Comparison of three techniques for paravertebral brachial plexus blockade in dogs

Eva Rioja*, Melissa Sinclair*, Heather Chalmers*, Robert A Foster† & Gabrielle Monteith*

* Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Canada.

† Department of Pathobiology, Ontario Veterinary College, University of Guelph, Canada.

Correspondence: Eva Rioja, Department of Companion Animal Clinical Studies, Faculty of Veterinary Sciences, University of Pretoria, Private bag X04, Onderstepoort, 0110, Pretoria, South Africa.

Tel: +27 125298200

Fax: +27 125298307

E-mail: eva.riojagarcia@up.ac.za

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Abstract

Objective To compare success and complication rates, based on staining of nerves and other structures, among three techniques of paravertebral brachial plexus blockade (PBPB) in dogs.

Study design Prospective randomized design.

Animals A total of 68 thoracic limbs from 34 dogs.

Methods Limbs were randomly assigned to blind (BL) (n=24), nerve stimulator-guided (NS) (n=21) or ultrasound-guided (US) (n=23) technique. Injections were made with 0.3 mL kg^{-1} of lidocaine mixed with new methylene blue. Time to perform each block and current used during NS technique were recorded. Dogs were anesthetized during the blocks and euthanized once completed. Dissections were performed to evaluate staining of nerves, spinal cord, mediastinum, pleura and vessels. An ANOVA and Tukey adjustment for time, logistic regression for association between current and nerve staining and a generalized linear mixed model for staining of different structures were used. Significance was considered when $p \leq 0.05$.

Results The median (range) number of nerves stained was 2 (0-4) with BL, 1 (0-3) with NS and 1 (0-4) with US guided technique. No significant differences in staining of C6, C8 and T1 or other structures were found among techniques. Nerve C7 was more likely to be

stained by BL (p=0.05). Time to perform the blocks was significantly different among techniques, with mean ± SD duration in minutes of 3.6 ± 1.8 with BL, 6.3 ± 2.7 with US and 12.2 ± 5 with NS. The most common complication was staining of the spinal cord (29%, 38% and 39% with BL, NS and US, respectively).

Conclusions Success rates were low and complication rates were relatively high, based on staining, with the three techniques.

Clinical relevance The use of more advanced techniques for PBPB in dogs is not justified according to this study. Clinical significance of the complications encountered in this study should be evaluated.

Keywords: paravertebral brachial plexus block, ultrasound guided, nerve stimulator, dog

Introduction

Over the past several years, regional anesthetic techniques (RAnT) are increasingly being used in combination with systemic analgesics during small animal anesthesia and surgery in order to provide multimodal analgesia, which reduces intra-operative anesthetic requirements and improves post-operative pain relief (Wenger et al. 2005; Mosing et al. 2010). Decreasing the doses of general anesthetics and other drugs may allow more stable cardio-respiratory function during anesthesia and the development of fewer side effects. In humans, it has been shown that RAnT provided superior pain relief and improved perioperative outcomes, including a shortened hospital length of stay and a significant

reduction in postoperative urinary retention and ileus formation, compared to systemic opioids (Singelyn et al. 1998; Capdevila et al. 1999; Hebl et al. 2008).

However, performing RAnT is not free of risks. These techniques may be associated with neurological complications such as neuropathy due to intra-neural injection or non-neurological complications, such as systemic toxicity due to intra-vascular injection or hematoma formation. In humans, the reported incidence of neuropathy ranges from 0.22% to 2.84% after peripheral nerve blocks (Brull et al. 2007; Watts & Sharma 2007) and from 0.022% to 0.038% after epidural or spinal block, respectively (Brull et al. 2007). The incidence of systemic toxicity after peripheral nerve blocks is reported to be 0.075% (Faccenda & Finucane 2001). No such information is available in the veterinary literature.

There are three techniques to perform RAnT: blind needle placement using anatomical landmarks (BL), using a peripheral nerve locator or nerve stimulator (NS) and ultrasound (US) guided needle placement. In humans, introduction of US guidance for RAnT has greatly improved the success rates, shortened performance and onset time, prolonged duration of the block and decreased the incidence of block-related complications, such as inadvertent vascular puncture, compared to other techniques (Liu et al. 2005; Chan et al. 2007; Koscielniak-Nielsen 2008; Abrahams et al. 2009). The use of US allows clear visualization and localization of nerves, provides real-time guidance for needle movement and allows observation of local anesthetic spread around the nerve, enabling a decrease of the total dose of local anesthetic used (Marhofer et al. 2005).

Brachial plexus nerve blockade (BPB) is performed in small animals undergoing surgery of the thoracic limb in order to desensitize the nerves that provide sensory and motor innervation. The brachial plexus may be desensitized once the individual nerves have been formed close to the axillary artery at two different levels, either at the level of the scapulo-humeral joint (Futema et al. 2002; Campoy et al. 2008) or cranial to the acromion (Mahler & Adogwa 2008). These two techniques are usually referred to as axillary BPB (ABPB). A more distal technique has been described to desensitize the individual nerves that innervate the thoracic limb (radial, ulnar, median and musculocutaneous nerves) at the level of the mid-humerus, called RUMM block (Trumpatori et al. 2010). Another technique blocks the spinal nerves that form the brachial plexus as they emerge from the spinal cord at the level of the intervertebral foramina (IVF), which is called paravertebral BPB (PBPB) (Lemke & Dawson 2000). Anesthesia distal to mid-humerus is theoretically obtained with both ABPB techniques and the RUMM block, whereas the PBPB will theoretically anesthetize the whole limb, allowing surgery of the humerus and shoulder joint.

In the veterinary literature there are only a few published studies on the use of US during BPB in dogs. One study describes the ultrasonographic appearance of the brachial plexus (Guilherme & Benigni 2008). Another describes the appearance and approach for PBPB with US guidance (Bagshaw et al. 2009). Another study describes the US guided technique for ABPB, femoral and sciatic nerve blocks (Campoy et al, 2010).

To our knowledge, no canine studies have compared the success rates and/or incidence of block-related complications among the three described techniques to achieve PBPB. In addition, no data currently exists to demonstrate any benefit of the more

advanced regional anesthesia techniques compared to the BL technique for PBPB in dogs. The objectives of the present study were to compare the success rates of nerve staining and complication rates, based on staining of other structures, with a new methylene blue solution of the BL, NS guided and US guided techniques for PBPB in dogs. Our hypothesis was that more advanced techniques, such as NS and US guided techniques, would have a greater success rate and lower incidence of complications compared with the BL technique.

Material and methods

A total of 34 isoflurane-anesthetized adult dogs, used in student wet-labs for unrelated purposes and scheduled to be euthanized, were used in this study. The protocol was approved by the institutional animal care committee and animals were maintained in accordance with animal care guidelines prior to anesthesia. Both thoracic limbs were used for the study; therefore, a total of 68 limbs were blocked. The side where the block was performed was randomly selected by block randomization, so approximately the same number of times the right and left sides were blocked with one of three techniques of PBPB, including BL (n=24; right=11; left=13), NS guided (n=21; right=11; left=10) and US guided (n=23; right=12; left=11).

Two board certified anesthetists performed the BL, one board certified anesthetist performed the NS guided and one board certified radiologist performed the US guided technique. The anesthetists performing the BL and NS techniques had clinical experience with this and other blocks in dogs. The radiologist performing the US technique had clinical experience in musculoskeletal ultrasonography and had practiced on three dog cadavers previous to the study to become familiarized with the landmarks and technique.

For blockade of the left side dogs were placed in right lateral recumbency and vice versa. The ventral branches of the cervical nerves 6 (C6), 7 (C7) and 8 (C8), and the thoracic nerve 1 (T1), were blocked using a total volume of 0.3 mL kg^{-1} of a solution consisting of lidocaine 20 mg mL^{-1} (Xylocaine, AstraZeneca, Canada) mixed with the same volume of new methylene blue 10 mg mL^{-1} (Methylene blue 1%, Omega Laboratories Ltd., Canada), which resulted in a final concentration of 10 mg mL^{-1} for lidocaine and 5 mg mL^{-1} for methylene blue. Blockade of nerves C6 and C7 was performed with individual injections of 0.1 mL kg^{-1} each at the level of the IVF and blockade of nerves C8 and T1 was performed with a third injection of 0.1 mL kg^{-1} cranial to the first rib. With any technique, aspiration before injection of the solution was performed to avoid intravascular injection and if resistance to injection was encountered, as assessed subjectively by the person performing the block, the needle was slightly repositioned to avoid intrafascicular injections. Time taken to perform each block was recorded for later analysis. Time taken to perform the blocks was defined as the interval in minutes from palpating landmarks (BL and NS) or placement of US probe (US) to completion of all the injections.

For the BL technique, the modified technique for PBPB described by Lemke and Creighton (2008) was used. Briefly, for this technique the transverse process of the sixth cervical vertebra was identified by palpation and a regular hypodermic needle (22-gauge 3.8 cm for dogs < 13 kg, 20-gauge 7.6 cm for dogs > 13 kg) was inserted dorsoventrally at a 30-45 degree angle with respect to the sagittal plane of the dog, parallel to a transverse plane of the dog (perpendicular to the sagittal plane that divided the dog into cranial and caudal parts), until the needle contacted the transverse process. The needle was then

reoriented to become parallel to the sagittal plane and advanced cranially to the transverse process, where an injection was made in order to block C6. The needle was then reoriented caudally to the transverse process and a second injection was made to block C7. For blockade of C8 and T1, the first rib was palpated and the needle was inserted parallel and cranial to it, slightly dorsal to the spine of the scapula, and was directed ventrally at a 30° angle with respect to the sagittal plane of dog. Injection was made when the needle was at a level dorsal to the costochondral junction.

For the NS technique, the same landmarks as for the BL technique were used. An insulated needle (22-gauge 5 cm for dogs < 13 kg, 21-gauge 10 cm for dogs > 13 kg) (Pajunk GmbH, Germany) connected to a nerve stimulator (Innervator 242, Fisher and Paykel Healthcare Ltd., New Zealand) was inserted at each of the three sites described for the BL technique, using the same landmarks, and electrical stimuli of 1 mA current, 0.1 msecond duration, at a frequency of 1 Hz were delivered. When typical muscle contractions for each of the nerves occurred, the current was decreased in decrements of 0.2 mA until contractions disappeared. Once muscle contractions disappeared, the current was increased by 0.2 mA to obtain muscle contractions again and the solution was injected. No minimum current was set for the injection. The current when muscle contractions disappeared for the first time was recorded for statistical analysis.

An US machine with a 12.5 MHz linear array transducer (Phillips HDI 5000, Phillips Medical Systems, WA, USA) was used for the US technique. Isopropyl alcohol (70%) was used as an acoustic coupling agent. A non-sterile probe cover was used to prevent damage to the transducer by the new methylene blue solution. The transverse

process of the sixth cervical vertebra was used as the initial ultrasound landmark in all dogs, which was identified by scanning in a cranial to caudal direction centered on the ventrolateral aspect of the vertebrae, with the probe parallel to the dorsal plane of the dog (perpendicular to the sagittal plane that divided the dog into dorsal and ventral parts) (Figure 1). Once this transverse process was identified, the probe was moved slightly dorsally in order to identify the IVF between the fifth and sixth cervical vertebrae in a longitudinal plane. At this site, a pulsating vessel (artery) was typically seen and a hypoechoic rounded structure without color Doppler flow signal was sometimes seen (nerve). The needle was inserted at this level in order to block nerve C6 and the injection was performed close to the nerve and avoiding penetration of the artery. From this probe position, the scan plane was then moved slightly ventrally to relocate the transverse process of the sixth cervical vertebra and the probe was rotated 90 degrees to image the IVF between the sixth and seventh cervical vertebrae in a transverse plane. The artery and nerve at this site were identified and the injection to block nerve C7 was performed close to the nerve and avoiding the artery. The probe was then repositioned for the blockade of nerves C8 and T1 as follows: in the first 8 dogs the probe was placed on the cranial aspect of the thoracic inlet, just medial and ventral to the shoulder, parallel to the first rib. The first rib was identified as a flat echoic linear interface that did not move with respiration and cast a complete shadow. The needle was inserted from dorsal to ventral and was aimed at the mid third of the first rib until a bony resistance was felt, and then it was retracted slightly for injection. For the remainder dogs (n=15), the approach to this area was altered due to the low success rate for staining of nerves C8 and T1 on the other dogs. The probe was aligned at the cranial aspect of the thoracic inlet in a slightly oblique direction and the axillary

artery and vein were identified and followed until a bundle of hypoechoic rounded structures with echoic septations (having a honeycomb appearance) consistent with the nerves as described by Guilherme and Benigni (2008) were identified (Figure 2). The needle was guided from craniodorsolateral to caudoventromedial to the "honeycomb" region and the injection was performed with the needle adjacent to, but not touching, the nerve bundle. The nerves were not always visualized. The needle was in plane with the US beam for the C6 and C7 nerves. For nerves C8 and T1, it was in plane for the first 8 dogs and out of plane for the following 15 dogs.

When the blocks in both limbs were completed, dogs were euthanized with an overdose of pentobarbital sodium and cooled to 4°C in a refrigerator overnight. Approximately 12-14 hours post-euthanasia, the nerves of the brachial plexi were dissected close to the IVF and first rib by a board certified pathologist, who was blinded to the technique used. The number of spinal nerves stained by the solution of new methylene blue was recorded. Additionally, presence of new methylene blue stain in the visceral pleura, inside the mediastinum, around the spinal cord and around blood vessels in the area was recorded.

Statistical analysis

Data were analyzed using statistical software (SAS, version 9.1.3, SAS Institute Inc, NC, USA). Normal distribution of data was checked using a Shapiro-Wilk test. Descriptive statistics were performed and presented as mean ± SD and as median (range) for normally and non-normally distributed parameters, respectively. Time taken to perform each block was analyzed with an ANOVA and post hoc Tukey adjustment. A generalized linear mixed

model (GLIMMIX), accounting for the random effect of dog and the fixed effect of side and technique, was used to evaluate possible differences among techniques in the number of nerves stained and staining of individual nerves, spinal cord, mediastinum, pleura and vessels. Exact conditional logistic regression was used to evaluate a possible association between current used during the NS technique and successful nerve staining. The possible association between dog breed (large versus small) or weight and the number of nerves stained was tested using exact conditional logistic regression and Spearman correlation analyses, respectively. A Fisher exact test was used to compare the rate of staining of the C8-T1 nerves with the two different ultrasound approaches. A standard t-test was performed to compare the number of nerves stained by both anesthetists performing the BL technique. Statistical significance was considered when $p \leq 0.05$.

Results

The mean ± SD weight of the dogs was 14.6 ± 9.9 kg. Breeds of dogs consisted of 10 Hounds and one Labrador weighing 26.8 ± 6.2 kg and 23 Beagles weighing 9.5 ± 2.7 kg. The BL technique was performed in 9, NS technique in 5 and US technique in 8 of the big dogs. Dog breed (large versus small) or weight did not have a significant effect on number of nerves stained with all techniques pooled or individual techniques.

The mean ± SD time taken to perform each technique was 3.6 ± 1.8 minutes for BL, 6.3 ± 2.7 minutes for US and 12.2 ± 5 minutes for NS guided technique. Time to perform BL was significantly shorter than to perform NS ($p<0.0001$) and US ($p=0.0083$) techniques, and to perform US technique was significantly shorter than to perform NS technique ($p<0.0001$).

The visualization of the ultrasonographic landmarks was as follows: the C5-C6 IVF was seen in all but two dogs (both of these were large breeds), the C6 transverse process was seen in all dogs and in one very small dog the C6-C7 IVF was not visualized. The first rib was visualized in all dogs (n=8), as were the axillary vessels (n=15); however, the nerve plexus at C8-T1 was visualized in only four dogs (all small breeds). When the two different ultrasound approaches to the C8-T1 region were compared, the first group of dogs having the first rib used as the landmark had staining of the nerve C8 3/8 times and of the nerve T1 3/8 times, and the second group having the vessels used as a landmark had staining of the nerve C8 5/15 times and of the nerve T1 3/15 times; however, the rate of staining of both nerves was not statistically significant between approaches ($p=0.52$). Therefore, data from both US approaches were pooled for further analysis.

No significant difference in the number of nerves stained was found between the two anesthetists performing the BL technique ($p=0.7$); therefore, data from both anesthetists were pooled for further analysis.

The median (range) number of nerves stained with each technique was 2 (0-4) with BL, 1 (0-3) with NS guided and 1 (0-4) with US guided technique. The effect of side (right versus left) where blocks were performed was not significant in the test of fixed effects for number of nerves stained. There was a trend towards significance for effect of technique in the test of fixed effects for number of nerves stained ($p=0.06$). The results of the GLIMMIX procedure for nerve staining are summarized in Table 1. Briefly, the BL technique was 2.37 and 2.25 times more likely to stain a greater number of nerves than the NS and US techniques, respectively.

For the staining of individual nerves, there was no significant effect of side or technique for nerves C6, C8 and T1. For nerve C7, there was a significant effect of side ($p=0.047$) and technique ($p=0.05$). The left nerve C7 was 2.78 times more likely to be stained than the right, and BL technique was 3.69 and 4.2 times more likely to stain nerve C7 compared to NS and US techniques, respectively. For staining of other structures (pleura, mediastinum, spinal cord and vessels), there was no significant effect of side or technique. Nerves C3 and C4 were occasionally stained only with US technique. Nerve C5 was stained frequently with all techniques and there was a significant effect of side ($p=0.003$) and technique ($p=0.001$). The left nerve C5 was nine times more likely to be stained than the right. Nerve C5 was stained significantly fewer times with the NS guided technique. The rates for staining of individual nerves and other structures with each technique are summarized in Table 2.

The median (range) current when muscle contractions ceased before injection was performed during the NS guided technique was 0.4 (0.0-0.8) mA for C6 and C7 and 0.6 (0.0-0.8) for C8 and T1. There was a significant association between current and probability of nerve staining ($p=0.0014$), with lower currents being associated with greater chances of nerve staining (Figure 3). Eight dogs developed diaphragmatic contractions (hiccups) while searching for nerves C6 or C7 during NS guided technique. The needle was redirected and injections made when no diaphragmatic contractions were obtained.

Discussion

The present study compares, for the first time in the veterinary literature, the success and complication rates, based on staining of nerves and other structures, of three different

techniques to perform PBPB in dogs. The success rates for staining all 4 targeted nerves of the brachial plexus were low for the three techniques; specifically they were 17% with BL, 0% with NS and 9% with US guided techniques. The rates of staining of other structures were similar for the three techniques, with staining of the spinal cord being the complication with the highest incidence. Cervical nerve 5 was frequently stained with all techniques, but BL had the highest incidence.

Paravertebral BPB is important to obtain better pain relief and to improve post-operative patient comfort when surgeries of the shoulder or humerus are performed. There are only a few studies in the veterinary literature describing the PBPB in dogs (Lemke & Dawson 2000; Hofmeister et al. 2007; Lemke & Creighton 2008; Bagshaw et al. 2009; Guilherme & Benigni 2008). In a previous study, the reported rate of successful staining of the four nerves with the BL technique was 33% (Hofmeister et al. 2007), which is also low. However, in that same study the nerve C6 was successfully stained 100% of the times. The difference in success rates of nerve staining between our study and the one by Hofmeister et al. (2007) with the BL technique could be due to several factors. Firstly, in their study the volume of injectate used was fixed to 3-5 mL per site in dogs weighing 10-30 kg. This volume was much higher than the volume we used, especially in small dogs, and therefore the spread of the local anesthetic was probably much greater in their study. A total injectate volume of 0.3 mL kg^{-1} was used in the present study as this is the volume of local anesthetic recommended in clinical practice for ABPB (Campoy et al. 2008). Using a fixed volume of injectate regardless of weight is not clinically applicable as the local anesthetics have the potential to cause systemic toxicity if administered at high doses. Secondly, the

technique used to block the nerves C8 and T1 differed from our technique in that they injected at the level of the IVF in all 4 nerves, yielding 4 separate injections, whereas we injected cranial to the first rib to block C8 and T1 in a combined approach as described by Lemke and Creighton (2008). Thirdly, it is possible that the person performing the blocks had more experience in Hofmeister et al's study than in our study. The person performing the BL approach in our study was always an anesthesiologist with experience doing these blocks in clinical practice. The first 8 BL blocks were performed by ER and the rest by MS; however, there was no significant difference in the success rate for nerve staining between the two anesthetists and therefore this was likely not an important source of variability. The PBPB has been introduced into clinical practice quite recently; therefore, it is possible that as this block is performed more often, the skills and the success rates will improve.

The NS guided technique for PBPB has been previously described (Lemke and Creighton 2008), but no reports of success rates have been published to our knowledge. The use of NS during ABPB in dogs and cats has been also described in several research and clinical studies with high staining (Campoy et al. 2008) and clinical (Futema et al. 2002; Wenger et al. 2005; Mosing et al. 2010) success rates. However, when the NS guided technique was compared to the BL technique for ABPB, both had similar rates of staining of nerves with no significant differences between them (Ricco et al. 2008; Wilson et al. 2008). In humans, a meta-analysis showed that NS guided techniques for ABPB improved the success rate when three or more nerves were stimulated and it decreased the incidence of systemic local anesthetic toxicity compared to BL techniques (Guay 2005). One possible explanation for the low rate of nerve staining obtained in our study with the NS technique

during PBPB is that an inappropriate endpoint was used to determine correct needle placement as we did not set a minimum target current for injection. In our study, injections were made even at currents up to 0.8 mA if the muscle response was good. In the statistical analysis of our data, the logistic regression analysis showed that there was an inverse association between current and probability of nerve staining, which is in accordance with a previous study in humans (Carles et al. 2001). Therefore, it is likely that the rate of nerve staining with NS guidance would have improved if the injections were made at current thresholds of ≤ 0.4 mA. This has been shown in studies in humans, where they demonstrated a high degree of successful block with motor endpoints of ≤ 0.5 mA using an insulated needle and a pulse frequency of 1-2 Hz and 0.1 milliseconds duration (Neuburger et al. 2001, Lang 2002). However, in another study in humans they determined that the sensitivity of a motor response to electrical nerve stimulation at ≤ 0.5 mA was only 74.5% for detection of needle-to-nerve contact, which was confirmed by US imaging (Perlas et al. 2006). In contrast, low current endpoints between 0.2-0.4 mA are also associated with a high frequency of intraneural needle placement, which could lead to neural injury (Robards et al. 2009). It is also important to note that in humans these techniques are usually performed in conscious individuals that can describe their sensations, and the presence of paresthesia alone or in combination with NS guidance has been used in some studies as an endpoint for injection, which may have improved their success rates. Another possible explanation for the low rate of nerve staining with NS guidance in our study, despite successful muscle contractions obtained, is that the needle tip may have been in a different fascial plane than the nerve, and therefore strong muscle contractions could be still elicited but there was a significant diffusion barrier between the point of injection of the solution

and the targeted nerve (Lang 2002). Overall, the time and degree of NS needle movement required to locate the nerves in our study would make this technique the least appropriate of the three in clinical anesthetized canine patients.

There are two studies in dogs that describe the ultrasonographic anatomy of the nerves that form the brachial plexus at their exit from the IVF (Guilherme & Benigni 2008, Bagshaw et al. 2009). In the study by Bagshaw et al. (2009) they also determined the precision and spread of US guided injections of contrast medium around the nerve roots of C6, C7 and C8 with computed tomography; however, they did not visualize or evaluate the nerve T1, which also contributes to the brachial plexus in dogs.

The low success rates found in our study were unexpected, especially for the US guided technique. In humans, the use of ultrasound guidance during regional anesthesia has improved the clinical success rates and decreased the complication rates compared to neuro-stimulator guidance (Liu et al. 2005; Chan et al. 2007; Koscielniak-Nielsen 2008; Abrahams et al. 2009). In cadaveric dogs, the US guided technique for PBPB resulted in 100% successful staining of the nerve roots C6, C7 and C8 (Bagshaw et al. 2009). This difference between studies could be due to ultrasonographer experience, ultrasound machine and /or type of probe used. It is likely that as the operator performing US guided PBPB becomes more experienced with this block the rate of nerve staining will improve. Nonetheless, in a study in humans they observed that the success rates of block when anesthesia residents perform an US guided interscalene block, which is analogous to the PBPB in dogs, was similarly high (97%) at the beginning and the end of a 4-week supervised rotation, and that the only parameter that improved was the time needed to

image the nerves and to perform the block (Orebaugh et al. 2009a). In order to avoid a possible decrease in success due to lack of experience with the US technique, a board certified radiologist with experience in musculoskeletal US and US guided injections performed all the US blocks in the present study.

In the present study some of the challenges found during the US technique included: 1) the US transducer footprint was large and necessitated shallow and long angles, especially in small dogs, 2) the head and the shoulder prevented movement of the US transducer, especially in small dogs, 3) the shoulder prevented caudal injection at the IVF between the 6^{th} and 7^{th} vertebrae, making it necessary to use a ventrodorsal approach, and 4) the first rib may be easy to confuse with the medial aspect of the scapula especially in small dogs where they are closely situated. It is possible that some of these challenges prevented us from obtaining a higher rate of nerve staining with this technique, especially since most of the dogs were small. In humans, many clinical studies report problems in obtaining satisfactory nerve US images in some patients (Koscielniak-Nielsen 2008). The concomitant use of a nerve stimulator to confirm nerve location is used in some human studies and is reported to be especially useful for residents being trained in US guided blocks (Koscielniak-Nielsen 2008). This combination of techniques could also prove useful in veterinary patients and warrants further investigation.

The time needed to perform the BL technique in the present study was much shorter than to perform the other two techniques, especially the NS guided technique, which is an important factor in clinical practice, added to the fact that no specialized equipment is required. The NS guided technique proved to be the longest to perform and the most

challenging of the three in the present study, as it required multiple needle passes until the desired motor response was elicited. In humans, US guidance to perform peripheral blocks significantly shortened the time needed to complete the block and reduced the number of needle passes required to reach the target in all comparative studies with NS guided blocks (Koscielniak-Nielsen 2008; Abrahams et al. 2009).

Some potential clinical complications associated with PBPB in animals extrapolated from complications observed in humans following an analogous block include: epidural or spinal anesthesia, Horner's syndrome, diaphragmatic hemiparesis secondary to phrenic nerve block and hiccups (Dutton et al. 1994, Aramideh et al. 2002, Gomez and Mendes 2006, Riazi et al. 2008). In the present study, the most common complication was presence of dye around the spinal cord (29%-39%) with all techniques, especially close to the exit of C6 and C7 nerves, where the injections were made at the level of the IVF. In humans, cervical epidural or spinal anesthesia (Dutton et al. 1994; Aramideh et al. 2002; Gomez and Mendes 2006) and brainstem toxicity (Durrani & Winnie 1991) have been reported after a brachial plexus block using a similar approach. Epidural and/or spinal anesthesia at the cervical level could lead to life-threatening respiratory and cardiovascular depression in clinical cases (Aramideh et al. 2002). Intra- or post-operative respiratory and cardiovascular functions were not evaluated in this study as dogs were euthanized immediately after the blocks, but it is recommended that they be closely monitored in clinical cases.

In the present study, staining of the phrenic nerve was not evaluated. In dogs the phrenic nerve originates from ventral branches of C5, C6 and C7 nerves and runs medial to

the brachial plexus (Lemke and Creighton 2008). Therefore, blockade of this nerve is very likely whenever the PBPB is performed in clinical cases. Diaphragmatic paralysis occurs in 100% of humans following the interscalene block using high volumes of local anaesthetic and the incidence is reduced to 45% when the volume is decreased (Riazi et al. 2008). Similarly in cadaveric dogs, the incidence of phrenic nerve staining with US guided PBPB was 20% when 3 mL of solution was used versus 0% when 0.3 mL was injected at each nerve root (Bagshaw et al. 2009). Acute phrenic nerve blockade does not seem to impair ventilation in awake or sleeping dogs when it is unilateral, but it could potentially lead to hypoxia and respiratory distress, especially in patients with limited respiratory reserve or when the block is bilateral resulting in complete diaphragmatic paralysis (Stradling at al. 1987; Riazi et al. 2008).

Seizures have also been reported after brachial plexus blockade in humans (Orebaugh at al. 2009b), which was probably due to intravascular injection of the local anesthetic. Perivascular staining was observed in two dogs in the present study, but no neurologic signs were observed. It is likely that the solution had not been injected intravascularly as aspiration before injections were performed. Less frequent is the occurrence of pneumothorax following brachial plexus blockade in humans, which has been reported even with the use of US guidance (Bhatia et al. 2010). In the present study, presence of stain in the visceral pleura was observed in 4%-13% of dogs, indicating that thoracic puncture had occurred, which could lead to pneumothorax in clinical cases. Presence of stain inside the mediastinum was also observed in some dogs, although the clinical significance of this finding remains unclear.

Even though none of the previous adverse events have been reported to date in veterinary clinical cases, human reports together with our and Bagshaw et al.'s (2009) results suggest that careful technique, the use of low volumes and close monitoring of the cardiorespiratory function are essential whenever PBPB is performed in clinical practice with any technique.

In most dogs the brachial plexus is formed by ventral branches of C6, C7, C8 and T1, which are the nerves blocked during the PBPB technique; however, in some dogs it also receives innervation from C5 and T2 (Allam et al. 1952). Therefore, the clinical efficacy of PBPB might be decreased in dogs with contribution from nerves C5 and T2, which are not targeted in this block. In the present study many dogs had inadvertent staining of nerve C5, especially with BL and US techniques, which could potentially lead to an increased clinical efficacy of the block in dogs with contribution from this nerve. Nonetheless, the clinical importance of this finding as well as staining of C3 and C4 nerves remains unclear.

Some limitations of this study include: 1) small sample size; 2) limited experience performing NS and US guided PBPB; 3) post-mortem evaluation of success and complications based on staining of nerves and other structures; 4) animals kept refrigerated for a few hours before post-mortem evaluation; 5) no evaluation of phrenic nerve staining. Post-hoc power calculation showed that for a two-tailed α of 0.05 the power of this study was approximately 75% to detect a difference in the mean number of nerves stained by techniques. It is possible that with a greater sample size more differences among techniques could have been found.

Success rates in humans are based on presence of clinical block as described by the patient; however, a limitation in veterinary medicine is the impossibility of the patient to communicate verbally whether the block has been successful or not. Therefore, in veterinary medicine the success is based on staining of desired nerves evaluated at post-mortem, experimentally in live dogs with assessment of sensory and motor deficits post injections or clinically by assessing intra-operative anesthetic sparing effect and post-operative pain. In the present study, several factors may have affected the spread of the solution until post-mortem evaluation of nerve staining was performed, such as position and temperature at which the animals were kept and time from injection of the solution until dissections were made. A previous study evaluating the radial, ulnar, median and musculocutaneous nerve blocks showed greater successful staining of the nerves in cadavers compared to the clinical success rates on live dogs (Trumpatori et al. 2010). This was also observed in two studies of the pelvic limbs in dogs, which showed that successful staining of nerves at post-mortem did not correspond to clinical efficacy (Rasmussen et al. 2006a and 2006b).

In conclusion, this study shows that performing PBPB with any of the three studied techniques is associated with low success rates and potentially with a high degree of complications based on staining of the four main cervical nerves that form the brachial plexus and other structures. Close cardiorespiratory monitoring is recommended when performing this block in clinical cases. Further studies are needed, especially looking at the clinical efficacy of different PBPB techniques and incidence of complications in patients undergoing surgery.

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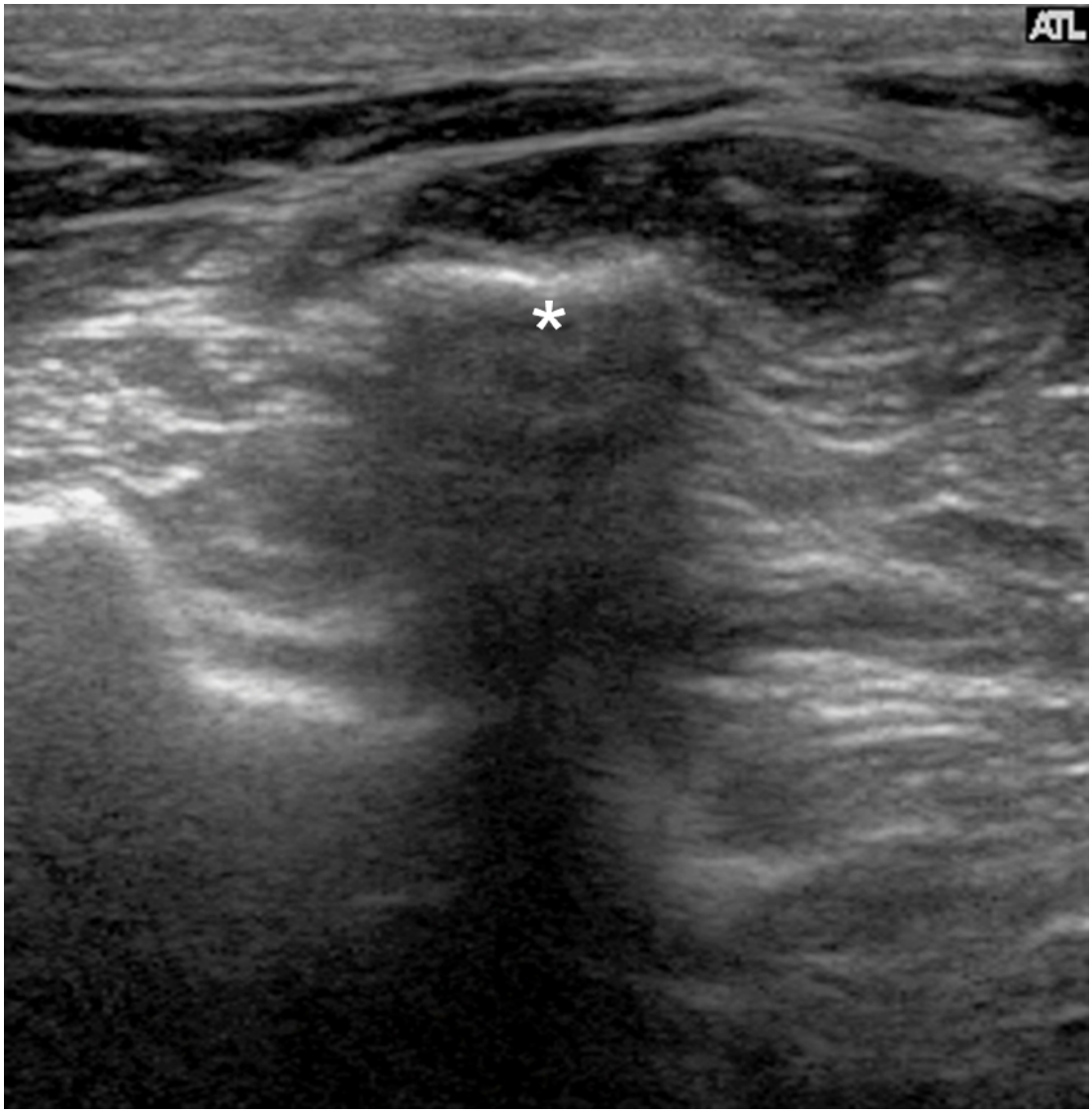

Figure 1. Longitudinal (cranial to caudal) ultrasound image of the lateral aspect of the neck of a 10 kg dog. The large transverse process of C6 (*) casts an acoustic shadow and is identified by its size and location relative to the vertebral body. This serves as an initial landmark to identify the sites for injection.

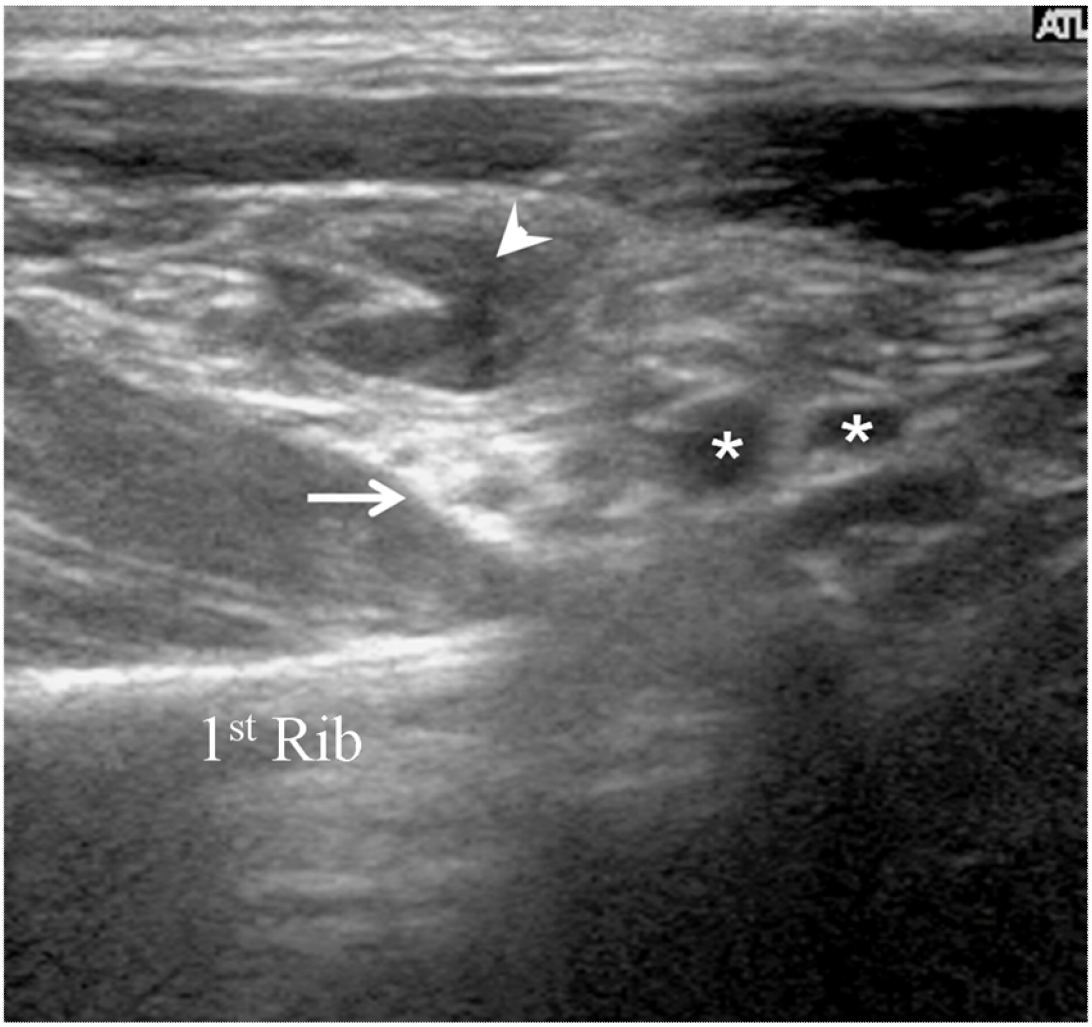

Figure 2. Ultrasound image of landmarks used in the injection of the C8-T1 nerves at the level of the first rib, seen as a hyperechoic interface that casts an acoustic shadow. The blood vessels are seen as hypoechoic rounded structures (*) and recognizable with color Doppler signal, and the plexus was typically adjacent (white arrow) and having a slightly honeycomb appearance (as described by Guilherme & Benigni 2008). The needle tip is surrounded by a hypoechoic bleb of local anesthetic and is positioned adjacent but not in contact with the nerves (white arrow head).

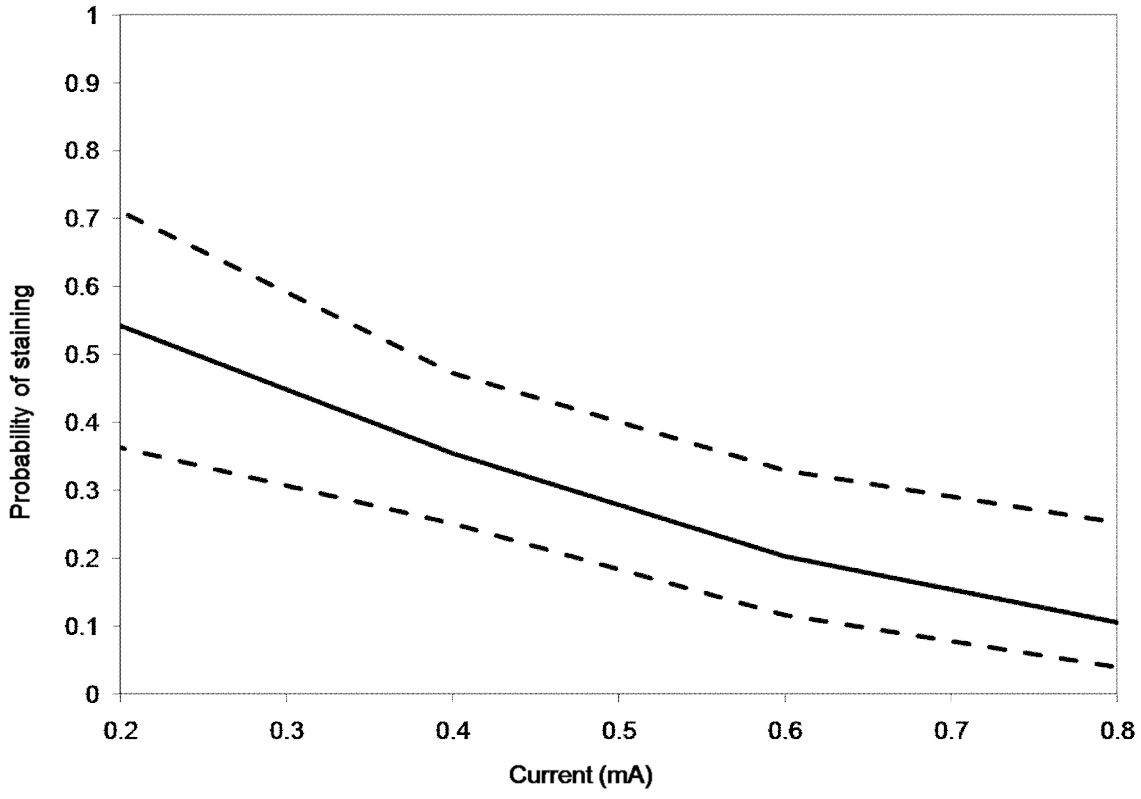

Figure 3. Exact conditional logistic regression graph for the association between probability of staining and current used during the NS guided paravertebral brachial plexus block in dogs. The continuous line shows the predicted probabilities and the dashed lines show the 95% confidence intervals.

Table 1. Results of the GLIMMIX procedure for nerve staining comparisons among 3 techniques to perform the paravertebral brachial plexus block in dogs.

	Techniques	Odds Ratio (OR)	OR 95% Confidence interval	P value
Number of nerves stained	BL vs NS	2.37	1.04 - 5.39	0.04
	BL vs US	2.25	1.02 - 4.96	0.04
	NS vs US	0.95	0.41 - 2.19	0.9
Stain at C6	BL vs NS	2.85	0.71 - 11.48	0.13
	BL vs US	4.14	1.06 - 16.17	0.04
	NS vs US	1.45	0.41 - 5.15	0.55
Stain at C7	BL vs NS	3.69	1 - 13.57	0.05
	BL vs US	4.2	1.19 - 14.84	0.03
	NS vs US	1.14	0.31 - 4.18	0.84
Stain at C8	BL vs NS	2.97	0.7 - 12.57	0.13
	BL vs US	2.28	0.64 - 8.07	0.19
	NS vs US	0.76	0.19 - 3.02	0.69
Stain at T1	BL vs NS	3.43	0.62 - 19.13	0.15
	BL vs US	2.86	0.72 - 11.3	0.13
	NS vs US	0.83	0.16 - 4.22	0.82
Stain at C5	BL vs NS	12.6	1.91 - 83.58	0.01
	BL vs US	0.16	0.03 - 0.74	0.02
	NS vs US	0.01	0.001 - 0.11	0.0003

Table 2. Rate of staining of individual nerves that form the brachial plexus and other structures with 3 different techniques to perform the paravertebral brachial plexus block in dogs.

	BL	**NS**	**US**
C6	19/24 (79%)	12/21 (57%)	11/23 (48%)
C7 *	15/24 (62.5%)	7/21 (33%)	8/23 (35%)
C8	8/24 (33%)	5/21 (24%)	8/23 (35%)
T1	5/24 (21%)	3/21 (14%)	6/23 (26%)
C3 §	0/24 (0%)	0/21 (0%)	1/23 (4%)
C4 §	0/24 (0%)	0/21 (0%)	5/23 (22%)
C5 *	10/24 (42%)	6/21 (29%)	8/23 (35%)
Spinal cord	7/24 (29%)	8/21 (38%)	9/23 (39%)
Pleura	1/24 (4%)	1/21 (5%)	3/23 (13%)
Mediastinum	1/24 (4%)	2/21 (9.5%)	2/23 (9%)
Vessels	1/24 (4%)	0/21 (0%)	1/23 (4%)

* Significant effect of technique in the GLIMMIX procedure ($p \leq 0.05$); § Statistical analysis was not performed on these nerves as they were stained only by the US guided technique.