue is present freely in the lateral and dorsal aspect of the vertebral canal, only surrounded by a thin smooth capsule. The fat present ventrally in the canal is attached to adjacent structures such as lumbar and sacral spinal nerves and epidural blood vessels. The epidural fat provides a smooth sheath for the movement of the dural surface within it by filling irregularities of the vertebral canal walls and helps protect the spinal cord against mechanical injury from bony prominences within the canal, especially in the caudal parts of the vertebral column (Ramsey 1959a; Ramsey 1959b).

A vascular network of veins and arteries is also present in the epidural space. The internal ventral vertebral venous plexus or epidural venous vessels, consists of a valveless system that
communicates with vessels from the spinal cord and bones (basivertebral veins) and drain into intervertebral veins that travel with spinal nerves through the intervertebral foramina, before communicating with the azygos and hemiazygos veins and the vena cava (Groen & Ponssen 1991).

The arterial blood supply to the epidural space includes vessels to the meninges and to the spinal cord. For the meninges and the epidural space, branches of the thyrocervical, subclavian, intercostal, lumbar, and sacral arteries enter the space through the intervertebral foramina (Groen & Ponssen 1991). The arterial blood supply of the spinal cord varies according to the region of the spine, the cervical spinal cord is supplied by branches from the posterior inferior cerebellar arteries, and from vertebral arteries that originate from subclavian arteries. The thoracic spinal cord, has branches of the dorsal intercostal arteries, paired with branches of the subclavian artery and thoracic aorta. In the lumbar region, arterial blood supply is from branches of lumbar arteries that originate from the dorsal surface of the abdominal aorta, but not all branches from the aorta reach the spinal cord; some spinal branches divide into dorsal or ventral branches, and in some situations in both, after entering the intervertebral foramen, and they can supply a nerve root or the dura mater, without reaching the spinal cord; some do not penetrate beyond the surrounding arterial system of the spinal cord; and finally some branches supply the spinal cord (Mazensky et al. 2017).

1.2.3 Spinal Cord

Along with the brain, the spinal cord constitutes the central nervous system. The spinal cord is present in most of the length of the vertebral canal at birth canines, originating at the brainstem, passing through the foramen magnum and into the vertebral canal all the way to the sacrum. Following postnatal development, it ends in the caudal lumbar region (two last lumbar vertebrae, sixth or seventh), where the filum terminale or cauda equina originates (Evans & de Lahunta 2013).
During growth, the spinal cord shrinks into the growing vertebral column, which results in the *filum terminale* being present at approximately 1 cm caudal to the fifth lumbar vertebra in large breed dogs and at the lumbosacral vertebral junction in small breed dogs (Valverde 2008; Evans & de Lahunta 2013).

From the spinal cord, dorsal and ventral spinal nerve roots emerge to form the peripheral nervous system. Dorsal roots convey sensory (afferent) input to the spinal cord, whereas the ventral roots carry motors (efferent) output from the spinal cord to muscles and glands (Evans & de Lahunta 2013).

**1.2.3.1 Spinal Cord Membranes**

The spinal cord is covered by three membranes, the meninges, which are fibrous membranes that surround and protect the spinal cord and the brain (Jones 2001). Intimately adhered to the spinal cord and nerve roots of the spinal nerves (forming epineural sheaths) is the first membrane, *pia mater* or leptomeninx (due to its thinness) (Jones 2001; Evans & de Lahunta 2013). The *pia mater* is a highly vascularized membrane, through which blood vessels supply nutrients and oxygen to the brain and spinal cord, externally it is surrounded by CSF, which is present in the subarachnoid space (Jones 2001; Evans & de Lahunta 2013).

The subarachnoid space extends caudally to the caudal segments of the spinal cord to form the lumbar cistern, which contains the CSF. The inner limit of the subarachnoid space is the *pia mater* and its outer limit is the next membrane, the *arachnoid mater*. (Evans & de Lahunta 2013). The terms subarachnoid, spinal, and intrathecal injection are all synonyms that refer to an injection in this space (Bernards & Hill 1992). The *arachnoid mater* is a thin, almost transparent layer, with tight intercellular junctions and a spider-web appearance. It is composed of fibrous tissue and
trabeculae, which extend from the *arachnoid* to the *pia mater* to secure the spinal cord in the CSF and, also has tubular extensions that surround the spinal nerve roots. The outer surface of the *arachnoid mater* maintains a close contact with the inner surface of the *dura mater*, due the pressure of the CSF (Jones 2001). The *arachnoid mater* through its intercellular junctions is the barrier that determines the passage of drugs into the CSF, based on the drug’s lipophilicity (Bernards & Hill 1992; Evans & de Lahunta 2013).

The outer layer of the meninges is the *dura mater* or pachymeninx, which is separated from the periosteum by the epidural space and epidural fat (Jones 2001). The dura mater in human infants is composed by two well defined laminae, however, in adults, the external lamina cannot be identified as a separate layer and is represented by the periosteum of the vertebral canal (Groen & Ponssen 1991). At the skull level, the *dura mater* is closely adhered as a fused double layer, that is separated in the vertebral column. Only its internal lamina is made of fibrous tissue, and surrounds the spinal cord, providing rigidity to help support the blood vessels that supply the spinal cord. The epidural space, formed between the *dura mater* and the periosteum of the vertebral canal, represents an intradural space, because the location between the two dural laminae (Groen & Ponssen 1991). The epidural space has a tubular shape, with lateral tubular extensions to cover the spinal nerve roots as it accompanies the spinal nerve roots to the intervertebral foramen and blends to form a single sheath where ventral and dorsal nerve roots join to form a spinal nerve (Jones 2001). The subarachnoid space and dural sac extend a few centimeters beyond the end of the spinal cord, and the cauda equina, is formed by sacral and caudal spinal roots, that lie caudal to the dural sac (Evans & de Lahunta 2013).

Meningovertebral ligaments provide support and contribute to the structural arrangement of the spinal cord and its membranes within the vertebral canal. At the lumbar region, these ligaments
consist of fibroelastic fibres surrounded by fat lobules and anchor the outer surface of the *dura mater* from the ventral, dorsal and lateral epidural spaces to the osteofibrous walls of the lumbar canal (Geers et al. 2003). The ligaments have an irregular and discontinuous arrangement, and are not present in all vertebrae, which cause partial partitioning of the epidural space, because of the vertebral growth and progressive stretching along axial spinal vessels (Geers et al. 2003). This compartmentalization is suggested as a cause of epidural injection failure because it could cause heterogeneous spread of the injectate and interfere with the introduction of an epidural catheter. The trauma caused to these ligaments during epidural injection or catheter placement, can result in injury of epidural vessels in proximity of the ligament (Geers et al. 2003).

### 1.2.3.2 Spinal Cord Anatomy

The spinal cord consists of white and gray matter. The white matter (*substantia alba*) is the superficial “layer” of the spinal cord and surrounds the gray matter. It is characterized by the presence of packed myelinated axons, although nonmyelinated axons are also present. The white matter can be further divided in three parts for each half of the white matter. Medial to the dorsolateral sulcus, where dorsal rootlets enter the spinal cord, it is called the dorsal funiculus. Medial to the where the ventral rootlets exit from the spinal cord, is the ventral funiculus. Lastly, the part located in between dorsal and ventral root attachments is the lateral funiculus (Evans & de Lahunta 2013). Some myelinated axons cross from one half to the other of the spinal cord, this constitutes the white commissure, ventral to the gray commissure and connect right and left ventral funiculi (Evans & de Lahunta 2013).

The gray matter (*substantia grisea*) forms the core of the spinal cord around the central canal (*canalis centralis*), located in the center of the spinal cord, which is filled with CSF and lined by
ependymal cells. This canal is slightly enlarged at the caudal aspect of the spinal cord, giving origin to the *ventriculus terminalis* (Evans & de Lahunta 2013). The gray matter is rich in capillary supply, composed of cell bodies and processes of neurons and glial cells, and only sparse myelinated axons (Evans & de Lahunta 2013).

The gray matter, when observed in a transverse section of the spinal cord, has a butterfly shape, with bilateral “wings”, also called lateral intermediate substance, connected across the midline by the central intermediate substance. The central intermediate substance surrounds the central canal. The lateral intermediate substance, project into adjacent white matter as lateral horns that project dorsally and ventrally into dorsal and ventral horns, respectively (Evans & de Lahunta 2013).

The spinal cord includes two areas where its relative diameter increases. The cervical enlargement occurs from C₆ to T₁ to give origin to the brachial plexus and innervation of the thoracic limb. The lumbar or lumbosacral enlargement occurs from L₅ up to S₁ for the innervation of the pelvic cavity and pelvic limbs (Fletcher & Kitchell 1966). Caudal to the lumbar enlargement, the spinal cord tapers into a cone shape, also known as conus medullaris, where segments from S₂ and S₃ are present. The terminal filament, is the part of the spinal cord where it is reduced to a uniform strand of glial and ependymal cells, encased on a layer of *pia mater*, this filament extends caudally as the cauda equina to attach to sacral and caudal vertebrae (Fletcher & Kitchell 1966).

The roots originating from the spinal canal are mainly divided in dorsal and ventral roots, before forming the spinal nerve and further divisions (Evans & de Lahunta 2013). The spinal cord segments, or spinal nerves are identified numerically; dogs have: 8 cervical, 13 thoracic, 7 lumbar, 5 sacral, and several caudal nerves (Evans & de Lahunta 2013).
The dorsal and ventral root fibres of the spinal cord consist of thousands of axons, with different amounts of Schwann cell myelin, and enveloped by the meninges. The axons of each root are bound laterally, where dorsal and ventral roots form the spinal nerves. When approaching the spinal cord, these axons regroup into separate bundles, also called rootlets. On the cervical region, in addition to dorsal and ventral roots, the eight cervical segments, have rootlets that emerge midlaterally from the spinal cord to form the spinal root of the accessory nerve.

The dorsal root fibres are primary afferent axons, while the ventral root fibres are efferent. The afferent roots have mainly sensorial function, and include temperature, touch, noxious stimuli, at the somatic level; organ content, distention, chemicals at the visceral level; and muscle and joint movement at the proprioception level. The efferent roots have mainly motor function, with the somatic action on striated skeletal muscle, but also visceral components on smooth muscle, cardiac muscled, glands (mainly by sympathetic innervation) (Evans & de Lahunta 2013).

The nerve fibres can be further classified, based on the presence of myelin, their diameter and conduction velocity. The A-α fibres, are myelinated, with a large diameter of 15 to 20 µM and fast conduction speed of 30 to 120 (meters per second; m second⁻¹); these fibres are located in afferent and efferent nerves of muscles and joints, and their main function is related to motor and proprioception. The A-β fibres, are also myelinated, slightly smaller (5–15 µM) and slower conduction speed of 30–70 m second⁻¹; they are present in efferent roots to muscle nerves and afferent sensory nerves and participate in motor and sensory (touch and pressure) function. The A-γ fibres, are myelinated, have a diameter of 3–6 µM and a conduction speed of 15–35 m second⁻¹; they are located in efferent nerves to muscle spindles to provide muscle tone. The A-δ fibres are the smaller (2–5 µM) and slower (5–25 m second⁻¹) of the A fibres and are located in afferent sensory nerves, responsible for pain (fast), touch and temperature. The B fibres are myelinated,
part of the sympathetic nerves, small (1–3 µM) and slow (3–15 m second⁻¹); they are present on preganglionic sympathetic nerve fibres for autonomic function. The C fibres are small (0.4–1.5 µM), the only types of fibre that is not myelinated and have the slowest conduction of all the nerve fibre types (0.7–1.3 m second⁻¹); they are located in postganglionic sympathetic nerve fibres to participate in autonomic functions, pain (slow) and temperature (Liu & Joseph 2006; Skarda & Tranquilli 2007).

The autonomic nervous system involves sympathetic nerves that originate between segments T1 and L2, and receive rami from the sympathetic chain, before reaching the tissue or organ (Guyton 1991; Wilson-Pauwels et al. 1997) and this location makes them susceptible to the effects of epidural injections. Sympathetic nerve fibres are composed of two neurons (a preganglionic and a postganglionic), the cell body of the preganglionic lies in the intermediolateral horn of the spinal cord, and the fibres pass through the ventral spinal root of the corresponding spinal nerve (Guyton 1991; Wilson-Pauwels et al. 1997).

The parasympathetic nerve fibres, leave the CNS through cranial nerves (III, VII, IX and X), and second and third sacral spinal nerves (sometimes also first and fourth sacral nerve). Most of the parasympathetic activity is mediated through the vagus nerve (X) (Guyton 1991; Wilson-Pauwels et al. 1997), therefore the importance of the parasympathetic nerve fibres during epidural blocks is limited but can still affect the sacral segments.

1.2.4 Cerebrospinal Fluid

The cerebrospinal fluid (CSF) or *liquor cerebrospinalis*, is a clear, colorless liquid that circulates through the ventricular system and the subarachnoid space at the brain and descends into the spinal cord level (Cook & DeNicola 1988). The CSF is the product of ultrafiltration of plasma
and active transport mechanisms across its semipermeable membrane (Cook & DeNicola 1988; Di Terlizzi & Platt 2006). In general, the CSF is almost acellular, normally there are no erythrocytes (Bailey & Higgins 1985) and a few small lymphocytes (Cook & DeNicola 1988). It is a liquid with low protein concentration, ranging from 10 to 40 mg dL\(^{-1}\), compared to serum proteins at 5 to 7 g dL\(^{-1}\) (Crone 1965). Other components of the CSF include some electrolytes and glucose, and when compared to plasma, the levels of chloride, sodium and magnesium are slightly higher, and the levels of potassium, calcium and glucose are slightly lower (Cook & DeNicola 1988). Other enzymes, neurotransmitters and substances that can be present in the CSF include creatine kinase, aspartate transaminase, lactate dehydrogenase, lactate, glutamate, pyruvate, and Gamma aminobutyric acid (Di Terlizzi & Platt 2006).

The CSF physically suspends the brain and protects it from injury, it modulates normal variations in the intracranial pressure, provides a stable, closely controlled and chemically responsive ionic environment to the brain parenchyma. Its pH has direct effect on brain function, has antibacterial properties and contains antibodies, and is a medium for transport and exchange of different substances between extracellular fluid of nervous tissue and \textit{pia mater} or ependyma, acting similar to a lymphatic drainage of the central nervous system (Cook & DeNicola 1988; Di Terlizzi & Platt 2006; Evans & de Lahunta 2013).

In dogs, the CSF production rate is approximately 0.05 mL min\(^{-1}\) or 3 mL h\(^{-1}\) (Evans & de Lahunta 2013). The rate of formation changes among species, and size of the animal, it is also related to the weight of the choroid plexus, and to the rate of sodium and bicarbonate ion exchange (Di Terlizzi & Platt 2006). Changes in blood pressure or CSF pressure do not impact the CSF formation rate, but acute changes in plasma osmolality have a direct and linear relationship with
CSF production, hypoosmolality increases CSF production and hyperosmolality decreases it (Di Terlizzi & Platt 2006).

1.3 EPIDURAL DRUGS

A wide variety of drugs have been administered by the epidural route. Drugs with actions on different receptors, through agonism or antagonism, can act on receptors, channels and/or ions, located in the spinal cord and the nerves that emerge from it. These drugs have included NMDA antagonists, alpha-2-agonists, opioids, benzodiazepines, NK-1 antagonists, cholinergic agonists, nonsteroidal anti-inflammatory drugs, and especially local anesthetics. Each drug may be used for different reasons, but ultimately analgesia is the goal. Local anesthetics are the most common epidural drugs, and their use and importance are described in the following sections.

1.3.1 Distribution of Drugs in the Epidural Space

The uptake of drugs within the epidural space is subject to several mechanisms: vascular absorption, leakage through the intervertebral foramen, epidural fat uptake and diffusion through the spinal cord’s arachnoid mater (Bromage 1967). The extent to which these routes affect the different drugs, depends on the physicochemical properties of the drug (Valverde 2008). For local anesthetics it is believed that the most important site of action is due to the dural cuffs surrounding the nerve roots (Bromage 1967). The local anesthetic penetrates the ascending and descending nerve axons, and blocks sodium and potassium channels (Bradley et al. 1980). The absorption of drugs by the epidural vessels can be affected by potential transmission of intra-abdominal and intrathoracic pressure to this vascular network.
One of the physicochemical properties that can affect the distribution and uptake of the drug, is its lipophilicity. It can enhance systemic absorption, sequestration to the fat tissue, and free movement between epidural and intrathecal space. On the other hand, hydrophilicity has the opposite effects. The lipophilicity implies that drugs administered epidurally can have spinal and systemic or supraspinal effects. Clinically, lipophilic drugs, have marked systemic effects, and limited rostral spread due to fast vascular absorption and fat trapping. In addition, the dose for epidural administration is similar to the systemic dose and the duration of action is similar for both routes (Yaksh et al. 1990; Bernards et al. 2003). The opposite happens with hydrophilic drugs, a lower epidural dose is required compared to systemic dose, which limits its systemic effects, but allows a better rostral spread and a more prolonged effect due to its longer presence within the spinal cord (Gourlay et al. 1987).

The spinal absorption of drugs is the result of their movement across the meninges, from the epidural space (Valverde 2008). The knowledge of the specific gravity of the CSF is important for intrathecal injections since it would contribute to the distribution of the drug within the CSF but is not important for the epidural route (Valverde 2008). In dogs, the CSF specific gravity ranges from 1.005 and 1.017 (Mosing et al. 2006). For epidurals, isobaric or hypobaric solutions are generally used (Valverde 2008).

In general, the concentration of the local anesthetic used for an epidural injection needs to be higher, than what would be used within a nerve to cause blockade, to counteract the routes of uptake from the epidural space (Valverde 2008). The drugs commercially available are lipophilic and have similar permeability coefficients, which allows to be readily absorbed systemically (Bernards & Hill 1992), and even that the epidural doses are similar to systemic dose, they are less
likely to produce supraspinal analgesia, with the exception of lidocaine that causes systemic analgesia (Valverde et al. 2004).

The duration of action of these drugs is related to the protein binding of the drug at the receptor (sodium channel). Local anesthetic drugs with lower protein binding have shorter duration (e.g., lidocaine and mepivacaine), in contrast to bupivacaine with a higher protein binding and longer duration of action. The onset of action is related to the constant of dissociation of the drug, and the closer to the physiologic pH, the faster the onset (Valverde 2008).

1.3.2 Local Anesthetics

1.3.2.1 Physiology of the Sodium Channel

Sodium voltage gated channels (Navs) transmit rapid depolarizing impulses throughout cells, and are present in the nervous system, skeletal muscle, and heart cells of mammals (Marban et al. 1998). The Na⁺ channels consist of various subunits; the α subunit is the most relevant for the channel’s function, with a modular architecture, composed by four homologous domains (I – IV), each containing six transmembrane (S1 to S6) segments, bearing resemblance to the α subunit of a voltage-dependent K⁺ channel. The domains create a central pore, which have a similar structure to a Ca²⁺ channel. Between the subunits five and six there is a structure called the pore-lining or P segment (Marban et al. 1998). A smaller auxiliary β-subunit influences the activation-inactivation states of the channel (Catterall 2000), which determines if a local anesthetic can or cannot have an action on the channel. Local anesthetics, antiarrhythmic and anticonvulsant binding site are located at the S6 of the IV domain in the α subunit, (Catterall 2000), within the pore, and accessible from the intracellular side only (Narahashi & Frazier 1971).
The channels are a gated conduit of Na⁺ ions, and exist in three different states, resting (closed), open and inactivated. The generation and propagation of an action potential is dependent on the activation of the channels. During the resting membrane potential, the channels are predominantly in the closed state. During depolarization, the channel opens when the membrane potential increases from -60 mV to above -55 mV to allow an influx of Na⁺ ions through the channel pore, resulting in further depolarization of the cell membrane and channel activation (positive feedback loop) (Catterall 2000; Wann 1993). The membrane potential increase is based on the amount of Na⁺ influx and can reach 40 mV, which is close to the Na⁺ equilibrium potential (Ramahi & Ruff 2014). The activation period is very short lived and after a few milliseconds the depolarization triggers the channel to become inactivated. After some delay, K⁺ channels become activated, leading to efflux of K⁺ ions, which restores the resting membrane potential to -60 mV, and the Na⁺ closes spontaneously, reverting to the resting closed state. (Catterall 2000; Wann 1993). During the repolarization, the channels are inactivated, and cannot be activated until return to resting state, this period is called refractory period of the cell (Butterworth & Strichartz 1990; Wann 1993).

Besides a common general functional mechanism, there are nine different Navs, which are nominated from Nav1.1 to Nav1.9 (Goodwin & McMahon 2021). Not all Navs are found in neurons. For example, the Nav1.4 is found in the skeletal muscle cells, and the Nav1.5 in cardiac cells, therefore are not important for action potential conduction and firing in neurons (Devor 2006; Goodwin & McMahon 2021). From the remaining channels, the ones that are mainly correlated with pain are the Navs1.1, 1.6, 1.7, 1.8 and 1.9 (Goodwin & McMahon 2021). The Nav1.2 is found in the CNS and dorsal root ganglia (DRG), but its effects are mainly correlated to excitatory neurons, increase in its activity is a cause of seizure and loss of its function is related to autism in
humans or seizure as well (Ogiwara et al. 2018). Nav1.3 can be found in the CNS, but its expression occurs during embryonic development or after nerve injury (Waxman et al. 1994). Regarding the location, Navs 1.1, 1.6 and 1.7 can be found in the CNS cells, 1.1, 1.6 to 1.9, in the DRG cells, 1.7 can also be found in glia cells, and within the DRG and peripheral nervous system 1.8 and 1.9 are found in small diameter neurons (Devor 2006; Goodwin & McMahon 2021).

Other important differences in neurons, when compared to other excitable cells, is how the membrane resting potential can be restored, for that cell to be susceptible to be excited again. Each cell type has different ion-specific membrane permeabilities. Neurons in general have the greatest permeability to K⁺, due to the activity of several and distinct potassium channels. Skeletal muscle cells, also have permeability to K⁺, however, the channel-mediated permeability to Cl⁻ exceed the K⁺, and therefore is the most important electrolyte for the resting potential in that cell. Analogous to cardiac cells where the Ca²⁺ is of importance for it resting membrane potential (Wright 2004).

1.3.1.2 Mechanism of Action of Local Anesthetics in the Epidural Space

The main mechanism of action of the local anesthetics in the epidural space is due to its high binding affinity for the Na⁺ channel in the open and/or inactivated states, and a low affinity in the resting state (Wann 1993; Fozzard et al. 2005). The common binding site for local anesthetics in the Navs, is in the pore lining of the S6, from the DIV, on the center of the pore. To access the binding site, the drug can enter by the pore on its open state, when drug is on it charged form, or cross the lipid membrane of the neurons, when on its uncharged form, turn into ionic form in the intracellular space, and them bind within the pore (Narahashi & Frazier 1971; Butterworth & Strichartz 1990; Catterall et al. 2019). The blockade is going to be reversible, and its duration is based on drug properties.
These two pathways to the binding site, are possible due to the physicochemical characteristics of these drugs. Local anesthetics contain hydrophilic and hydrophobic domains that are separated by an intermediate linkage containing an amide or an ester group. When in solution, at equilibrium, local anesthetics exist in their ionized or charged form that is water-soluble, and the non-ionized or uncharged form that is lipid soluble, with the first one being the active form of the drug (Narahashi & Frazier 1971; Butterworth & Strichartz 1990). The amount of each form of the drug is determined by the pKa, that is the pH at which both fractions of the drug are present in a 50:50 ratio. The commonly used local anesthetics pKa range from 7.72 to 9.06 (weak bases), so at physiological pH the drug with the lower pKa will have the higher fraction of uncharged drug, and therefore more lipid soluble, when compared to a drug with a highest pKa. (Strichartz et al. 1990).

At the epidural space, local anesthetics inhibit other ions channels besides the sodium, such as potassium and calcium, at the level of the dorsal horn of the spinal cord (Olschewski et al. 2002; Ku & Schneider 2011). These actions affect the central neuroprocessing of sensory information, contributing to antinociceptive effects (Olschewski et al. 2002; Ku & Schneider 2011). The complete understanding of the K+ channel functions are still not clear and can be diverse but include the modulation of complex firing patterns in peripheral axons and spinal neurons, therefore the potential blockade of this ion channels by local anesthetics can influence sensory inputs or their processing in the spinal cord (Olschewski et al. 2002). The nociceptive transmission at the dorsal horn is mediated by other neurotransmitters such as tachykinins (substance P), and local anesthetics can inhibit substance P binding and evoked increase intracellular calcium (Li et al. 1995). An extra mechanism is the inhibition of the glutamatergic transmission in the spinal dorsal
horn neurons, reducing N-methyl-D-aspartate and neurokinin-mediated postsynaptic depolarization (Nagy & Woolf 1996; Furutani et al. 2010).

1.3.1.1 Effects

Local anesthetics block the action potential in different types of nerve fibres, which can result in a differential block (Gasser & Erlanger 1929). The block includes initial vasodilation, followed by loss of sensation of temperature, sharp pain and light touch, and at the last stage loss of motor activity (Nathan 1976). Factors that can change this effect include type of fibre (size and myelination), frequency of stimulation, length of the nerve exposed to the local and choice and concentration of the local anesthetic drug (Garcia 2015). Local anesthetics can block all different nerve fibres in the following sequence: B; C and A-δ; A-γ; A-β; and A-α. Clinically autonomic function is lost prior to the blockade of nerve fibres associated with pain sensation and motor function (Liu & Joseph 2006; Skarda & Tranquilli 2007).

The spread of the block depends on the dermatomes of the spinal cord that are reached by the local anesthetic. For a pelvic limb, the local anesthetic should reach nerve roots from L3 to S1 (Lumbosacral plexus) (Otero & Campoy 2013); for analgesia/anesthesia of the abdominal wall and peritoneum, should reach nerve roots from T11 to L3 (Evans & Miller 1993).

The rostral spread of the local anesthetic is affected by leakage through the intervertebral foramen, especially for a LS injection and the presence of the lateral sacral foramina; therefore, higher volumes may be needed, when compared to other sites of injection (Burn et al. 1973; Park et al. 1980). Also, the spread of the injectate tends to be predominantly cranial when the administration occurs cranial to the LS space (Vas et al. 2003; Freire et al. 2010), whereas a LS injection can spread both cranially and caudally (Otero & Campoy 2013). Other factors include
the volume and concentration, speed and pressure during injection, site of injection, direction of the needle bevel, position of the animal, size and permeability of the intervertebral foramen, amount of fat in the epidural space, size of the associated venous and lymphatic plexus, age and physical condition, baricity and specific gravity of the injected solution, lipophilicity and hydrophilicity of the drug used, meningovertebral ligaments and compartmentalization, increased epidural space pressure, and leakage (Valverde 2008; Otero & Campoy 2013).

1.3.2 Other Drugs

After local anesthetic, opioids are the most common drug used in epidural injections. The spinal cord has high concentration of µ, κ and δ opioid receptors at the dorsal horn (Yaksh 1984). When these receptors bind with opioids, there is presynaptic inhibition of substance P release from the C nociceptive fibres, and in a smaller extent, A- δ fibres (Yaksh 1984). Opioids do not block A-β or B-fibres, and the blockade of A-α is described, but motor weakness is rarely seen (Valverde 2008).

The pharmacological property that helps to explain the duration of the opioid in the epidural space is the lipophilicity of the drug. The higher the lipophilicity, the faster the onset, but the duration of action and dose required is similar to a systemic dose. The descending order of lipophilicity for opioids is: fentanyl and sufentanil; butorphanol, oxymorphone, hydromorphone, buprenorphine and methadone; morphine. Hydrophilic opioids, have a slower onset of action but a longer duration, with a lower dose required than for systemic use (Valverde 2008). The rostral spread of the drugs depends on the lipophilicity, the higher the lipophilicity the lower the rostral spread, due to vascular absorption and sequestration by fat, whereas hydrophilic drugs have an extensive rostral spread due to the prolonged presence in the CSF, facilitating the action on dermatomes cranial to the injection site (Valverde 2008).
Different studies in dogs with different drug combinations that included epidural opioids have demonstrated the analgesic effects of opioids. Lipophilic epidural opioids, such as fentanyl and oxymorphone, resulted in cardiovascular depression, including a decrease in heart rate, arterial blood pressure, stroke volume and cardiac output, and an increase in PaCO₂ (Duke et al. 1994; Torske et al. 1999; Naganobu et al. 2004), which were not observed with morphine; conversely, the decrease in inhalant end-tidal requirements (Minimum alveolar concentration; MAC) resulted in better arterial blood pressures (Valverde et al. 1991; Naganobu et al. 2004). Other beneficial effect of epidural morphine includes superior gut motility after abdominal surgery in dogs (Nakayoshi et al. 2007).

Adverse effects from epidural opioids reported in human and veterinary medicine include pruritus, urinary retention, nausea and vomiting, respiratory depression, and delayed hair growth at the LS intervertebral space (Valverde 2008).

Another class of drugs commonly used in epidural injections are the α2-agonists, which act through presynaptic binding sites on afferent nerves and postsynaptic action on the dorsal horn and result in similar actions to opioids on the inhibition of the release of substance P from C fibres (Valverde 2008). The clinical effects of α2-agonists coexist with systemic effects, including sedation, analgesia (supraspinal), cardiovascular effects (bradycardia, vasoconstriction), and vomiting (Valverde 2008). Xylazine is different than other drugs from this group because it can also block A-δ and A-α fibres, which can result in better analgesia, but also motor blockade, although B sympathetic fibres are not affected (Valverde 2008).

Clinically, a reduction in MAC and an increase in electrical and thermal thresholds was observed in dogs and horses, with the use of xylazine (Greene et al. 1995; Keegan et al. 1995;
Soares et al. 2004). Also, the potential of α2-agonists to increase the duration of epidural opioid analgesia has been documented (Branson et al. 1993).

Another receptor present in the spinal cord is the N-methyl-D-aspartate (NMDA), which inhibits the excitatory action of glutamate receptors and reduces sensitization and nociception. Ketamine antagonizes this receptor (Lizarraga et al. 2006) and has resulted in variable times and degree of analgesia/anesthesia (5 to 720 minutes), when administered epidurally, depending on the type of stimulus used (Amarpal et al. 2003; Duque et al. 2004; Acosta et al. 2005; Hamilton et al. 2005). This wide variability and systemic effects due to a high dose requirement have prevented it routine use for epidural administration (Valverde 2008).

Benzodiazepines, such as midazolam, and NSAIDs have being used and studied in different veterinary species, but the lack of advantages compared to local anesthetics, opioids and α2-agonists in the epidural space or the same drugs used in systemic routes, makes them less used in the clinical settings (Valverde 2008).

1.4 EPIDURAL INJECTION

1.4.1 Lumbosacral Technique

The LS space is the most common site of access to the epidural space in small animals, because it has the larger intervertebral space (Valverde 2008) and the dural sac tappers off before this space (Bradley et al. 1980).

To perform a lumbosacral epidural injection, the animal is positioned in sternal or lateral recumbency, the area over the LS space should be clipped and aseptically prepared. The LS space is located by palpation with the non-dominant hand, using the thumb and middle finger, to feel the anterior aspect of both iliac crests. The index finger is then used to palpate the spinous process of
the vertebrae and identify the LS space. Tracing an imaginary line between the iliac crests, coincides with the location of the L₆-L₇ space and from this point the index finger is directed caudally to fall into the L₇-S₁ space, at the midline of the patient (Valverde 2008). In cadavers, positioned in sternal recumbency with the pelvic limbs extended caudally, dissection of the LS space shows the diameter to be relatively small (2–4 mm) (Valverde 2008); however, CT images of live dogs positioned in this same fashion showed the diameter to be 4 ± 1 mm in dogs ≤ 10 kg, 6 ± 1 mm in dogs 15 – 20 kg, and 8 mm ± 3 mm in dogs ≥ 25 kg, and those measurements increased when the pelvic limbs were directed cranially to 8 ± 1 mm, 11 ± 1 mm, and 14 ± 2 mm, respectively (Concetto et al. 2012). These differences are probably related to the presence of tissue (e.g., fascia, ligaments, insertion points) in the cadaver study versus clean edges in the imaging study (Valverde 2008; Concetto et al. 2012). Because of these differences in diameter between both positions, some authors prefer the pelvic limbs rostral (Jones 2001; Concetto et al. 2012), although others prefer them caudal to maintain a parallel plane of the dog on the surface and facilitate the rostral spread of the injectate without the incline elicit by having the pelvic limbs cranially (Valverde 2008).

After the space is localized, still using the index finger as a guide for the midline and the space, under sterile conditions, a needle is introduced perpendicular to the skin. A spinal needle is ideal for epidural injections because of the presence of a stylet that protects the lumen of the needle until it is properly placed. The needle is held with the bevel facing forward and the angle of insertion of the needle can be adjusted to optimize its direction into the epidural space. As the needle advances, a “pop” sensation can sometimes be felt (not always) when the needle pierces the flavum ligament. At this point, the needle is further advanced into the epidural space (Valverde 2008; Otero & Campoy 2013; Campoy et al. 2015). In dogs, it is possible to advance the needle
all the way to the floor of the epidural space, and then withdrawn 1 to 2 mm, this would ensure that needle is in the epidural space (Valverde 2008).

Correct placement of the needle can be confirmed in several ways. The lack of resistance upon injection of air is commonly performed using a glass syringe lubricated with saline or a low-resistance plastic syringe, both of which offer minimal friction and are preferred over a regular plastic syringe. Only small volumes are needed (0.25–2 mL, depending on the dog’s size) since large volumes can compromise the spread of the solution, cause uneven cranial distribution, and also cause occasional compression of the spinal cord (Iseri et al. 2010). The technique consists of gentle injection of the volume of air without any resistance, which confirms correct positioning. Any resistance is indicative of malposition (Valverde 2008).

Other techniques include the hanging drop technique; for which once the spinal needle has advanced through the skin, the stylet is removed and the hub of the needle hub is filled with saline, the needle is slowly advanced into the epidural space until the saline is suctioned into the space by the subatmospheric pressure of the epidural space (Otero & Campoy 2013; Campoy et al. 2015). This technique is only effective with the animal in sternal position, so gravity can contribute to the subatmospheric pressure (Naganobu & Hagio 2007; Otero & Campoy 2013; Campoy et al. 2015). Positive epidural pressures have also been reported and one study provided a range from -6 to +15 mmHg (Iff et al. 2007). False negatives can occur with dogs in lateral recumbency but also in sternal recumbency (Naganobu & Hagio 2007). Factors that were described to influence the success of this technique are the gravity force, subatmospheric pressure within the epidural space, recoil of the flavum ligament when pierced by a blunt needle, and filling the needle bore with foreign material (blood clots, fat, periosteum, and skin) (Campoy et al. 2015).
An alternative method initially described in humans consists of the use of a constant fluid infusion or running drip that can be used with the patient in lateral or prone position. For this method, a Tuohy needle is advanced towards the epidural space, the stylet removed, and the needle connected to a three-way stopcock, a fluid set and bag of saline solution. Once the needle reaches the epidural space, there is an increase in the dripping rate (Baraka 1972). This method has also been described in dogs in lateral recumbency and allowed for lower number of attempts than the hanging drop technique (Martinez-Tabloada & Redondo 2017); however, it seems less practical.

Electrolocation has been used to identify the epidural space, as twitches of the pelvic limbs and tail can be observed when the needle is in the epidural space in proximity of the spinal cord (Read 2005; Garcia-Pereira et al. 2010). The minimal electric threshold in dogs is set at 0.3 mA, and a greater sensitivity and specificity was observed when compared to the lack of resistance technique (Garcia-Pereira 2010; Garcia-Pereira 2018), but it does not rule out an intrathecal positioning; therefore, the need to inspect for the presence of CSF or blood prior to injection of the local anesthetic (Campoy et al. 2015).

Other sophisticated technique includes identifying extradural pressure waves in synchrony with the heart rate, which consists of connecting a sterile, fluid-filled, non-distensible pressure line to a Tuohy needle without stylet and connected to a pressure transducer and multiparametric monitor. The transducer is zeroed at the level of the transverse process of the last lumbar vertebra and the wave scale adjusted to be able to observe positive and pulsatile waves, indicative of the needle being in the epidural space (Adami et al. 2013; Coccoluto et al. 2021). This technique has 100% specificity and 89% sensitivity; however, the absence of a pressure wave does not have a good negative predictive value (Adami et al. 2013).
Ultrasound-guided techniques can facilitate the localization of the epidural space, especially in obese dogs, using an in-plane parasagittal technique that allows for identification of anatomical structures and visualization of the spinal needle in the epidural space (Iff & Moens 2010; Liotta et al. 2015; Silva et al. 2020).

1.4.2 Spinal Needle

Spinal needles have a removable, close-fitting stylet, ideal for epidural injections (Valverde 2008; Campoy et al. 2015). Their tip is blunter than hypodermic needles, allowing safer penetration of the needle in different tissues, and the stylet prevents obstruction of the lumen during placement (Campoy et al. 2015). These needles are for single injection and available in different sizes and lengths. Clear needle hubs are helpful to allow the visualization of blood or CSF from the puncture (Campoy et al. 2015).

1.4.3 Epidural Catheter

An epidural catheter can be placed in the epidural space, to allow a more cranial blockade, or to perform continuous or repetitive administration of epidural drugs. The preferred position for the dog is in sternal recumbency.

The technique is the same as for a standard epidural injection; however, a Tuohy needle is used instead of a spinal needle. The bevel of the Tuohy needle is specially designed with a curvature and angle that facilitates the passage of a catheter through it. The bevel is placed to face forward for the advancement of the catheter. Once in the epidural space, correct placement is verified by one of the described techniques (e.g., lack of resistance or hanging drop technique), the Tuohy needle should be held with the non-dominant hand, while the dominant hand advances the
catheter through the needle into the epidural space. Catheters have length markings that allow for knowledge of how far the catheter is advanced within the epidural space (Martin-Flores 2019). In most cases, the catheter is advanced no more than 5 cm to avoid the risk of catheter curling or trauma of the spinal cord. If resistance is encountered during advancement, then the needle can be angled to rule out that the catheter is rubbing on the bevel of the needle; if the resistance persists, the catheter may have reached a narrow point in the canal and is rubbing on the spinal cord, so it should not be forced further cranial. Once in place, the needle is gently removed without dragging the catheter out with it. After confirmation of no presence of CSF or blood, the catheter connector and bacterial filter are connected, and the catheter flushed with 0.1-0.2 mL of preservative free saline (Campoy et al. 2015).

The catheter allows the use of continuous delivered epidural or multiple injections. Complications are similar to those of the epidural injection and include accidental drug administration, neural damage/neurotoxicity, infections, inadvertent subarachnoid/spinal or subdural injection, inadvertent intravascular injection, hypotension, bradycardia, Horner’s syndrome, respiratory depression, total spinal anesthesia, and urinary retention (Otero & Campoy 2013; Campoy et al. 2015).

An advantage of the epidural catheter is the possibility to reduce the volume/dose, if positioned closer to the spinal root desired, or with repeated use (Martin-Flores 2019). In addition, they can be left in place for days to weeks when aseptic technique is applied.

1.5 EPIDURAL DOSE AND VOLUME

There are variations in the dose, volume, and concentration of local anesthetic used for epidural injections. There are two main methods to calculate the volume of the epidural injectate.
1) based on body weight and 2) based on the length of the vertebral column from the occipital crest to the sacrococcygeal space (Strande 1968; Johnson et al. 1996; Gorgi et al. 2006; Iseri et al. 2010; Freire et al. 2010; Son et al. 2011; Vesovski et al. 2019; Valverde & Skelding 2019). Most published studies have used the former approach and it is not yet known if both methods are interchangeable. A recent study compared both methods of calculating epidural volume in a mathematical model and determined that small and medium size dogs (< 25 kg) would receive larger calculated volumes based on length than weight and this difference tends to disappear or revert as size increases (> 25 kg). In that study, the calculated epidural volume was also influenced by the body condition score (BCS) of the dog, since as BCS increases (obesity) a smaller volume is required when calculated using length compared to weight (Valverde & Skelding 2019).

The rostral spread of volume has been assessed in cadaver models and live animals and can be determined by injecting dye or contrast medium, and later assessed by imaging (radiographs, computer tomography [CT]) or necropsy (Strande 1968; Johnson et al. 1996; Gorgi et al. 2006; Iseri et al. 2010; Freire et al. 2010; Son et al. 2011; Kawalilak et al. 2015; Vesovski et al. 2019). It has also been assessed clinically by injection of local anesthetics and determination of the extent of blockade by applying noxious stimuli to corresponding dermatomes (Freire et al. 2010; Leite et al. 2017). When comparing similar studies using different methods of evaluation (imaging, dye or clinical) it appears that there is a more extensive rostral spread for the imaging and dye methods than what would be seen clinically for the number of dermatomes blocked, so care should be taken when extrapolating data from different studies.

A linear relationship exists between the volume of injectate and level of rostral spread (Strande 1968; Johnson et al. 1996; Freire et al. 2010; Vesovski et al. 2019). To exert its analgesic/anesthetic effects, the drug(s) injected in the epidural space spreads rostrally and
interacts with receptors and sensory fibres of the spinal cord and emerging spinal nerves, and the magnitude of effect is related to the dermatomes innervated by those spinal nerves. Therefore, the volume of injectate can be adjusted to the area of blockade desired, which results in smaller volumes for the pelvis and pelvic limbs than for the abdomen (Bromage 1962; Strande 1968; Vesovski et al. 2019), due to the linear relationship between volume and rostral spread.

The degree of spread of a specific volume of injectate is influenced by two physical factors, gravity and friction (Valverde & Skelding 2019), which in turn are also influenced by inherent factors of the patient, such as BCS, weight, size, and length of the vertebral column, body position of the patient at the time of injection and thereafter, whether the patient is conscious or under general anesthesia or heavy sedation, elapsed time from injection to assessment, site of injection and equipment used (spinal needle versus epidural catheter), local anatomy of the vertebral column (fat and normal or not anatomy of the intravertebral space), and imaging technique used to assess the rostral spread (Strande 1968; Gorgi et al. 2006; Iseri et al. 2010; Freire et al. 2010; Son et al. 2011; Vesovski et al. 2019; Valverde & Skelding 2019).

It is important to prevent excessive rostral spread of local anesthetics that may reach high dermatomes of the spinal cord (cervicothoracic) and interfere with diaphragmatic function and breathing or cause excessive sympathetic blockade. Therefore, adequate knowledge of both methods of calculation for epidural doses should be established. The hypothetical study (Valverde & Skelding 2019) showed that mathematically both techniques are not always interchangeable and that in small and medium size dogs they can result in extreme opposites and could result in inadequate anesthesia/analgesia or overdosing.
1.5.1 Epidural Dose Based on Weight

When the volume was calculated by mL kg\(^{-1}\), different volumes with different local anesthetics and other drugs have been reported in dogs. Volumes of injectate of local anesthetic, contrast medium, or dye have ranged between 0.1–0.8 mL kg\(^{-1}\) (Gorgi et al. 2006; Freire et al. 2010; Son et al. 2011; Kawalilak et al. 2015; Vesovski et al. 2019). At 0.1 mL kg\(^{-1}\), rostral spread was observed from 0 to 3 vertebral bodies in Greyhound cadavers (Vesovski et al. 2019). At 0.2 mL kg\(^{-1}\), rostral spread reaches the thoracolumbar area, which corresponds to 6 to 10 vertebral bodies (T\(_{10}\)-L\(_2\)) (Freire et al. 2010; Son et al. 2011; Kawalilak et al. 2015; Vesovski et al. 2019) and as high as 12–21 vertebral bodies (C\(_7\)-T\(_9\)) (Iseri et al. 2010; Son et al. 2011). Differences in the degree of spread are related to the factors mentioned above that interfere with the behaviour of the injectate in the epidural space due to gravity and friction. For example, 0.2 mL kg\(^{-1}\) of bupivacaine combined with methylene blue demonstrated clinical signs of blockade up to L\(_1\), although the spread of dye was up to T\(_3\) ± 7 vertebrae, after necropsy in the same dogs (Freire et al. 2010). Larger volumes of 0.4 to 0.8 mL kg\(^{-1}\) of methylene blue spread rostrally up to 22 and 26 vertebral bodies (C\(_2\)–C\(_6\)) in a dose-related fashion at necropsy (Freire et al. 2010), whereas for the same dose and assessed by CT rostral spread reached to T\(_{12}\)–T\(_1\), also in a dose-related fashion (Vesovski et al. 2019). Therefore, it appears that dye can be recognized more rostral at necropsy than contrast medium during imaging, but the implication of this for the effectiveness of the clinical block has not yet been determined.

A study with 20 mongrel female dogs, weighting around 9.9 kg, with a vertebral column length close to 53.4 cm, used a solution of 0.25% bupivacaine and 0.5% methylene blue, at volumes of 0.2-, 0.4-, 0.6- and 0.8-mL kg\(^{-1}\) (5 animals per group). The epidural was performed through an epidural catheter (5 cm inside the lumbar-sacral space), with the animals awake and
standing. The solution was administered at a rate of 1 mL minute\(^{-1}\). The epidural cranial spread increased with increasing volumes of injectate up to 0.6 mL kg\(^{-1}\), but no further advantages were seen by increasing from 0.6–0.8 mL kg\(^{-1}\). Volumes of 0.2 mL kg\(^{-1}\), which are commonly used in a clinical setting, resulted in an average sensory loss up to L\(_3\) observed by pinching the skin. Sensory loss, above the spinous process up to the T\(_6\) vertebrae was observed with a volume of 0.6 mL kg\(^{-1}\). After euthanasia and dissection of the spinal cord, the methylene blue had a spread much further than the clinical evaluation indicated, going up to T\(_2\) and C\(_1\) for 0.2- and 0.6 mL kg\(^{-1}\), respectively (Freire et al. 2010).

A different study using healthy Beagles of different gender, with a body weight around 7.6 kg, were anesthetized with propofol and kept in sternal position, with the hind limbs flexed facing forward. The epidural injection was performed with a Tuohy needle in the LS space, with a final volume of 0.2 mL kg\(^{-1}\), of a solution made with 100 mL of iohexol radiographic contrast medium and 1 gm of crystalline new methylene blue. The solution was administered at a rate of 1 mL per minute. Spread was evaluated with lateral radiographs projections (with animals in sternal), over 45 minutes, followed with euthanasia and dissection of the spinal canal. The peak of spread was observed radiographically at around 20–25 minutes ranging between the C\(_6\) and L\(_3\). At necropsy the spread ranged between C\(_6\) and L\(_2\) (Son et al. 2011).

In a study with 6 greyhound cadavers, around 28.7 kg and 88 cm of column length, in sternal recumbency, using a Tuohy needle, the LS space, was injected with iohexol contrast diluted in saline (1:3) to a total volume of 0.6 mL kg\(^{-1}\) and injected in fractions (0.1-, 0.2-, 0.4- and 0.6 mL kg\(^{-1}\)), followed by a CT scan to evaluate the spread. The median number of vertebrae’s spread with dye was 1, 3, 12, and 18 for the same volumes respectively (Vesvoski et al. 2019).
A study using 2% lidocaine, in healthy medium to large size dogs (13.2 to 22 kg), used a volume of 1 mL for each 3.5 kg (0.29 mL kg\(^{-1}\)), cranial spread was tested by pinching the skin (Leite et al. 2017). This block had an average duration of 63 minutes, and a peak spread of 13 cm. However, no data of the column length of the dogs or the corresponding vertebrae was reported, which makes difficult to evaluate the data described.

The cranial spread of 0.2 mL kg\(^{-1}\) of iohexol in adult healthy beagles, in sternal recumbency and under anesthesia with sevoflurane was evaluated by CT imaging in another study. Contrast was injected at the LS space using two different methods of lack of resistance technique (air or saline, 0.3 mL per dog). Cranial spread was evaluated over 20 minutes and resulted in statistically significantly higher spread when saline was used compared with air (Iseri et al. 2010). The peak average cranial vertebrae stained with CT contrast was C\(_7\) for saline, and T\(_5\) for air (Iseri et al. 2010). This shows a higher cranial spread than the clinically observed by Freire et al. (2010) of up to L\(_3\) using the same volume of injection.

The difference in methodology and results within the aforementioned studies makes direct comparisons difficult.

### 1.5.2 Epidural Dose Based on Length of the Vertebral Column

The information of the cranial spread of an epidural injectate when the volume is calculated by the vertebral column length is still limited. To use this method of calculation, it is necessary to measure the total vertebral column length, from the occipital crest to the sacroccogygeal space (Otero et al. 2010; Otero & Campoy 2013; Valverde & Skelding 2019). This is an alternative method which may be better for breeds with longer spinal lengths, when compared to breeds from the same size, or obese patients (Otero et al. 2010).
The first report of this method being used in veterinary medicine, was published in dogs and pigs (Strande 1968). Epidurals were performed in lateral recumbency and the injectate volume was increased in 2 mL increments over time, until a pre-determined final volume was reached, based on the size of the animal. The longer the vertebral column length, the more volume was needed to reach the same target vertebrae (T₁₁₀); T₁₁₀ was selected because by reaching this level it is possible to produce abdominal analgesia/anesthesia. Pigs and dogs had a similar volume requirement to reach T₁₁₀. A total volume of 1 mL was given for animals with column length < 40 cm, and an increment of 1.5 mL for each 10 cm increase in the vertebral column (Strande 1968).

An initial study used three different volumes (0.05-, 0.1- and 0.15 mL cm⁻¹) in dogs of different breeds and sizes, using an iopamidol-ropivacaine solution, and radiographically determined a rostral spread of 33%, 59%, and 73%, respectively, of the total length of the occipital-sacrococcygeal length (Otero et al. 2010). A mathematical model with these results predicted that for the same % of the column length with contrast, higher volumes were needed for larger lengths. However, the results were reported with the % of the column length and not the vertebrae, which makes difficult the clinical evaluation of the model (Otero et al. 2010).

Clinically, a dose of 0.1 mL cm⁻¹ of bupivacaine 0.5% in dogs undergoing orthopedic surgery with a mean weight of 33.4 kg (range 23–55.7 kg) and mean distance of 76.5 cm (range 60–90 cm), did not result in adverse effects and appears to be beneficial for surgery, but quantitative information on the degree of rostral spread was not reported (Hendrix et al. 1996). The protocol used seems to provide good postoperative analgesia, as evaluated in the study. However, to provide analgesia for the pelvic limbs, smaller volumes are needed in comparison to abdominal surgeries (up to T₁₁₀), which would make a larger volume unnecessary, since this volume would block around 60% of the column length (Otero et al. 2010).
In other study using 10 dogs with body weights between 13.2–22 kg, under general anesthesia, a dose of 0.15 mL cm\(^{-1}\) of lidocaine 2% was used and calculated based on the length from the occipital crest to the sacrococcygeal space (Leite et al. 2017).

### 1.5.3 Comparison Between Techniques

Two studies have compared epidural volumes with the two different calculation methods; one was a theoretical mathematical study (Valverde & Skelding 2019) and the other a randomized cross-over clinical trial (Leite et al. 2017).

The mathematical study (Valverde & Skelding 2019) evaluated the final volume of epidural injectate from the data obtained from 432 dogs of different breeds and sizes. For the comparison, the weight, vertebral column length and body condition score (BCS from 1 to 5; Baldwin et al. 2010) were obtained from the dogs, and the volume of injectate based on body weight and based on vertebral column length (from the occipital crest to the sacrococcygeal space) calculated for each dog for comparison. The dose for each method was based on the resultant volume reaching T\(_{10}\). For the volume based on the dose by weight (VW), 0.2 mL kg\(^{-1}\) was used and for the volume based on vertebral column length (VL) 0.05, 0.07, 0.08, and 0.11-mL cm\(^{-1}\) for dogs with lengths of \(\leq 49\), 50 to \(< 70\), 70 to \(< 80\), and \(\geq 80\) cm, respectively. The dogs were further divided in groups of size, based on body weight: small (\(< 10\) kg), medium (10 to \(< 25\) kg), large (25 to \(< 45\) kg) and giant (\(\geq 45\) kg). The results of the study showed that if the BCS was not considered, small, medium, and large dogs had a statically significantly larger calculated volume when calculated by VL when compared to VW, and giant dogs had a smaller calculated volume when calculated by VL when compared to VW. If the BCS, was considered, small and medium dogs had a larger calculated volume using VL when compared to VW for all BCS; the same findings occurred in large dogs.
with BCS of 2 or 3, but no difference in calculated volumes if BCS were 4 or 5. Giant dogs behaved differently in that larger calculated volumes were obtained for VW than for VL if BCS were 4 or 5, but no differences between methods for BCS of 2 or 3 (Valverde & Skelding 2019).

The clinical trial included 10 dogs with body weights between 13.2–22 kg, in a crossover, randomized design (Leite et al. 2017). Dogs received on separate occasions 0.15 mL cm\(^{-1}\) or 0.29 mL kg\(^{-1}\) of lidocaine 2% under general anesthesia. The final volume was approximately 50% higher when dogs were dosed based on the length of the vertebral column but no specific information on the length was reported. In addition, the sensory blockade was more rostral and longer lasting for the dose by length than by weight, which prompted the authors to suggest that when a longer block or further rostral spread is desired, the volume should be based on the length of the vertebral column (Leite et al. 2017).

1.5.4 Potential Complications of Epidurals

Epidurals can cause different types of complications or disadvantages, including occurrence of motor block (when undesired), potential toxicity of local anesthetics, need for training, potential damage to the spinal cord, hypotension, cardiovascular depression, urinary retention, delayed fur regrowth on the injection site (Troncy et al. 2002; Kona-Boun et al. 2006; Ferreira 2018). Some of the complications can be due the type, concentration or amount of drug used.

Based on the principles of epidural anesthesia and the anatomy of the epidural space, the same factors that affect uptake and rostral spread of a local anesthetic within the epidural space can result in underdosing or overdosing. Additionally, miscalculations in the dose may happen. These factors are discussed below.
1.5.4.1 Underdosing

There is limited information regarding underdosing from epidurals and lack of precise information on failure rate. Animal anatomical variability and physicochemical properties of the drug that limit rostral spread and incomplete effects include highly lipophilic drugs, meningovertebral ligaments that cause compartmentalization, increased epidural space pressures, positioning that increases frictional and gravity forces against the injectate, and leakage from the epidural space through intervertebral foramina (Valverde 2008). In addition, insufficient dose and inappropriate technique may be contributing factors (Hermanides et al. 2012; Martin-Flores et al. 2021).

The epidural route can be used to achieve analgesia in different parts of the body (pelvic limbs, abdomen, and thorax). For pelvis and pelvic limbs analgesia/anesthesia it is desired that the local anesthetic reaches L₃, so all the nerve roots in charge of innervation of these areas are blocked (Bailey & Kitchell 1987). For abdominal procedures, the local anesthetic should reach at least to T₁₀, although complete innervation of abdominal organs can include up to T₂, so that cranial organs such as the stomach, duodenum, pancreas, liver, and spleen are also included (Cleland & Tait 1927; Berthoud 2004). If the volume or profile of the drugs used does not reach this area, underdosing occurs and the patient has an incomplete block and analgesia.

1.5.4.2 Overdosing

Overdosing can also result from anatomical and physicochemical properties of the drug that specifically increase rostral spread. This include hydrophilic drugs that can have more prolonged effects if absorbed into the CSF and are not dosed correctly, decrease in the epidural space (e.g.,
pregnancy or obesity), positioning that decreases frictional forces and helps gravity favour rostral spread, and above all, a miscalculation that results in a larger volume of injectate (Valverde 2008).

Overdosing can result in similar effects to a total spinal block. When local anesthetics spread rostrally to the thoracic vertebral area, it can result in hypotension due to vasodilation and decreased vascular resistance from sympathetic blockade (ß fibres) from the sympathetic trunk located from T2 to L2(3) (Vagts et al. 2003; Schwarte et al. 2004). The hypotension can be more severe in patients with a compromised circulatory system and lead to cardiac arrest (Schwarte et al. 2004; Savvas et al. 2006). If the local anesthetic solution spreads to the cervical spine, it is possible to have respiratory failure due to blockade of the phrenic nerve, which originates from nerve branches from C5 to C7, and is the only nerve providing motor function to the diaphragm (Evans & de Lahunta 2013).

The spread of the local anesthetic can also affect the vagosympathetic trunk, composed by preganglionic cell bodies that originate from C8 to T7 spinal nerves, a plexus that is located close to some of the cranial nerves, formed by a combination of sympathetic nerve fibres and parasympathetic nerve fibres (vagus nerve) (Evans & de Lahunta 2013). Also, the cervicothoracic ganglia, which receives nerve branches from T1 to T3(4), and the heart, which receives sympathetic innervation from T1 to T6 (Evans & de Lahunta 2013) may be affected, resulting in a decrease in heart rate and contractility by blockade of the fibres responsible for cardiac acceleration and inotropy.

1.5.4.3 Epidural and Spinal Anesthesia

Epidural anesthesia consists in the injection of the analgesic drugs on the epidural space, which is the space between dura mater and periosteum, whereas spinal anesthesia consists of
injection into the subarachnoid space, between the *dura mater* and *arachnoid mater* (Campoy et al. 2015). An epidural injection at the LS space reduces the chance of a spinal injection since the spinal cord tapers into the *filum terminale* around the 5th to 7th lumbar vertebrae (Evans & de Lahunta 2013). However, it is still possible to penetrate the *dura mater* and perform a spinal injection.

Spinal anesthesia is used less frequently in veterinary medicine; however, it offers advantages compared to the epidural anesthesia. The confirmation of spinal anesthesia starts with the presence of CSF, due to the proximity to the spine the onset of anesthesia is faster, the degree of anesthesia is more profound, and the level and lateralization can be managed by using solutions with different baricity and changes in body position. However, the risk of serious complications is higher than for epidural, and include cardiac arrest, neurologic injury, and radiculopathy (Aldrete 2003). Because the local anesthetic does need to penetrate the *dura mater* during spinal anesthesia, lower volumes and doses are required (Otero & Campoy 2013).

Accidental spinal injection is more frequent in cats than dogs, because of the presence of the spinal cord at the LS and can occur in up to 27% in cats having epidural injections (Hansen 2001). Unintended injection, with the epidural drugs and volumes, may cause total spinal anesthesia or death (Otero & Campoy 2013).

### 1.6 RATIONALE, HYPOTHESIS AND OBJECTIVES

It is important to prevent excessive rostral spread of local anesthetics that may reach high dermatomes of the spinal cord (cervicothoracic) and interfere with diaphragmatic function and breathing or cause excessive sympathetic blockade. Therefore, adequate knowledge of the rostral spread of the epidural injection, using both methods of calculation for epidural doses should be
established. The theoretical study (Valverde & Skelding, 2019) shows that mathematically both techniques are not always interchangeable and that in small and medium size dogs they can result in extreme opposites that could result in inadequate anesthesia/analgesia or overdosing.

The objectives of this study are to:

1) Assess and compare in cadavers the rostral spread of epidural volumes that contain dye mixed with contrast medium, calculated based on body weight (0.2 mL kg\(^{-1}\)) or vertebral column length (0.05 mL cm\(^{-1}\) [< 50 cm], 0.07 mL cm\(^{-1}\) [50 to < 70 cm], 0.08 mL cm\(^{-1}\) [70 to < 80 cm], and 0.11 mL cm\(^{-1}\) [≥ 80 cm]) in paired individuals of similar weight and body conformation, by using CT, radiographs, and necropsy findings.

2) To apply the findings from the first objective to a group of small size research dogs (< 10 kg), to determine if rostral spread of local anesthetic combined with contrast medium differs from findings of cadavers of similar size and body conformation. Cranial the spread will be assessed with CT and radiographs, after injection of the volume calculated by weight and length in a crossover study.

3) To determine the rostral spread of local anesthetic in the same group of small size research dogs, by applying a noxious stimulus and correlate it to the corresponding spinal dermatome.

Hypothesis:

The calculation of epidural volumes based on body weight would have less rostral spread than the spread from volumes calculated on vertebral column length, especially in small size dogs.

Expected Results and Impact:
This study will determine if calculations based on body weight or vertebral column length are interchangeable. In addition, it will allow to determine which method is preferable according to the size of the dog, so that a better epidural blockade and less adverse effects result from it.
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CHAPTER 2

COMPARISON OF ROSTRAL SPREAD OF LUMBOSACRAL EPIDURAL VOLUME CALCULATED BY BODY WEIGHT OR LENGTH OF THE VERTEBRAL COLUMN IN DOGS
2.1 SUMMARY

Objective – To assess and compare in cadavers paired by similar weight and body conformation, the rostral spread of lumbosacral (LS) epidural volumes that contain dye mixed with contrast medium, calculated based on body weight (BW) or vertebral column length (LE), by using computerized tomography (CT) and necropsy findings. The next objective was to determine in live small size research dogs (≤ 10.2 kg), if rostral spread of local anesthetic combined with contrast medium differs from findings of the cadaver, by using CT and noxious stimulation, after injection of the volume by BW or LE.

Study Design – Cadavers were paired based on similar weight, length of the vertebral column, and body conformation. Live dogs were used in a prospective, randomized, crossover experimental trial.

Animals – Twenty-two cadavers (4.6–52.0 kg [BW]; 39–99 cm of vertebral column length [LE]) and six live female Beagles (BW: 7.5–10.2 kg; LE: 46–56 cm).

Methods – Within each cadaver pair, dogs were randomly assigned to receive a LS epidural volume based on BW (0.2 mL kg⁻¹) or LE (0.05 mL cm⁻¹ [< 50 cm], 0.07 mL cm⁻¹ [50 to < 70 cm], 0.08 mL cm⁻¹ [70 to < 80 cm], and 0.11 mL cm⁻¹ [≥ 80 cm]), using contrast medium (iopamidol 30%) mixed with yellow tissue dye in a 5:1 ratio and injected through an epidural catheter. The rostral spread was evaluated by anatomical dissection and CT to determine the number of vertebrae reached by the solution. The live dogs were randomly assigned in a crossover block design to the
two treatment groups (BW and LE). Group BW received 0.2 mL kg\(^{-1}\) and group LE 0.05 mL cm\(^{-1}\) (< 50 cm) or 0.07 mL cm\(^{-1}\) (50–69 cm) at LS. Dogs were mask-induced with isoflurane, positioned in sternal recumbency and an epidural catheter advanced 1–2 cm into the LS. The volume of injectate (1:1 mixture of bupivacaine 0.5% and iopamidol) was administered over 20 seconds, and within 5 minutes the vertebral column was scanned to determine the number of vertebrae reached by the contrast. The dog was recovered and kept in sternal recumbency until fully conscious to start assessing analgesia from the tail to the thoracic limb using a hemostat or clamp, motor function, indirect blood pressure, heart rate, respiratory rate and rectal temperature until return to normal. Comparisons between groups were completed with a mixed linear model (\(p < 0.05\)).

**Results** – In cadavers, the volume of injectate was always larger for LE than BW but not significantly different for all 11 pairs of dogs. For both contrast and dye, there was no difference in the number of vertebrae reached by the indicator between LE and BW; however, the number of vertebrae reached by the dye was larger than for contrast (\(p = 0.0041\)). In live dogs, the volume of injectate was significantly larger for LE than BW (3.29 ± 0.74 mL \textit{versus} 1.81 ± 0.21 mL; \(p = 0.0012\)), which resulted in a significantly higher rostral spread (22 ± 2 vertebrae \textit{versus} 19 ± 2, respectively; \(p = 0.0488\)). Response to nociception for all dermatomes and time to presence of pain was similar between groups (37.7 ± 20.4 minutes for LE and 49.0 ± 20.4 minutes for BW). There were no differences in cardiorespiratory variables and rectal temperature.

**Conclusion and clinical relevance** – Dye spread is significantly higher than contrast medium spread and should not be considered interchangeable in research studies. Dosing based on LE results in higher rostral spread than BW in live dogs of small size.
Keywords bupivacaine, dog, epidural, iopamidol, length, weight.
2.2 INTRODUCTION

Epidural lumbosacral (LS) injection of a local anesthetic with or without an opioid is one of the most common regional techniques used in clinical practice in dogs to provide anesthesia and/or analgesia to the pelvic limb, pelvic area, and abdomen (Valverde 2008; Garcia-Pereira 2018; Martin-Flores 2019).

The rostral spread of a local anesthetic drug injected into the LS space allows its interaction with the spinal cord and emerging spinal nerves, to exert its analgesic/anesthetic effects. For a local anesthetic, the relationship between volume of injectate and rostral spread is linear (Strande 1968; Johnson et al. 1996; Freire et al. 2010; Vesovski et al. 2019) and determines the extent of blockade. A small volume of injectate acts close to the site of injection and may only involve sensory blockade of the pelvic area, but a larger volume can be adjusted to reach higher lumbar and lower thoracic segments of the spinal cord in the epidural canal to include the abdomen (Bromage 1962; Strande, 1968; Vesovski et al. 2019). Volume is less important for an opioid injected epidurally at the LS since its analgesic actions result from binding to opioid receptors in the spinal cord, and the drug is made available to them throughout the length of the spinal cord by mixing with the CSF to achieve relatively uniform concentrations, especially for hydrophilic opioids such as morphine (Valverde et al. 1992).

In addition to volume of injectate, the degree of epidural rostral spread is influenced by two physical factors, gravity and friction (Valverde & Skelding 2019), which in turn are also influenced by inherent factors of the patient, such as body condition score (BCS) and size, position of the patient at the time of injection and thereafter, injection at the site of entry of the needle or in a more rostral position with an epidural catheter, and whether the patient is conscious, sedated or under...
general anesthesia (Bromage 1962; Strande 1968; Gorgi et al. 2006; Iseri et al. 2010; Freire et al. 2010; Son et al. 2011; Vesovski et al. 2019; Valverde & Skelding 2019).

The epidural volume of injectate can be calculated in two described methods, based on body weight (BW; mL kg$^{-1}$) or on the length of the vertebral column (LE; mL cm$^{-1}$) from the occipital crest to the sacrococcygeal space (Strande 1968; Johnson et al. 1996; Gorgi et al. 2006; Iseri et al. 2010; Freire et al. 2010; Son et al. 2011; Vesovski et al. 2019; Valverde & Skelding 2019); however, it has not been determined if both methods are interchangeable. In a recent study, the hypothetical comparison of both methods of calculation determined that the calculated volume of injectate was not interchangeable in small and medium size dogs (< 25 kg); larger volumes were obtained for LE than BW and this difference tended to disappear or revert as size increased (≥ 25 kg) (Valverde & Skelding 2019). A higher BCS (obesity) resulted in a smaller volume for LE than BW (Valverde & Skelding 2019).

If both methods of calculation are not always interchangeable, the volume of injectate calculated from either method could result in inadequate anesthesia/analgesia or overdosing; however, at this time it is not known which method should be the gold standard. Therefore, the first objective of this study was to assess and compare in a wide range of paired cadavers matched to be similar for BW, LE and BCS, the rostral spread of LS epidural volumes that contain dye mixed with contrast medium, using computerized tomography (CT) and necropsy findings. The second objective was to use live research dogs (< 10.2 kg) in a crossover randomized study, to determine the rostral spread using CT of an epidural volume of local anesthetic combined with contrast medium, based on BW or LE, and to assess the response to a noxious stimulus by correlating it to the spinal dermatome.
2.3. MATERIALS AND METHODS

2.3.1 Animals

A total of 22 dog cadavers (six mixed breed, four Hounds, three German Shepherds, two Poodles, two Shih Tzu, one Chihuahua, one Beagle, one Rottweiler, one Mastiff and one Great Dane), corresponding to clinical cases that were euthanized and donated for teaching laboratories, were used and divided into four categories according to body weight: small (< 10 kg; eight cadavers), medium (10 to < 25 kg; six cadavers), large (25 to < 45 kg; six cadavers), and giant (≥ 45 kg; two cadavers). All cadavers were kept frozen until the start of the study, then thawed completely prior to use. Institutional Animal Care Committee was not required for the use of the cadavers.

For the clinical study, a total of six purpose-bred research, spayed female Beagles, mean ± standard deviation (SD) age of 6.1 ± 1.3 years, considered healthy based on physical exam, complete blood count and biochemistry profile, were used. The study was carried out in accordance with the guidelines of the Canadian Council on Animal Care and was approved by the Institutional Animal Care Committee at the University of Guelph (AUP #4347).

2.3.2 Study design

2.3.2.1 Cadaveric study

Within each body weight category, cadavers were paired according to body weight (BW; kg) and length of the vertebral column (LE; cm), measured from the sacrococcygeal space to the occipital crest using a metric tape, and had to comply with < 10% difference for BW and LE, and same body condition score (BCS scale of 1-5; where 1 = emaciated; 2 = thin; 3 = moderate; 4 = stout; and 5 = obese) (Baldwin et al. 2010) between the two cadavers. Each matched pair within
each category was then randomized (http://www.random.org) for an epidural injection according to BW (0.2 mL kg\(^{-1}\)) in one of the cadavers or according to LE (0.05 mL cm\(^{-1}\) (< 50 cm), 0.07 mL cm\(^{-1}\) (50 to < 70 cm), 0.08 mL cm\(^{-1}\) (70 to < 80 cm), and 0.11 mL cm\(^{-1}\) (≥ 80 cm) in the other cadaver (Valverde & Skelding 2019).

### 2.3.2.1.1 Epidural injection and evaluation

Each matched pair of cadavers was processed the same day. Each cadaver was thawed completely prior to the day of the experiment. The day of the experiment, each cadaver was positioned in sternal recumbency, with the hind limbs extended caudally and an epidural catheter was placed at the lumbosacral space (LS) and advanced rostrally through a Tuohy needle into the epidural space 1 cm per BW category beyond the tip of the needle (e.g., 1 cm for < 10 kg; 2 cm for 10 to < 25 kg; 3 cm for 25 to < 45 kg; and 4 cm for ≥ 45 kg). The epidural catheter used in the BW category of < 10 kg consisted of a 24 gauge, 25 cm catheter advanced through a 20 gauge, 4.5 cm Tuohy needle (MILA Epidural Pain Management Kit, MILA, KY, USA), whereas a 19 gauge, 90 cm catheter and 17 gauge, 8.9 cm Tuohy needle was used in the other three categories (Arrow Flextip Plus Epidural catheter; PA, USA). Once the epidural catheter was in placed, the Tuohy needle was removed, the catheter was flushed with 0.1–0.2 mL of saline and secured in place with a towel clamp.

Cadavers remained in sternal recumbency for the computed tomography (CT) (16-slice detector, GE Brightspeed CT scanner, GE Healthcare, WI, USA). The injection consisted of a solution of iopamidol contrast medium (IsoVue\(^{TM}\)-300, 300 mg mL\(^{-1}\), Bracco Imaging, QC, Canada) diluted to a final concentration of 240 mg mL\(^{-1}\) (5:1) by adding yellow tissue marking dye (Cancer Diagnostics Inc., NC, USA). The injectate volume included an additional 0.1 mL of
the solution for small epidural catheters and 0.2 mL for large epidural catheters, to prime the catheter. An initial scout was performed following a small test injection (0.3 mL) of contrast to confirm appropriate placement of the epidural catheter. After confirmation, the remaining volume was injected over 20 seconds and a CT exam of the entire spine completed within 5 minutes of the injection.

Images from the CT were acquired and evaluated from the cranial aspect of the cervical spine to just caudal to the LS space in both, soft tissue (standard) and bone windows, by a board-certified radiologist (MJ). From these images, the cranial spread of positive contrast medium was evaluated by the radiologist, blinded to the treatment. Assessment included both the number of vertebrae reached by the contrast and the LE (cm) to that specific vertebra from the LS space. A maximum of 27 vertebrae was possible (seven lumbar + 13 thoracic + seven cervical), and was always inclusive of the vertebra it reached, regardless of being whole or a fraction of it (Fig. 2.1). The LE measurement was obtained following reconstruction of the transverse images into a sagittal plane using multiplanar reformatting to make the spine as straight as possible.

Following the CT scan, cadavers were kept in sternal and transferred immediately to the necropsy room and dissected in this position, avoiding changes in the horizontal position during this phase. A longitudinal skin incision was made along the spinous processes of the vertebral column and all anatomical layers above the spinous processes reflected on each side until the vertebrae were exposed below the vertebral arches. A laminectomy was performed by removing each arch from C1 to the sacrum until the entire extent of the spinal cord was exposed. The cranial spread of dye was assessed similar to the CT, by recording the number of vertebrae and the LE to that specific vertebra from the LS space using a measuring tape.
2.3.2.2 Live study

This phase consisted of a prospective, randomized block, cross-over study design, in which each dog received two treatments with a washout period of at least 5 days. The day of procedure, the dogs were fasted for 12 hours for food, but water was available. Body weight was recorded each day before the experiment and LE was recorded under anesthesia while in sternal recumbency using a metric tape, during the first treatment. Three dogs received treatment 1 (epidural volume based on LE) the first time and the other three dogs received treatment 2 (epidural volume based on BW), followed by the reversed order on the second occasion (http://www.random.org). Volumes of injectate for the epidural injection were 0.2 mL kg\(^{-1}\) according to BW, and 0.05 mL cm\(^{-1}\) (< 50 cm) or 0.07 mL cm\(^{-1}\) (50 to < 70 cm) according to LE.

2.3.2.2.1 Epidural injection and evaluation

General anesthesia was induced on the CT table by delivering 5% isoflurane (Aerrane; Baxter Corporation, Mississauga, Ontario, Canada) in oxygen at 4–5 L minute\(^{-1}\) via facemask with a circle system. The trachea was intubated and dogs connected to the delivery system and mechanically ventilated with an electronically controlled, volume-cycled ventilator (S/5 Aespire 7900 Ventilator; GE Healthcare, Madison, Wisconsin, USA) at a rate of 10 breaths minute\(^{-1}\), a tidal volume of 15 mL kg\(^{-1}\) and an inspiration: expiration ratio of 1:3. The dogs were positioned in sternal recumbency with the hind limbs extended caudally, and anesthesia maintained with isoflurane in > 95% oxygen at 1–2 L minute\(^{-1}\) at an initial end-tidal isoflurane concentration (Fe’Iso) of 1.5% and adjusted accordingly for depth during the epidural catheter placement. The Fe’Iso was measured from the connection of the endotracheal tube with the circle system using a
multiparameter monitor (Datex-Ohmeda S/5 Anesthesia Monitor; GE Healthcare Finland, Helsinki, Finland), calibrated before each experiment with a standardized calibration gas mixture designed for the analyzer (755571-HEL, Calibration gas mixture, GE Healthcare Finland Oy, Helsinki, Finland). In addition, dogs were instrumented for indirect oscillometric arterial blood pressure using a cuff size approximately 40% of the limb circumference above the tarsal joint on the right hindlimb (size 2), rectal temperature, capnography, pulse oximetry, and sidestream end-tidal CO₂ (PETO₂). These variables were recorded at 5-to-10-min intervals throughout anesthesia during the CT and total time of anesthesia from intubation to extubation was recorded.

A five-by-five cm area over the LS space was clipped and aseptically prepared. A stab incision was made over the LS space and a 24 gauge, 25 cm catheter advanced through a 20 gauge, 4.5 cm Tuohy needle. The catheter was advanced one to two cm into the epidural space from the tip of the needle. The Tuohy needle was left in place and the catheter flushed with 0.1 mL of sterile saline, followed by the injection of a test dose of 0.2–0.3 mL of a 1:1 mixture of preservative free bupivacaine 0.5% (Bupivacaine Injection BP, SteriMax Inc., ON, Canada) and Iopamidol contrast medium. An initial scout was performed followed by a CT scan from the lumbar vertebral column, to confirm appropriate placement of the epidural catheter and presence of the contrast in the epidural space. Following confirmation, the remaining of the assigned randomized final volume was injected over 20 seconds and a CT exam of the entire spine completed within 5 minutes of the injection. Measurements were again obtained by the same radiologist, blinded to the treatment, as described for the cadavers.

Following the CT, the epidural catheter and needle were removed, isoflurane administration was discontinued, and dogs allowed to recover, and extubated when spontaneous breathing returned. Total anesthesia time, from induction to extubation, required for the epidural
catheterization and CT was recorded. Dogs were taken to a recovery area and maintained in sternal recumbency until full display of normal behavior conscious. During recovery, dogs were assessed for 30 minutes for post anesthesia sedation using a recovery score from 0 to 3, with 0–indicating no sedation and 3–indicating profound sedation (Appendix A; Skelding et al. 2019). Heart rate, assessed by auscultation, oscillometric blood pressure (Cardell Veterinary vital signs monitor, model 9403; Midmark Corporation, Versailles, OH, USA), and rectal temperature, were measured at 10 and 30 minutes during this period.

At 30 minutes post extubation, dogs were assessed for ambulation using a score from 0 to 3, with 0–indicating normal ambulation and 3–indicating unable to stand and/or ambulate (Appendix B), at 30, 45, 60 minutes and every 30 minutes thereafter or until a score of 0 was achieved. Time to full recovery was recorded as time from extubation to a score of 0 for both the sedation and ambulation score. Analgesia assessments were aimed at detecting the presence or not for pain and were completed by an investigator blinded to the treatments. These assessments started when the post anesthesia sedation score was 0 but not before 30 minutes post extubation.

The assessment consisted of applying a noxious stimulus to eleven different dermatomes, starting at the tail, and moving cranially to the thoracic limb paw. These included the base of the tail (AN1), the pelvic limb paw (AN2), the area surrounding the iliac crest (AN3), the inguinal area near the femoral nerve (AN4), the area close to the transverse process of L3 and flank (AN5), the paramedian area next to the umbilicus (AN6), along the thirteenth rib (AN7), the paramedian area to the xyphoid (AN8), along the ninth rib (AN9), along the second rib (AN10), and the thoracic limb paw (AN11). Noxious stimulation consisted of clamping of the tail or paws with a 24 cm sponge forceps with protective plastic tubing on each jaw (Sponge forceps, Robbins Instruments Inc, Chatham, NJ, USA) or the use of a 12.5 cm hemostat forceps for the remaining
dermatomes. The stimulation consisted of clamping the anatomical region with the instrument, without reaching the first notch, for a period of < 3 seconds. A positive response to pain consisted of gross purposeful movement, or a vocal response elicited during or before the period of stimulation. If the evaluator was uncertain of the response, electrical current was applied to the anatomical area tested with the surgical instrument and consisted of one to three single stimuli of 30–40 V at 50 cycles/s for 10 milliseconds (S48 Stimulator, Astro-Med Inc, West Warwick, RI, USA) using alligator clips 5 cm apart in the anatomical area previously tested with the instruments. If gross purposeful movement or a vocal response was elicited before the cycle was completed, the electrical stimulus was discontinued immediately, and the response assessed as positive. A second positive response obtained in the following assessment time was indicative of complete sensory recovery and the time to a positive response to noxious stimulation was recorded as the time when the first assessment was completed. All dermatomes were assessed until two consecutive positive responses were obtained and were no longer stimulated subsequently.

Dogs were administered SQ meloxicam (0.1 mg kg⁻¹; Metacam; Boehringer Ingelheim Canada Ltd, ON, Canada) at the end of each experiment and were monitored for normal behavior. Dogs were returned to the supplier after the last dog completed the study.

2.3.3 Statistical Analysis

All data was tested for normality using Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darlin procedures. UNIVARIATE and PLOT procedures were used to detect unequal variances, outliers, and other non-random patterns within the data for each group (Version 9.4; SAS Institute, Cary, North Carolina, USA). For the cadaver study, a mixed model of residual maximum likelihood was used for comparisons between pairs of dogs (LE versus BW dosing) for
each weight category for the use of contrast (CT) and dye (necropsy) and the number of vertebrae reached by each substance. When appropriate, multiple pairwise comparisons were done by use of the Tukey-Kramer test. Comparisons between the number of vertebrae reached by the contrast and dye in all dogs were completed with an unpaired $t$-test (GraphPad Software, version 9.3.1 for macOS, San Diego, California, USA). For the live study, a mixed model of residual maximum likelihood was used to compare the effect of method (LE versus BW dosing) and period of administration (first and second time) in the number of vertebrae reached by the contrast. Presence of pain was analyzed with a mixed model of residual maximum likelihood that considered the method and dermatome. The error structure was selected based on the Akaike Information Criteria among structures offered by SAS using for each treatment a random effect. Cardiorespiratory and temperature variables during and after anesthesia with the two methods were analyzed with a mixed model if there were missing values, or a two-way analysis of variance with repeated measures when all values were recorded, and multiple comparisons within treatments were performed with a Sidak test (Prism 9.3.1). Sedation and ambulation scores for both groups were compared between groups using a Wilcoxon matched pairs test. Results were considered statistically significant if the value of $p < 0.05$.

The sample size was calculated based on the mathematical model for the different methods of calculation for the epidural volume (Valverde & Skelding 2019), using the small size ($< 10$ kg) dog category data. Therefore, to compare the mean volume of injection of 2.2 mL (LE dose), and 1.1 mL (BW dose), with a standard deviation of 0.5, a power of 0.8 (Type II error) and alpha of 0.05 (Type I error), at least five dogs were required.
2.4 RESULTS

2.4.1 Cadaveric study

Demographic information of the dog cadavers and volume of injectate administered are provided in Table 2.1. All pairs were within the established limits of < 10% difference in BW and LE, and same BCS. The volume of injectate was always larger for LE than BW but not significantly different for all 11 pairs of dogs (5.47 ± 3.30 mL versus 4.15 ± 3.16 mL, respectively; mean ± standard deviation) (Fig. 2.2). For each contrast medium and dye, there was no difference in the number of vertebrae or distance reached by the indicator between LE and BW for all size categories included, although there was a trend for the small and medium size categories to have a more rostral spread with LE than BW (Table 2.2; Figs. 2.3–2.7). There was a strong correlation between volume of injectate and distance reached by both contrast medium and dye in both groups, with r values > 0.92 for all comparisons (p < 0.0001 for each comparison) (Fig. 2.8); however, this correlation was weaker between volume of injectate and number of vertebrae, with r = 0.48 with both contrast medium (p = 0.1335) and dye (p = 0.1366) in the LE group, and r = 0.83 for contrast medium (p = 0.0014) and r = 0.71 (p = 0.0136) for dye in the BW group (Fig. 2.9).

The number of vertebrae reached by dye (22 ± 6) was larger than for contrast medium (19 ± 5) (p = 0.0597), but this data had to be transformed to accept normality, which resulted in a significant difference (p = 0.0041) between the method of indicator used to assess rostral spread (Fig. 2.10). The distance reached by dye (51.8 ± 23.6 cm) was not significantly larger than for contrast medium (40.0 ± 18.6 cm). There was a strong correlation between dye and contrast medium for all dogs in both LE and BW groups for the number of vertebrae (r = 0.92; p < 0.0001) and for distance (r = 0.99; p < 0.0001) reached by the indicators (Fig. 2.10).
2.4.2 Live study

All six dogs completed the study without complication. Demographic information of the dogs is provided in Table 2.3. One of the dogs was 10.2 kg and was maintained in the study because findings of the cadaver phase supported that data from this dog was consistent with dogs < 10 kg. Total anesthesia time from intubation until extubation was 24.2 ± 2.1 minutes in the LE group and 23.7 ± 6.5 minutes in the BW group.

2.4.2.1 Cardiorespiratory variables

Cardiorespiratory values and rectal temperature during the epidural injection and CT under anesthesia, and during the assessment of presence of pain after anesthesia were not significantly different between groups (Fig. 2.11).

2.4.2.2 Epidural volume and rostral spread

The volume of injectate was significantly larger for LE than BW (3.29 ± 0.74 mL versus 1.81 ± 0.21 mL; \( p = 0.0012 \)), which resulted in a significantly higher \( (p = 0.0488) \) rostral spread for LE (22 ± 2 vertebrae; 95% confidence intervals: 20, 24) than BW (19 ± 2; 17, 21) (Fig. 2.12). The range of vertebrae for the LE dose was C\(_4\)--T\(_1\) and for BW was C\(_5\)--T\(_3\).

2.4.2.3 Response to noxious stimulation

Sedation scores of 0 (no sedation) were present in all dogs at 30 minutes post extubation and not significantly different between groups \( (p = 0.125) \). Ambulation scores were not significantly different between groups at any of the periods of assessment \( (p = 0.1094) \), and time for a score of
0 for both sedation and ambulation scales were 48.2 ± 18.8 minutes in the LE group and 35.5 ± 28.6 minutes in the BW group ($p = 0.2904$).

The time from recovery until the first assessment for the presence of pain was 36.3 ± 0.8 minutes in the LE group and 37.7 ± 1.9 minutes in the BW group, and not significantly different ($p = 0.2065$). Time to positive response to nociception was similar between individual dermatomes and groups ($p = 0.1140$) and overall time to presence of pain (37.7 ± 20.4 minutes for LE and 49.0 ± 20.4 minutes for BW) was also similar ($p = 0.3591$). The times for detection to positive response to nociception for each dermatome are shown in Table 2.4.
2.5 DISCUSSION

The design of this study allowed a quantitative determination of the rostral spread of an epidural LS volume of injectate based on BW or LE in cadavers and live dogs. However, antinociceptive and cardiorespiratory effects of that volume of injectate during the live dogs’ phase of this study had a major limitation with the use of general anesthesia because it interfered with the immediate post injection assessment of the degree of motor and sensory block, which delayed the assessments until the dog had fully recovered, and the possible lingering effects of isoflurane on cardiorespiratory function.

The results of the live study demonstrated that in small dogs (≤ 10.2 kg) the volume of epidural injectate is 1.82-fold larger with LE than BW, which results in a higher rostral spread (22 ± 2 versus 19 ± 2 vertebrae, respectively). This was also observed in small and medium size cadavers, but statistical comparisons were not performed between the individual weight categories due to the low number of dogs and the pilot nature of that phase of the study. Therefore, based on the differences between LE and BW in calculated volumes of injectate in small size dogs and the resultant degree of rostral spread, the two methods should not be considered interchangeable for that size category.

Results from this study point out differences between assessing contrast medium with radiographs or CT and dye with necropsy. In addition, there were differences between imaging techniques among cadavers and live dogs. In cadaver and live dogs’ studies there is also variation in the distribution of the indicator (contrast medium or dye) around the spinal cord on imaging or necropsy assessments, and the significance of this is not known. Some studies, including this one measured the rostral spread reached by the indicator without consideration for uniform spread (Gorgi et al. 2006; Iseri et al. 2010; Otero et al. 2010). Other studies calculated a mean of the dorsal
and ventral spread (Freire et al. 2010; Son et al. 2011), or by approximating the number to the nearest half vertebral body (Zhang et al. 2013; Kawalilak et al. 2015), or the number corresponding to the ventral column (Strande 1968), or the number according to a set percentage of coverage around the spinal cord (e.g., 90%) (Vesovski et al. 2019).

Regarding contrast medium, several studies have used the injection of contrast medium to determine rostral spread by counting number of vertebrae. In these studies, small to medium size live dogs, 7.6 ± 1.1 kg (Son et al. 2011), 12.8 kg (10–15 kg) (Iseri et al. 2010), and 12.0 kg (10.9–14.0 kg) (Zhang et al. 2013) were injected at the LS in sternal position under general anesthesia a dose of 0.2 mL kg⁻¹ of the contrast iohexol 30% combined with methylene blue 1% (1:1 mixture) at 1 mL minute⁻¹ (Son et al. 2011) or iohexol 14% at 0.6 mL minute⁻¹ after verification of the epidural space with the injection of 0.3 mL of saline or air, in a crossover study (Iseri et al. 2010) or 0.2 mL kg⁻¹ of iohexol 14% at 0.6 mL minute⁻¹ using an epidural catheter advanced 10 cm and injected with the dogs in dorsal position (Zhang et al. 2013). These studies performed time-dependent imaging of the rostral spread for up to 45 minutes using radiographic imaging (Son et al. 2011) or 20–30 minutes using CT (Iseri et al. 2010; Zhang et al. 2013) and demonstrated a gradual increase in the spread of the contrast medium and number of vertebrae involved over the first 20–30 minutes post injection (Son et al. 2011; Iseri et al. 2010; Zhang et al. 2013) and then a decrease in the number of vertebrae after 30 minutes post injection due to absorption of the contrast medium (Son et al. 2011).

In one study, the highest number of vertebrae reached by the contrast medium was in the thoracolumbar area (T₂–L₄) at 20 minutes for 12.0 ± 5.4 vertebrae (Son et al. 2011), in Iseri et al. (2010) study rostral spread at 5, 10, and 20 minutes post epidural had a median of 19.5, 20.5 and 21.0 vertebrae (e.g., T₁ = 20), respectively, for the group receiving the saline test, and 12.0, 15.0
and 16.0 vertebrae, respectively, for the group receiving the air test, and in Zhang et al. (2013) study rostral spread at 5, 10, 20, and 30 minutes post epidural had a median of 18.5, 19.75, 20.5 and 21.5, respectively. The method used for determination of number of vertebrae was only described as approximating the number to the nearest half vertebral body in one study (Zhang et al. 2013) but not in the other studies; therefore it is not possible to elucidate in all studies if a vertebra was counted to the nearest half or whole vertebral body, and if both dorsal and ventral column had to be present in relation to the contrast medium. However, one study clarified that if the spread of the contrast medium was not uniform between the dorsal and ventral column, it was counted as the mean of the two (Son et al. 2011).

In the current study the rostral spread assessed with CT within 5 minutes of injection in live dogs (9.0 ± 1.0 kg) was of 19 ± 2 vertebrae, from C5–T5, similar to findings in the group that received the saline test in Iseri et al. (2010) and the study by Zhang et al. (2013), but higher than the group that received the air test in Iseri et al. (2010) and the study by Son et al. (2011). The faster speed of injection (20 seconds), the placement of the epidural catheter one to two cm cranially in the epidural space, and the method of counting the number of vertebrae (inclusive of the vertebra reached by the contrast medium) could have resulted in the difference in rostral spread in this study, despite an earlier imaging evaluation. Other factors that affect rostral spread were similar in the current study to those studies (e.g., position, size, BCS, and general anesthesia).

Interestingly, cadavers in the small size group (< 10 kg) in this study had similar number of vertebrae with the contrast medium (13 ± 5 vertebrae, from T5–T11) to those two studies with live animals (Son et al. 2011; Iseri et al. 2010). Movement of a substance injected in the epidural space is influenced by the compliance of the spinal canal, physical activity and position of the individual, the cardiovascular system (blood pressure, cardiac output, heart rate) and the respiratory system
(rate and effort of breathing), all of which affect CSF motion and pressures in the epidural space (Higuchi et al. 2005; Sánchez et al. 2018). In addition, systemic absorption of the injected substance decreases the amount over time (Son et al. 2011). All these factors are altered or absent in cadavers. Therefore, findings from cadaveric studies cannot be assumed to represent findings from live studies regarding contrast medium and time for imaging, since cadavers are not subjected to the same conditions for the dynamic behaviour of the contrast medium, despite similar results obtained in this study to studies with live dogs (Son et al. 2011; Iseri et al. 2010). Furthermore, using the same methodology in both cadavers and live dogs in this study, rostral spread was higher for live dogs than cadavers.

Results from this study also demonstrated that in cadavers there is variation in the rostral spread of contrast medium according to the timing of imaging from other studies. In two studies where imaging (CT) was performed immediately after epidural injection, the number of vertebrae associated with the contrast medium was less than observed in this study where imaging was performed within 5 minutes of the epidural injection. In those studies, a dose volume of 0.2 mL kg\(^{-1}\) was injected LS in sternal recumbency, using the contrast ioxilan 30% in one study (Kawalilak et al. 2015) and a 1:3 mixture of iohexol 35% with saline in the other study (Vesovski et al. 2019). For dog cadavers of 21.4 to 35.8 kg and number of vertebrae counted to the nearest half vertebral body, a rostral spread of 7.2 ± 0.7 vertebrae was measured (Kawalilak et al. 2015), whereas in dog cadavers of 28.1 ± 2.6 kg and number of vertebrae counted to the nearest whole vertebrae when at least 90% of the spinal cord circumference was surrounded by the contrast medium, had a median rostral spread of 3 vertebrae (range of 0–10) (Vesovski et al. 2019). In the latter study, with a 50% coverage of the spinal cord circumference, the rostral spread was a median of 10 vertebrae (range of 6–17) (Vesovski et al. 2019).
In this study, cadavers in the 10 to < 25 kg category and in the 25 to < 45 kg category had a rostral spread of 16 ± 5 and 22 ± 5 vertebrae, respectively, which exceeded those of the above-mentioned studies for dogs of similar size. The speed of injection was similar between those studies and this study, but the wider variation in range of weight, the presence of the epidural catheter two to three cm cranially, and time to CT scanning (within 5 minutes) in this study are possible reasons for the differences in rostral spread. The small standard deviation (SD) in Kawalilak et al. (2015) compared to the wider SD of this study could indicate that it was still early during the imaging for the spread and distribution of the contrast medium in that study, as a time-dependent effect has been demonstrated for imaging of contrast medium in live dogs (Iseri et al. 2010; Son et al. 2011).

Regarding dye staining, live conscious dogs (9.9 ± 1.9 kg) with a vertebral column length of 53.4 ± 5.1 cm measured from the LS space to the occipital crest, injected 0.2 mL kg⁻¹ of a 1:1 mixture of bupivacaine 0.5% with methylene blue 1% at 1 mL minute⁻¹ in standing position through an epidural catheter advanced 5 cm into the epidural space, were assessed for dyeing of the spinal cord 30 minutes after injection plus time to euthanasia and to complete the necropsy, and a total of 18 ± 7 vertebrae were stained (Freire et al. 2010). Son et al. (2011) using the same volume dose with a 1:1 mixture of iohexol 30% and methylene blue 1% at 1 mL minute⁻¹ in anesthetized dogs (7.6 ± 1.1 kg) in sternal position, assessed the rostral spread after 45 minutes of imaging plus time to bleed the dogs out from the carotid artery, euthanasia and necropsy, and a total of 14.0 ± 5.4 vertebrae (range from C₆–L₂) were stained. In this study, the dye was injected in cadavers using a 5:1 mixture of iopamidol 30% and yellow tissue marking dye, the necropsy was carried out immediately after the CT, and resulted in staining of 16 ± 7 vertebrae (range from C₇–T₁₁) in small size dogs which is similar to the above studies, despite differences in timing to necropsy and the injections in cadaver dogs versus live dogs in those studies (Freire et al. 2010;
Son et al. 2011). Therefore, it appears that dye assessments during necropsy are less variable than contrast assessments with imaging, regardless of using cadavers or live dogs.

Rostral spread of an epidural volume of injectate dose using LE has not been extensively studied and although different studies have used it clinically, only one study has assessed its rostral spread with contrast medium. In sedated dogs (14.9 ± 5.2 kg) injected at LS 0.05-, 0.1- or 0.15 mL cm⁻¹ of iopamidol with ropivacaine 0.2% (low concentration) and 0.05- or 0.1 mL cm⁻¹ of iopamidol with ropivacaine 0.5% (high concentration) were assessed radiographically 10 minutes later to determine the percentage of the total LE reached by the contrast medium. Rostral spreads of 33.5 ± 6.9%, 59.5 ± 7.8% and 73.9 ± 1.7% for the low concentration doses, respectively, and 33.7 ± 6.7% and 57.7 ± 4.9% for the high concentration doses, respectively, were measured (Otero et al. 2010). Unfortunately, these results cannot be compared to the current study because the number of vertebrae or LE of the dogs was not reported.

The LE doses used in this study were based on studies in pigs and dogs in which a linear regression line was generated from injecting the contrast diatrizoate mixed with lidocaine 2% in 2 mL increments at the LS to determine the rostral spread radiographically immediately after the injection to help predict the volume of injectate to reach T₁₀ (vertebra 11) (Strande 1968). The latter rostral spread represents dermatomes that include abdominal analgesia/anesthesia in addition to the pelvis and pelvic limb, which only require the inclusion of up to L₃ (vertebra 5) (Bailey & Kitchell 1987). In the current study with live dogs, these doses spread the contrast medium 22 ± 2 vertebrae, from C₄–T₁, which exceeded the predicted site (T₁₀). Likewise, using the BW dose, the spread was also beyond T₁₀ (19 ± 2 vertebrae, from C₅–T₅). Similar findings occurred in the cadavers with the LE and BW doses spreading the medium contrast beyond T₁₀ in 20/22 dogs in all categories (19 ± 5 vertebrae, from C₄–T₁₁), except two dogs in the small size category.
Rostral spread of dye was of greater magnitude than contrast medium, with both dose methods (BW and LE) in this study. This agrees with the results of a previous study but using a BW dose (Son et al. 2011). Contrast medium and dye had a strong correlation between them for all dogs in both LE and BW groups for number of vertebrae ($r = 0.92$) and distance ($r = 0.99$), as they did for dogs dosed based on BW in other study for number of vertebrae ($r = 0.91$) (Son et al. 2011). The difference in rostral spread in live animals is likely due to systemic absorption of contrast medium, which decreases its presence post injection in the epidural space after 25 minutes (Son et al. 2011), whereas dye remains on the surface of the spinal cord membranes despite delayed assessment until the time of necropsy. Therefore, findings from studies using dye versus contrast medium should be interpreted accordingly and are not necessarily interchangeable. In humans, the mean radiographic spread of 5 mL of the contrast medium iotrolan injected in the epidural space through an epidural catheter advanced three to four cm, was highly correlated ($r = 0.97$) to the analgesic spread of 5 mL of lidocaine injected the previous day at the same site (Yokoyama et al. 2004).

In the present study 11 dermatomes were used to assess analgesic effects of epidural bupivacaine from its rostral spread. Epidural analgesia underestimates the magnitude of rostral spread indicated by dye, combined with local anesthetic, on the spinal cord. This discrepancy in number of vertebrae dyed and the sensory dermatome that corresponds to that level, decreased as the dose increased. In one study, 0.2 mL kg$^{-1}$ of a 1:1 mixture of bupivacaine 0.5% with methylene blue 1% had a rostral spread of dye of 18 ± 7 vertebrae (T₃), but the analgesia assessed for 30 minutes post injection with pinching of the spinous processes of the vertebrae with a Kelly forceps corresponded to a maximum of 5.0 ± 3.3 vertebrae (L₃) (Freire et al. 2010). In comparison, a dose of 0.4 mL kg$^{-1}$ resulted in a 2-fold increase in the maximum number of vertebrae blocked (14.2 ±
4.2; T\textsubscript{7}) while only four more vertebrae were dyed (22 ± 2; C\textsubscript{6}); whereas higher doses of 0.6- and 0.8 mL kg\textsuperscript{-1} quadruples the maximum number of vertebrae blocked with respect to the low dose (20.2 ± 1.3 and 21.0 ± 0; T\textsubscript{1} and C\textsubscript{7}, respectively for each high dose) while only 8 more vertebrae were dyed (26 ± 2 and 26 ± 1; C\textsubscript{2}, respectively) (Freire et al. 2010). This discrepancy is likely more pronounced for dye than for contrast medium, as it was demonstrated in this and other study (Son et al. 2011) that dye has a higher rostral spread than contrast medium.

Based on the discrepancy between dye and analgesia, means that a dose of 0.2 mL kg\textsuperscript{-1} of local anesthetic is likely insufficient to block up to T\textsubscript{10} to produce cranial abdominal analgesia/anesthesia since it only blocks to L\textsubscript{3}, useful for pelvis and pelvic limb analgesia/anesthesia. Abdominal analgesia requires of T\textsubscript{2-11} dorsal roots to include most of the cranial viscera (Cleland & Tait 1927; Berthoud 2004). A dose of 0.4 mL kg\textsuperscript{-1} was necessary to reach that dermatome upon sensory assessment (Freire et al. 2010), and although the current study and others (Freire et al. 2010; Iseri et al., 2010; Son et al. 2011) have demonstrated sufficient rostral spread with dye or contrast medium, assessment of analgesia in research or clinical conditions differs from necropsy and imaging findings for the extent of the sensory block in relation to the rostral spread of those indicators.

Onset of analgesia/anesthesia for epidural local anesthetics can be rapid and is determined by the ionization constant (pKa), the closer the value to physiological pH, the faster the onset (Becker & Reed 2006), but it also depends on the dose (volume) and concentration of the local anesthetic, and the dermatome. Sensory and motor block is faster, of longer duration and of higher degree closer to the site of injection; in dogs, increasing the volume administered at LS (0.2-, 0.4- and 0.6 mL kg\textsuperscript{-1} of bupivacaine 0.25%) had a dose-dependent effect on rostral spread for number of vertebrae and corresponding dermatomes that resulted in decreased sensory and motor function
within 2 minutes post injection and reached the maximum effect for each dose at 10 minutes post injection; however, although diminished, sensory responses were never absent (Freire et al. 2010). Similarly, increasing the concentration of bupivacaine (from 0.25% to 0.5% or 0.75%; 0.2 mL kg$^{-1}$), administered at LS in dogs, resulted in faster onset and duration of block for sensory and motor function, but sensory responses were never absent with the 0.25% concentration dose and almost absent with the higher concentrations (Gómez de Segura et al. 2000). Clinically, higher concentrations of bupivacaine increase the success of the block in dogs (Duke et al. 2000).

In this study, analgesia was assessed approximately 35 minutes post extubation in both the LE and BW group; therefore, not earlier than 40 minutes after the epidural injection of bupivacaine 0.25%. Due to the study design, the immediate effects on sensory and motor function were missed with the current study design since positive responses to nociceptive stimulation were already present at 37.7 ± 20.4 minutes for LE and 49.0 ± 20.4 minutes for BW post extubation. These results and those of Gómez de Segura et al. (2000) and Freire et al. (2010) indicate that bupivacaine 0.25% is not effective for a complete sensory block (anesthesia) and provides analgesia of short duration. Epidural bupivacaine 0.25% is commonly used to provide analgesia during labour in women, but frequent top-ups are required to maintain the degree of analgesia (Eddleston et al. 1996), whereas epidural bupivacaine 0.5% alone can be used to provide anesthesia for caesarean section (Crosby et al. 1998).

The methods for analgesia assessment used in the current study consisted of clamping of the tail or paws and pinching with a hemostat and were similar to other studies (Gómez de Segura et al. 2000; Freire et al. 2010). In certain dermatomes the use of electrical current was easier to interpret when responses to the mechanical stimulation were not definitive. This occurred for anatomical areas that were more diffusely innervated such as the flank and the inguinal area. The
main difference in assessment of the response to nociception in this study with other studies was that the observer was blinded to the treatments, and it was an all or none response, which is more representative of detecting the presence or not of nociception, rather than degree of analgesia.

In the current study, dogs were fully ambulatory at 48.2 ± 18.8 minutes in the LE group and 35.5 ± 28.6 minutes in the BW group post extubation, and this difference, although not significantly different was most likely related to the higher volume of bupivacaine in the LE group. In the study by Gómez de Segura et al. (2000), 0.2 mL kg⁻¹ of bupivacaine 0.25% caused complete motor block in only 33% of the dogs at 10 minutes post injection and the duration of the motor block for ataxia or complete paralysis was 72 ± 55 minutes (Gómez de Segura et al. 2000); Freire et al. (2010) reported similar results.

It is possible that the effects of isoflurane anesthesia during the recovery phase in the current study could have influenced the coordination of the dogs and prolonged the duration of motor block; however, times of motor block from the current study were shorter than in those studies for the same dose and bupivacaine concentration in the BW group and the larger volume administered in the LE group (Gómez de Segura et al. 2000; Freire et al. 2010). Differences in these times are due to the endpoints for assessment of motor block since in both of those studies, ataxia was considered as part of the motor block, whereas in this study dogs had to be completely unable to ambulate to consider the motor block as present.

Results from this and those studies indicate that bupivacaine 0.25% at a dose of 0.2 mL kg⁻¹ is not an effective epidural dose in dogs if anesthesia is desired. In addition, in two studies in dogs, the duration of motor block was longer than the sensory block for this and higher doses (0.4- and 0.6 mL kg⁻¹) (Freire et al. 2010). The same effect was reported with higher concentrations of bupivacaine (0.5% or 0.75%) at 0.2 mL kg⁻¹ (Gómez de Segura et al. 2000). However, this is
contrary to studies in people where epidural bupivacaine 0.5% injected at L2–L3 had a significantly longer sensory than motor block. The motor block, when assessed by a scale that considers the ability of the patient to flex or extend different joints of the leg and ambulation (Axelsson et al. 1989), which can be considered similar to the assessments in those studies for ambulation and ataxia (Gómez de Segura et al. 2000; Freire et al. 2010). Furthermore, when the motor block was assessed in people by a quantitative method that considered isometric measurements of force of extension and flexion of the leg joints to 90% of the control value (before epidural), there was no difference in the return to sensory and motor block for bupivacaine (Axelsson et al. 1989). This result is more in accordance to findings of the current study, where return of complete recovery of motor function was used for timing of the block.

Cardiorespiratory values and rectal temperature monitored during anesthesia and post epidural injection were within acceptable values for anesthetized and conscious dogs (Merin et al. 1991; Haskins et al. 2005), independent of the volume of injectate used for each treatment. In dogs (18.9 ± 3.3 kg) administered LS bupivacaine 0.25% at 0.2- or 0.4 mL kg⁻¹ under isoflurane anesthesia, mean arterial pressure values were acceptable but lower than before injection with both doses for up to 60 minutes, although the decrease occurred earlier and was lower with the higher volume dose within five minutes post injection and for up to 25 minutes (Dias et al. 2018). Cardiac output did not change with either dose, but hypoventilation from a decrease in tidal volume, and Horner’s syndrome in 2/6 dogs occurred in the high-volume dose (Dias et al. 2018).

Cardiorespiratory complications from epidural local anesthetics include hypotension, usually associated to the degree of rostral spread and the effects it causes at the associated dermatome(s) on blocking preganglionic sympathetic outflow and causing paralysis of cardiac sympathetic fibres (Greitz et al. 1983), and hypoventilation from blocking thoracic motor fibres to
intercostal muscles and cervical fibres to the phrenic nerve and diaphragm (Lebeaux 1973; Freire et al. 2010; Dias et al. 2018). In this study, anesthesia time was short, and dogs were discontinued from isoflurane once the CT was completed within 5 minutes of the epidural injection; therefore, the effects of bupivacaine 0.25% were not assessed during anesthesia. Subsequent cardiorespiratory variables in the recovery period were not affected by the effects of isoflurane on systemic vascular resistance and values for indirect measurement of arterial blood pressure were within normal range.

In this study, respiratory function was only assessed by respiratory rate and none of the dogs appeared to have negative effects of the rostral spread of bupivacaine 0.25%, most likely because at this concentration and dose (BW: 0.2 mL kg\(^{-1}\)) the effects on motor fibers to the respiratory muscles is minimal for 0.2 mL kg\(^{-1}\) (Dias et al. 2018). The approximate volume dose used in the LE group was 0.37 mL kg\(^{-1}\); a dose of 0.4 mL kg\(^{-1}\) resulted in hypoventilation in anesthetized dogs in one study (Dias et al. 2018), but only with higher volumes (0.6- and 0.8 mL kg\(^{-1}\)) and not 0.2- or 0.4 mL kg\(^{-1}\) in other study with conscious dogs (Freire et al. 2010).

The rostral spread obtained in this study was facilitated by the presence of the epidural catheter one to two cm in the epidural space in the live dogs, and one to four cm according to the size category in the cadavers. This approach guaranteed the injection in the epidural space and the fenestrations from the catheter to be beyond the Tuohy needle, to avoid leakage of the injectate through the lumen of needle or into the canal made by the needle as it has been reported in other study (Son et al. 2011).

Epidural catheters offer advantages for rostral spread; in one study, advancing an epidural catheter at L₁₋₂ two to three cm in women undergoing gynecologic surgery and injecting 12 mL of lidocaine with epinephrine over 50 seconds (14.4 mL minute\(^{-1}\)) achieved more analgesic rostral
spread than injection with a needle at that same rate, but if the rate with the needle was increased to deliver that volume in 10 seconds (72 mL minute$^{-1}$), there was no difference in the degree of analgesic spread except for discomfort from the injection with the faster rate. The use of an epidural catheter does not guarantee a uniform distribution of the injectate since during placement of the catheter its tip can travel in unexpected directions, including retrograde, towards an intervertebral foramen, in the paravertebral tissue lateral to the intervertebral foramen, and ventrally placed under the spinal cord (Hogan 1999; Yokohama et al. 2004; Freire et al. 2010); however, despite this uneven distribution, an adequate anesthetic effect can still be obtained whether the injection was completed with a needle or epidural catheter (Hogan 1999).

In conclusion, calculated volumes of epidural injectate in small size dogs are larger with LE than BW, which results in more rostral spread and the methods cannot be considered interchangeable. Cardiorespiratory function was similar for dogs between methods, but the design of this study did not allow to demonstrate if the difference in volume had an impact on sensory blockade because recovery from anesthesia delayed the start of this assessment. Results from this study have also shown that results from cadaver dogs differ from live dogs for contrast medium, and that rostral spread of dye is more extensive than for contrast medium. One additional finding was that bupivacaine 0.25%, administered epidurally, provided short and unreliable sensory block with both methods of volume calculation.
2.6 REFERENCES


Appendix A  Sedation score used during the first thirty minutes (recovery period), after epidural injection, in dogs anesthetized with isoflurane.

<table>
<thead>
<tr>
<th>Sedation score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Bright and alert, no apparent sedation</td>
</tr>
<tr>
<td>1</td>
<td>Calm, minimal sedation (quite but still alert an aware of surroundings, mild resistance to restraint for lateral recumbency, moderate response to voices and touch; mild resistance to catheterization)</td>
</tr>
<tr>
<td>2</td>
<td>Very calm, with moderate sedation (quiet, relaxed, minimal restraint required or lateral recumbency, mild response to voices or touch; no resistance to catheterization)</td>
</tr>
<tr>
<td>3</td>
<td>Profound sedation (quiet, very relaxed, no restraint necessary for lateral recumbency, does not respond to voice or touch)</td>
</tr>
</tbody>
</table>

Modified from Skelding et al. (2019).

Appendix B  Ambulation score used at the end of recovery phase (30- minutes mark), after the epidural injection, in dogs anesthetized with isoflurane.

<table>
<thead>
<tr>
<th>Ambulation Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal ambulation</td>
</tr>
<tr>
<td>1</td>
<td>Able to walk but shows ataxia and mild proprioceptive deficit of hind limbs</td>
</tr>
<tr>
<td>2</td>
<td>Drags one or both hind limbs when walking; marked proprioceptive deficit</td>
</tr>
<tr>
<td>3</td>
<td>Unable to stand and/or ambulate</td>
</tr>
</tbody>
</table>
Figure 2.1 Diagram used for number of vertebrae reached by the contrast or dye in dog cadavers and live animals after lumbosacral epidural injection. A maximum of 27 vertebrae was possible (seven lumbar + 13 thoracic + 7 cervical).
Figure 2.2 Volume of lumbosacral epidural injectate in 22 dog cadavers matched by < 10% difference in both weight (BW) and length of the vertebral column (LE), and same body condition score, where one cadaver received an epidural injection of 0.2 mL kg\(^{-1}\) for BW and the paired cadaver received 0.05 mL cm\(^{-1}\) (< 50 cm), 0.07 mL cm\(^{-1}\) (50 to < 70 cm), 0.08 mL cm\(^{-1}\) (70 to < 80 cm), or 0.11 mL cm\(^{-1}\) (≥ 80 cm mL cm\(^{-1}\)) for LE, of a 5:1 volume mixture of iopamide 30% and yellow tissue marking dye.
Figure 2.3 Number of vertebrae reached by a 5:1 volume mixture of iopamid 30% (A) and yellow tissue marking dye (B) in 22 cadavers matched by < 10% difference in body weight (BW) and length of the vertebral column (LE), and same body condition score, where one cadaver received a lumbosacral epidural injection of 0.2 mL kg⁻¹ for BW and the paired cadaver received 0.05 mL cm⁻¹ (< 50 cm), 0.07 mL cm⁻¹ (50 to < 70 cm), 0.08 mL cm⁻¹ (70 to < 80 cm), or 0.11 mL cm⁻¹ (≥ 80 cm mL cm⁻¹) for LE, of the mixture.
**Figure 2.4** Rostral spread of dye in pair #2 of dogs from the small size category (< 10 kg) that received a lumbosacral epidural injection of 0.2 mL kg\(^{-1}\) for BW or 0.05 mL cm\(^{-1}\) (< 50 cm) for LE of a 5:1 volume mixture of iopamide 30% and yellow tissue marking dye. Dog BW spread 19 vertebrae (T\(_2\)) and dog LE spread 26 vertebrae (C\(_2\)).
Figure 2.5 Rostral spread of dye in pair #3 of dogs from the medium size category (10 to < 25 kg) that received a lumbosacral epidural injection of 0.2 mL kg\(^{-1}\) for BW or 0.08 mL cm\(^{-1}\) (70 to < 80 cm) for LE of a 5:1 volume mixture of iopamide 30% and yellow tissue marking dye. Dog BW spread 23 vertebrae (C\(_5\)) and dog LE spread 26 vertebrae (C\(_2\)).
Figure 2.6 Rostral spread of dye in pair #3 of cadaver dogs from the large size category (25 to < 45 kg) that received a lumbosacral epidural injection of 0.2 mL kg\(^{-1}\) for BW or 0.11 mL cm\(^{-1}\) (≥ 80 cm mL cm\(^{-1}\)) for LE of a 5:1 volume mixture of iopamide 30% and yellow tissue marking dye. Dog BW spread 24 vertebrae (C\(_4\)) and dog LE spread 27 vertebrae (C\(_1\)).
Figure 2.7 Rostral spread of dye in the pair of cadaver dogs from the giant size category (≥ 45 kg) that received a lumbosacral epidural injection of 0.2 mL kg⁻¹ for BW or 0.11 mL cm⁻¹ (≥ 80 cm mL cm⁻¹) for LE of a 5:1 volume mixture of iopamidol 30% and yellow tissue marking dye. Dog BW spread 27 vertebrae (C₁) and dog LE spread 27 vertebrae (C₁).
Figure 2.8 Correlation between distance (cm) (A) or number of vertebrae (B) reached by a volume of injectate of a 5:1 mixture of iopamide 30% and yellow tissue marking dye (B) in 22 cadavers matched by < 10% difference in body weight (BW) and length of the vertebral column (LE), and same body condition score, where one cadaver received a lumbosacral epidural injection of 0.2 mL kg\(^{-1}\) for BW and the paired cadaver received 0.05 mL cm\(^{-1}\) (< 50 cm), 0.07 mL cm\(^{-1}\) (50 to < 70 cm), 0.08 mL cm\(^{-1}\) (70 to < 80 cm), or 0.11 mL cm\(^{-1}\) (≥ 80 cm mL cm\(^{-1}\)) for LE, of the mixture.
Figure 2.9 Comparison of number of vertebrae reached by a 5:1 volume mixture of iopamidol 30% and yellow tissue marking dye in 22 cadavers matched by < 10% difference in body weight (BW) and length of the vertebral column (LE), and same body condition score, where one cadaver received a lumbosacral epidural injection of 0.2 mL kg\(^{-1}\) for BW and the paired cadaver received 0.05 mL cm\(^{-1}\) (< 50 cm), 0.07 mL cm\(^{-1}\) (50 to < 70 cm), 0.08 mL cm\(^{-1}\) (70 to < 80 cm), or 0.11 mL cm\(^{-1}\) (≥ 80 cm mL cm\(^{-1}\)) for LE, of the mixture. A) Data without normality and B) Data transformed to achieve normality.
Figure 2.10 Correlation between number of vertebrae or distance (cm) reached by a volume of injectate of a 5:1 mixture of iopamide 30% and yellow tissue marking dye (B) in 22 cadavers matched by < 10% difference in body weight (BW) and length of the vertebral column (LE), and same body condition score, where one cadaver received a lumbosacral epidural injection of 0.2 mL kg<sup>-1</sup> for BW and the paired cadaver received 0.05 mL cm<sup>-1</sup> (<50 cm), 0.07 mL cm<sup>-1</sup> (50 to <70 cm), 0.08 mL cm<sup>-1</sup> (70 to <80 cm), or 0.11 mL cm<sup>-1</sup> (≥80 cm mL cm<sup>-1</sup>) for LE, of the mixture.
Figure 2.11 (a) Heart rate (HR), (b) respiratory rate ($f_R$), (c) systolic arterial pressure (SAP), (d) diastolic arterial pressure (DAP), (e) mean arterial pressure (MAP), (f) rectal temperature (TEMP), (g) end-tidal CO$_2$ (PE’CO$_2$), and (h) end-tidal isoflurane concentration (FE’Iso) in six dogs initially under general anesthesia (GA) for placement of an epidural lumbosacral catheter and injection of a 1:1 volume mixture of iopamide 30% and bupivacaine 0.5%, and after anesthesia (RE). The injection consisted of 0.2 mL kg$^{-1}$ for body weight (BW) on one occasion, or 0.05 mL cm$^{-1}$ (< 50 cm) or 0.07 mL cm$^{-1}$ (50 to < 70 cm) for length of the vertebral column (LE) on a different occasion in a randomized crossover fashion. Data are presented as mean ± standard deviation.
Figure 2.12 Comparison of number of vertebrae reached by a 1:1 mixture of iopamide 30% and bupivacaine 0.5% in six dogs administered a lumbosacral epidural injection of 0.2 mL kg\(^{-1}\) for body weight (BW) and 0.05 mL cm\(^{-1}\) (< 50 cm) or 0.07 mL cm\(^{-1}\) (50 to < 70 cm) for length of the vertebral column (LE) in a randomized crossover fashion, of the mixture.
Table 2.1 Demographic data of 22 dog cadavers matched by < 10% difference in both weight (BW) and length of the vertebral column (LE), and same body condition score (BCS), where one cadaver received an epidural injection of 0.2 mL kg\(^{-1}\) for BW and the paired cadaver received 0.05 mL cm\(^{-1}\) (< 50 cm), 0.07 mL cm\(^{-1}\) (50 to < 70 cm), 0.08 mL cm\(^{-1}\) (70 to < 80 cm), or 0.11 mL cm\(^{-1}\) (≥ 80 cm mL cm\(^{-1}\)) for LE, of a 5:1 volume mixture of iopamide 30% and yellow tissue marking dye, respectively.

<table>
<thead>
<tr>
<th>Size</th>
<th>Pair</th>
<th>Treatment</th>
<th>Breed</th>
<th>BW (kg)</th>
<th>LE (cm)</th>
<th>BCS</th>
<th>Volume (mL)</th>
</tr>
</thead>
<tbody>
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<td>3/5</td>
<td>0.92</td>
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<tr>
<td></td>
<td></td>
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<tr>
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<td>Hound</td>
<td>21.5</td>
<td>73</td>
<td>3/5</td>
<td>5.84</td>
</tr>
<tr>
<td>Large (25 to &lt; 45 kg)</td>
<td>1</td>
<td>BW</td>
<td>German Shepherd</td>
<td>25.0</td>
<td>75</td>
<td>3/5</td>
<td>5.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LE</td>
<td>German Shepherd</td>
<td>25.1</td>
<td>79</td>
<td>3/5</td>
<td>6.32</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>BW</td>
<td>Mixed breed</td>
<td>33.0</td>
<td>79</td>
<td>3/5</td>
<td>6.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LE</td>
<td>Mixed breed</td>
<td>34.0</td>
<td>80</td>
<td>3/5</td>
<td>8.80</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>BW</td>
<td>Rottweiler</td>
<td>40.0</td>
<td>91</td>
<td>3/5</td>
<td>8.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LE</td>
<td>German Shepherd</td>
<td>37.0</td>
<td>91</td>
<td>3/5</td>
<td>10.01</td>
</tr>
<tr>
<td>Giant (&gt; 45 kg)</td>
<td>1</td>
<td>BW</td>
<td>Mastiff</td>
<td>52.0</td>
<td>90</td>
<td>3/5</td>
<td>10.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LE</td>
<td>Great Dane</td>
<td>48.0</td>
<td>99</td>
<td>3/5</td>
<td>10.89</td>
</tr>
</tbody>
</table>
**Table 2.2** Rostral spread measured by number of vertebrae and distance (cm) from the lumbosacral space (LS) of a LS epidural dose calculated by body weight (BW; 0.2 mL kg\(^{-1}\)) or by length of the vertebral column (LE; 0.05 mL cm\(^{-1}\) [< 50 cm], 0.07 mL cm\(^{-1}\) [50 to < 70 cm], 0.08 mL cm\(^{-1}\) [70 to < 80 cm], 0.11 mL cm\(^{-1}\) [≥ 80 cm mL cm\(^{-1}\)]) using of a 5:1 volume mixture of iopamide 30% and yellow tissue marking dye, respectively, in dog cadavers matched according to < 10% difference in BW and LE, and same body condition score. Spread measured by CT imaging (iopamide) and necropsy of the spinal cord (dye).

<table>
<thead>
<tr>
<th>Size</th>
<th>Pair</th>
<th>Treatment</th>
<th>Number of Vertebrae</th>
<th>Distance from LS (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CT</td>
<td>Necropsy</td>
</tr>
<tr>
<td>Small (&lt; 10kg)</td>
<td>1</td>
<td>BW</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LE</td>
<td>21</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>BW</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LE</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>BW</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LE</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>BW</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LE</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>BW</td>
<td>13 ± 5 (T(_5)–T(_11))</td>
<td>16 ± 7 (C(_7)–T(_11))</td>
</tr>
<tr>
<td>(Range)</td>
<td></td>
<td>LE</td>
<td>18 ± 5 (C(_5)–T(_11))</td>
<td>24 ± 7 (C(_1)–T(_4))</td>
</tr>
<tr>
<td>Medium</td>
<td>1</td>
<td>BW</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>(10 to &lt; 25kg)</td>
<td></td>
<td>LE</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>BW</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LE</td>
<td>22</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>BW</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LE</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>BW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>(Range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large (25 to &lt; 45 kg)</td>
<td>1</td>
<td>BW</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LE</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>BW</td>
<td>21</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LE</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>BW</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LE</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td>Giant (≥ 45 kg)</td>
<td>1</td>
<td>BW</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LE</td>
<td>22</td>
<td>27</td>
</tr>
<tr>
<td>Overall mean ± SD</td>
<td></td>
<td>BW &amp; LE</td>
<td>19 ± 5</td>
<td>22 ± 6*</td>
</tr>
</tbody>
</table>

* Significantly different from CT ($p = 0.0041$).
Table 2.3 Demographic data of female Beagle dogs that received a lumbosacral epidural injection of 0.2 mL kg\(^{-1}\) for body weight (BW) and 0.05 mL cm\(^{-1}\) (< 50 cm) or 0.07 mL cm\(^{-1}\) (50 to < 70 cm) for the length of the vertebral column (LE) in a randomized crossover fashion of an equal volume mixture of iopamidol 30% and bupivacaine 0.5%.

<table>
<thead>
<tr>
<th>Dog (n)</th>
<th>FT</th>
<th>Age (years)</th>
<th>LE (cm)</th>
<th>BW1 (kg)</th>
<th>BW2 (kg)</th>
<th>Volume for BW (mL)</th>
<th>Volume for LE (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LE</td>
<td>5.3</td>
<td>52</td>
<td>10.2</td>
<td>10.2</td>
<td>2.04</td>
<td>3.64</td>
</tr>
<tr>
<td>2</td>
<td>BW</td>
<td>7.1</td>
<td>52</td>
<td>9.6</td>
<td>9.5</td>
<td>1.92</td>
<td>3.64</td>
</tr>
<tr>
<td>3</td>
<td>LE</td>
<td>5.3</td>
<td>46</td>
<td>7.5</td>
<td>7.5</td>
<td>1.50</td>
<td>2.30</td>
</tr>
<tr>
<td>4</td>
<td>LE</td>
<td>5.3</td>
<td>48</td>
<td>7.6</td>
<td>8</td>
<td>1.60</td>
<td>2.40</td>
</tr>
<tr>
<td>5</td>
<td>BW</td>
<td>8.3</td>
<td>55</td>
<td>9.6</td>
<td>9.6</td>
<td>1.92</td>
<td>3.85</td>
</tr>
<tr>
<td>6</td>
<td>BW</td>
<td>5.3</td>
<td>56</td>
<td>9.4</td>
<td>9.4</td>
<td>1.88</td>
<td>3.92</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Year</th>
<th>±</th>
<th>±</th>
<th>±</th>
<th>±</th>
<th>±</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT</td>
<td>6.1 ± 1.30</td>
<td>51.5 ± 3.9</td>
<td>9.0 ± 1.1</td>
<td>9.0 ± 1.0</td>
<td>1.81 ± 0.21</td>
<td>3.29 ± 0.74</td>
<td></td>
</tr>
</tbody>
</table>

FT = first assigned treatment. BW1 = first occasion. BW2 = second occasion. * Significantly different from volume for BW \((p = 0.0012)\).
Table 2.4 Time to presence of a positive response to noxious stimulation, consisting of clamping with a sponge forceps or a hemostat or electrical stimulation, of different dermatomes starting from caudal to cranial in six female Beagle dogs that received a lumbosacral epidural injection of 0.2 mL kg\(^{-1}\) for body weight (BW) and 0.05 mL cm\(^{-1}\) (≤ 50 cm) or 0.07 mL cm\(^{-1}\) (50 to < 70 cm) for the length of the vertebral column (LE) in a randomized crossover fashion of an equal volume mixture of iopamide 30% and bupivacaine 0.5%. AN1 = base of the tail, AN2 = pelvic limb paw, AN3 = area surrounding the iliac crest, AN4 = inguinal area near the femoral nerve, AN5 = area surrounding the transverse process of L\(_3\) and flank, AN6 = paramedian area next to the umbilicus, AN7 = along the thirteenth rib, AN8 = paramedian area to the xyphoid, AN9 = along the ninth rib, AN10 = along the second rib, and AN11 = thoracic limb paw.

<table>
<thead>
<tr>
<th>Dermatome</th>
<th>Time to presence of pain (minutes)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LE</td>
<td>55.2 ± 23.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BW</td>
<td>52.7 ± 23.4</td>
<td>52.7 ± 23.4</td>
</tr>
<tr>
<td>AN1</td>
<td>46.3 ± 23.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AN2</td>
<td>38.8 ± 23.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AN3</td>
<td>38.8 ± 23.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AN4</td>
<td>36.3 ± 23.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AN5</td>
<td>36.3 ± 23.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AN6</td>
<td>36.3 ± 23.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AN7</td>
<td>36.3 ± 23.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AN8</td>
<td>36.3 ± 23.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AN9</td>
<td>36.3 ± 23.4</td>
<td>40.2 ± 23.4</td>
<td></td>
</tr>
<tr>
<td>AN10</td>
<td>36.3 ± 23.4</td>
<td>37.7 ± 23.4</td>
<td></td>
</tr>
<tr>
<td>AN11</td>
<td>36.3 ± 23.4</td>
<td>37.7 ± 23.4</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>37.7 ± 20.4</td>
<td>49.0 ± 20.4</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 3

GENERAL DISCUSSION AND FINAL CONCLUSIONS
3.1 GENERAL DISCUSSION

This study had a main objective of investigating the differences in rostral spread between a LS epidural volume of injectate calculated on BW or LE in dogs of small size (BW ≤ 10.2 kg).

Epidural injections are used frequently in clinical practice in small animals to provide anesthesia and/or analgesia and often include a local anesthetic and an opioid. The rostral spread of the volume of injectate of those drugs in the epidural space will determine the number of dermatomes on which the specific drug will exert its effects, as dictated by the mechanism of action of the drug. Local anesthetics act on dural cuffs surrounding the nerve roots (Bromage 1967) as the spinal nerves emerge from the spinal cord; therefore, their action is highly dependent on reaching that site and their contact time, and involves complete block of all types of nerve fibres, including nociceptive fibres. The higher the rostral spread, the more numbers of dermatomes a local anesthetic can block, such that reaching lumbar segments can block pelvis and pelvic limb, whereas reaching low thoracic segments can also include the abdomen.

Conversely, opioids rely on absorption across the meninges into the CSF, which can occur all along the spinal cord and facilitates its actions on opioid receptors located at the dorsal horn, where it can block the actions of nociceptive fibres, mostly C fibres (Rawal & Sjörstrand 1986). For an epidural injection of small volume this absorption occurs at distal segments of the spinal cord and the distribution within the CSF is facilitated by any type of movement that agitates the CSF, such as breathing, the heartbeat, changes in body position, and coughing, which results in detectable CSF opioid concentrations throughout the length of the spinal cord (Strube et al. 1984; Valverde et al. 1992). Therefore, volume of injectate and rostral spread is less relevant for opioids.
Dosing using BW for epidural injections is more common and familiar to practitioners than using LE. Dosing with LE is less affected by body condition of the patient (BCS; i.e., obesity or thinness) because the length of the vertebral column is not affected by it, which can result in incorrect dosing if using BW. Although several studies have used LE dosing (Strande 1968; Hendrix et al. 1996; Otero et al. 2010; Leite et al. 2017), there have been no comparisons between the two methods to determine the degree of rostral spread and which method should be preferred.

A mathematical study compared final volumes of injectate for different dog size categories and BCS when using BW versus LE (from the occipital crest to the sacrococcygeal space); small (< 10 kg) and medium (10 to < 25 kg) size dogs had a larger calculated volume using LE than for BW, but these differences were not present for dogs > 25 kg (Valverde & Skelding 2019). In a clinical trial with dogs between 13.2–22 kg, using a crossover, randomized design, the volume of injectate was approximately 50% higher when doses were dosed based on LE versus BW, and the degree of sensory blockade was more rostral and longer lasting (Leite et al. 2017).

None of these studies has precisely determined the degree of rostral spread in the epidural space with the use of imaging or necropsy techniques using indicators, such as contrast medium or dye, to corroborate if differences in volumes of injectate between methods of dosing result in differences in rostral spread. Thus, this study aimed to investigate the differences in rostral spread between a LS epidural volume of injectate calculated on BW or LE in dogs of small size (< 10.2 kg), where resultant calculated volumes have been shown to be significantly different (Valverde & Skelding 2019).

In the first phase of the current study, cadaver dogs were used as a pilot study, in which dogs of different sizes were assigned to specific categories used in a mathematical study (Valverde & Skelding 2019), which included small dogs (< 10 kg), medium dogs (10 to < 25 kg), large dogs
(25 to < 45 kg), and giant dogs (> 45 kg), but special emphasis was placed in the small and medium dogs. Dogs within categories were paired for weight, length of the vertebral column and BCS, within < 10% difference in those characteristics, and within each pair one dog was dosed with a mixture of contrast medium and dye per LE and the other dog per BW. This was completed, to compare rostral spread using imaging (contrast medium) and necropsy (dye). Findings from this study demonstrated that the volume of injectate was larger for LE than BW and resulted in a trend for small and medium size dogs to have more rostral spread when dosed by LE versus BW. A limitation of this phase was that, as a pilot study, the sample size did not provide enough power to reach statistical significance.

In this phase of the study, it was also demonstrated that dye behaved differently from contrast medium because the rostral spread was significantly higher for dye than contrast medium, and that for both indicators, the distribution around the spinal cord is not uniform in most cases. These findings emphasize the idiosyncrasy of the epidural space and factors that interfere with the spread of any substance, including anatomical structures such as meningovertebral ligaments, epidural fat, presence of intervertebral foramina, differences in diameter along the spinal canal and differences in the diameter size of the spinal cord (Ramsey 1959; Burn et al. 1973; Park et al. 1980; Fletcher & Kitchell 1966; Geers et al. 2003). Based on our results, studies using dye or contrast should consider the differences between both indicators for rostral spread and that they are should not be used interchangeably.

The second phase of the study investigated the two methods in live dogs of small size. The objective was to determine if rostral spread of local anesthetic combined with contrast medium differs from findings of cadaver dogs in the first phase, by using a blinded, randomized, crossover design.
Results from this phase demonstrated that the volume of injectate for small size dogs was significantly larger for LE than BW, which also resulted in a significantly higher rostral spread of contrast medium. It was interesting to find out that, regardless of the method of dose calculation, the spread exceeded the predicted dermatomes of up to T_{10}, based on the original studies by Strande (1968). In this study, the range of vertebrae for the LE dose was C_{4}–T_{1} (22 ± 2 vertebrae; where 22 = C_{6}) and for BW was C_{5}–T_{5} (19 ± 2 vertebrae; where 19 = T_{2}), both groups with a higher rostral spread than what was previously predicted.

The degree of rostral spread for contrast medium was higher for live dogs than cadavers, from C_{5}–T_{11} (18 ± 5 vertebrae; where 18 = T_{3}) for LE, and from T_{5}–T_{11} (13 ± 5 vertebrae; where 13 = T_{8}) for BW, in the cadavers. Therefore, findings from cadaver and live dogs using contrast medium were not interchangeable. This discrepancy results from dynamic forces in live dogs that promote rostral spread of the contrast medium by affecting CSF motion and pressures in the epidural space, which include compliance of the spinal canal, physical activity and position of the individual, the cardiovascular dynamics (blood pressure, cardiac output, heart rate), respiratory function (rate and effort of breathing) and systemic absorption of the contrast medium (Higuchi et al. 2005; Son et al. 2011; Sánchez et al. 2018).

The high rostral spread with either dosing method, was expected to cause cardiorespiratory variables and rectal temperature changes. However, the values were within acceptable limits and similar in both groups. In one study, rectal temperature was lower in a group of conscious dogs dosed by LE when compared to dogs dosed by BW, but still ≥ 37°C and assumed to be the result of sympathetic block and vasodilation (Leite et al. 2017). It is likely that in this study no changes were detected because the resultant concentration of bupivacaine of 0.25% was ineffective in blocking sympathetic fibres. Concentrations of at least 0.5% have been shown to effectively cause
motor and sensory block (Axelsson et al. 1989; Gómez de Segura et al. 2000). Similarly, a bupivacaine concentration of 0.25% was also shown to increase its effectiveness if a higher dosing volume (0.4- and 0.6 mL kg⁻¹) was used (Freire et al. 2010). The analgesic effects of bupivacaine 0.25% in this study were probably missed, for the same reasons mentioned above, as well as the analgesia was only assessed after approximately 40 minutes post epidural injection, which likely resulted in missing the initial sensory blocking effects of a weaker bupivacaine concentration. Other studies using 0.25% bupivacaine have also demonstrated only an incomplete sensory block of short duration (Eddleston et al. 1996; Gómez de Segura et al. 2000; Freire et al. 2010).

This study had some limitations; the small number of cadavers used in the first phase of the study, limited the type of statistical comparisons between individuals and size categories. However, the purpose of this phase was to corroborate findings of the mathematical study (Valverde & Skelding 2019) and to provide basis to support the need of comparison of methods in dogs of small size, where greater differences in resultant volume of injectate could be of major impact on the resultant pharmacodynamic effects of epidural local anesthetics.

Other limitation was found in the live dog study with the time of assessment for the pharmacodynamic effects, regarding analgesia, motor block, and cardiorespiratory effects. In the design of the study, it was deemed necessary to have the dogs under general isoflurane anesthesia to place the epidural catheter, position the dogs in sternal recumbency, and obtain consistent CT images, which resulted in a recovery phase that did not allow for the prompt assessment of the effects of bupivacaine. The second limitation of this phase of the study was the need to dilute the bupivacaine 0.5% to 0.25% by adding an equal volume of the contrast medium. The latter resulted in appropriate imaging but decreased the effectiveness of bupivacaine’s actions. On the positive side, it allowed for this study to demonstrate that bupivacaine 0.25% does not cause a complete
sensory block, at least during the assessment approximately 40 minutes post injection, and that the effects in general are likely short lasting with this concentration.
3.2 FINAL CONCLUSIONS

The investigations performed and discussed here demonstrated the following results:

1. For small size dogs (< 10 kg), the volume of injectate for LS epidural injections was higher when calculated with LE than BW.

2. In cadaver dogs, dye had more rostral spread during necropsy than contrast medium during CT, regardless of the method of volume calculation.

3. Rostral spread of contrast medium assessed by CT was higher when dosing by LE than BW in small live dogs.

4. Rostral spread of contrast medium was higher in live dogs than in cadavers, regardless of the method of volume dosing.

5. Bupivacaine 0.25% was less effective than the reported effects of bupivacaine 0.5% for complete sensory block.

6. Cardiorespiratory function was similar for LE and BW after bupivacaine 0.25% injection, despite differences in the volume of injectate.
3.3 REFERENCES


