Veterinary and Animal Science 22 (2023) 100314

Contents lists available at ScienceDirect

Veterinary and Animal Science

journal homepage: www.elsevier.com/locate/vas

Differential blockade, comparative study of different ropivacaine concentrations (0.75%; 0.2%; 0.12%) for ultrasound guided sciatic and femoral nerve blocks in calves: Prospective cross-over study

Marcos Paulo Antunes de Lima [a,c,*], Renata Andrade Silva [a], Patrícia de Castro Duarte [a], Pablo Ezequiel Otero [b], Rafael Resende Faleiros [a], Suzane Lilian Beier [a]

[a] Department of Veterinary Clinic and Surgery, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[b] Department of Anesthesiology and Pain Management, Facultad de Ciencias Veterinarias, Universidade de Buenos Aires, Buenos Aires, Argentina
[c] Veterinary Medicine Course, Biological and Health Sciences Institute, Pontifícia Universidade Católica de Minas Gerais, Minas Gerais, Brazil

ARTICLE INFO

Keywords:
Bovine
Pressure algometry
Ultrasound
Neurostimulation

ABSTRACT

Pharmacodynamic understanding of the different local anesthetic concentrations allows adapting their use to diverse clinical/surgical procedures, such as intraoperative and/or postoperative analgesia. A crossover study was performed, where 6 calves (5 male and 1 female), weighing 120 ± 28 Kg, were subjected to combined sciatic and femoral nerve block using three ropivacaine concentrations. The treatments were: R0.75, using 0.75% ropivacaine; R0.2, 0.2% ropivacaine; and R0.12%, 0.12% ropivacaine. All treatments were performed with ultrasound and neurostimulation assistance, and a volume of 0.1 mL/kg of the respective local anesthetic solution was administered in each block point. The sites of mechanical nociceptive threshold (MNT) evaluation were based on the calf pelvic limb dermatomes. The proportion between desensitized areas, MNT elevation time and level of ataxia were registered. Elevation of MNT occurred in 100% of the tested areas in the R0.75 and R0.2 treatments, and in 82% of the R0.12 treatment. Mean MNT elevation times were 9.5 ± 0.7 h for R0.75, 6 ± 0.8 for R.02, and 2.4 ± 2.3 for R0.12, differing significantly between all treatments. No difference was observed between MNT elevation time and ataxia duration time, in each treatment. It is concluded that the duration of sensory-motor effects is dose-dependent, but there was not possible to detect block selectivity as the concentrations was reduced. More desensitized areas and extension were obtained with the use of higher concentrations.

Introduction

Success and safety during the execution of locoregional anesthesia techniques depend on the proper relationship of anatomical topography knowledge, expertise in the execution of these techniques with the help of tools such as ultrasound and neurostimulation, and also the pharmacological aspects of the drugs used based on their latency time and duration characteristics, and its selective motor or sensory blocks (Campoy & Schoroeder, 2013).

Ideally, the choice of local anesthetic should result in adequate intraoperative desensitization, adequate long-lasting postoperative analgesia, and minimal motor impairment during recovery. The effects mentioned above depend on the selected drug and the volume and concentration of the administered solution (Casati et al., 2004).

Ropivacaine is a long-acting local anesthetic from the amino-amide group, with intermediate vasoconstrictive proprieties. It is three to four times more potent than lidocaine and with a longer lasting effect. In low concentrations (e.g., ropivacaine 0,12%), it produces sensory analgesia without deep motor block (Kuthiala e Chaudhary, 2011). It is indicated for neuraxial and perineural blocks, for intraoperative management of nociception with single shots administration, as well to provide an adequate postoperative analgesia with continuous infusion via catheters (Kuthiala & Chaudhary, 2011).

Managing the volume and concentration of local anesthetics used during anesthesia are key factors to consistent and effective block, being determinants of sensory and motor effects (Taha & Abd-Elmaksoud, 2014). Besides that, the risk of systemic intoxication or nervous lesion are also reduced, since they are concentration dependent (Kuthiala &

* Corresponding author.
E-mail address: limampa@hotmail.com (M.P.A. de Lima).

https://doi.org/10.1016/j.vas.2023.100314

Available online 9 September 2023

Chaudhary, 2011; Yang et al., 2011; Hogan, 2008). In specific situations, such as in patients with peripheral neuropathies, as seen in diabetics, the use of lower concentrations is recommended, due to higher sensitivity and potential toxic effect (Kuthiala & Chaudhary, 2011).

The possibility of efficiently using a long-acting drug with analgesic effects and minimal motor implication is interesting in large animals, since it can allow the performance of procedures in the limbs with minimal propensity to ataxia, as well as facilitate the management during anesthesia recovery. Therefore, the purpose of this study was to assess the effects of the use of 0.75%, 0.2% and 0.12% ropivacaine solution for a combined sciatic and femoral nerve block, through the parasacral approach and a ventral to the ilium approach, respectively, on the mechanical nociceptive threshold, proprioception and ataxia of calves. We hypothesized that the sensory and motor effects are concentration dependent and that with reduced concentrations, such as 0.125% ropivacaine, the motor effects, ataxia and their duration are shorter compared to the elevated concentrations.

Material and methods

This study was approved by the Committee of Ethics in Animal Use (CEUA) of the Federal University of Minas Gerais (UFMG), protocol n^{o}. 116/2017.

Six healthy mixed-breed bovine calves (five males, one female), aged 5–8 months, weighing 120 ± 28 kg were used. Inclusion/exclusion criteria used consisted that all animals used were considered healthy (with the help of laboratory and physical exams). They had no scars or lesions in the pelvic limbs and none of them had history of lameness. Fifteen days before the study, the animals were kept in grazing areas or stables for acclimation, were they received corn silage, hay, and water ad libitum.

An experimental crossover design was used, in which each animal underwent all three proposed treatments. To avoid eventual residual effects between treatments, a period of 7 days was stablished from one treatment to the other. All animals were subjected to combined sciatic and femoral nerve block.

The proposed treatments consisted of the use of different ropivacaine concentrations (Ropi, Cristália, Brazil), administered in volumes of 0.1 mL/kg in each block point. Treatment R0.75 used 0.75% ropivacaine, while treatment R0.2% used 0.2% ropivacaine, and treatment R0.12 0.12% ropivacaine (solution prepared from 0.2% ropivacaine diluted with water for injection before injection). Perineural injections were performed over 1 min, in all treatments.

Before each experimental stage, the animals were subjected to food and water fasting during 24 and 12 h, respectively. They were sedated with 0.07 mg/kg of 2% xylazine (Xylasin, syntec, Brazil), intravenously (IV). After sedation was established, the animals were placed in right lateral recumbency on a padded surface. Hair was shaved from the areas of interest for the locoregional anesthesia techniques execution, followed by 2% chlorhexidine gluconate and 0.5% alcoholic chlorhexidine antisepsis. Perineural block was performed with the use of US and neurostimulation. The sciatic nerve block was performed with a parasacral approach described for sheeps (Waag, Stoffel, & Spadavecchia, 2014), and combined with the femoral nerve block with a ventral do the ilium approach, previously described for calves (DeVlamynck et al., 2013).

- Parasacral approach (sciatic nerve): through the dorsal aspect of the gluteal area, tracing a line between the dorso-cranial portion of the iliac crest and ischiatic tuberosity, the ultrasound probe is positioned in its medium portion and, through the superficial and middle gluteal muscles, the sciatic nerve is identified medially to the body of the ilium and close to the cranial gluteal artery and vein.
- Ventral to the ilium approach (femoral nerve): ventrally to the body of the ilium, the ultrasound probe is placed through the lateral aspect of the pelvic limb, approximately 3 to 4 cm caudal to the iliac wing, and in between the psoas major and minor muscles, near the external iliac artery and vein, the femoral nerve is located.

Ultrasound imaging were performed with of a linear 7.5 – 10 MHz (Gen 3 ultrasound Wi-fii, Beijing Konted Medical Technology, China) probe, always in the animals' left pelvic limb. After visualization of the nerve, 21 gauges, 100 mm neurostimulation needle (Locoplex, Vygon) was connected to the neurostimulation device (Plexygon, Vygon, France), and used for needling and posterior local anesthetic injection. The neurostimulator was set at 1 Hz and 0.1 ms. The correct position of the needle was confirmed by US visualization and muscular contraction at 0.5 mA at the neurostimulator, and no contraction at 0.2 mA. For the femoral nerve, there was search of contraction of the quadriceps femoris muscle and consequent extension of the stifle joint and, for the sciatic nerve, responses such as dorsal extension or plantar flexion of the tarsus and/or digits were sought. Treatments was always performed by the same researcher. After treatments, the animals received 0.01 mg/kg of atipamezole (Antisedan, Zoetis, Brazil) IV.

For assessment of the MNT on the block areas, a pressure algometry model was used, with the use of a portable dynamometer (Instrutemp 20 kgf ITFG – 5020, Brazil). The device has a stem with 12 cm of length, a conic tip with a 1 mm diameter, and readings made in kilograms. Pressure was applied in a 90° angle with the evaluated surface with maximal applied value of 3 kg, to avoid tissue injury. The stimulus was ceased when the animal showed any aversive movements, such as removing the limb or looking at the manipulated region. The points tested were based on the following calf's pelvic limb dermatomes, similar as proposed by *Re* et al. (2014): gluteal region; cranial, caudal and medial regions of the thigh; cranial, lateral and medial regions of the knee; medial and lateral regions of the leg; dorsal and plantar regions of the foot.

The degree of ataxia was characterized by the scale proposed by Bigham et al. (2010), where: 0 – No ataxia or proprioceptive deficit; 1 – Mild ataxia, mild proprioceptive deficit, able to walk; 2 – Moderate ataxia, marked proprioceptive deficit, evident difficulty to move, but can still stay in a standing position and walk; 3 – Severe ataxia, falling, isn't able to stay in a standing position or walk.

These evaluations were made always by the same researcher, blind to the treatments. The MNT was measured at the baseline (before sedating the animals), 30 min after atipamezole reversal, and every hour after atipamezole reversal, until total recovery of motor function and sensitivity in the pelvic limb (absence of motor and proprioceptive deficits; MNT values proximal to the baseline).

Statistical analysis

The Shapiro–Wilk test was performed to assess the occurrence of normal distribution of the data collected. The data with normal distribution were submitted to ANOVA with multiple repetitions, followed by Tukey test to evaluate the differences between times in the same treatment. To compare the means of the different treatments, the paired *t*-test was used. Data with non-normal distribution were submitted to Friedman analysis, followed by Dunn test, and Wilcoxon Rank test for comparison between treatments. Statistical difference was considered when $p < 0{,}05$. All analysis were performed using SigmaPlot 12.0 and graphics made with GraphPad Prism 7.0.

Results

In the R0.75 and R0.2 treatments, there was significant increase of the MNT in 100% (11/11) of the tested areas, while in the R0.12 treatment, significant increase, in at least one moment, occurred in 9/11 tested areas. The mean times of MNT increase, in all tested areas, were significantly different between all treatments ($p = 0.001$). Treatment R0.75 had mean time of MNT of 9.5 ± 0.7 h, while treatments R0.2 and R.012 had 6 ± 0.8 h and 2.4 ± 2.3 h, respectively. The mean values of

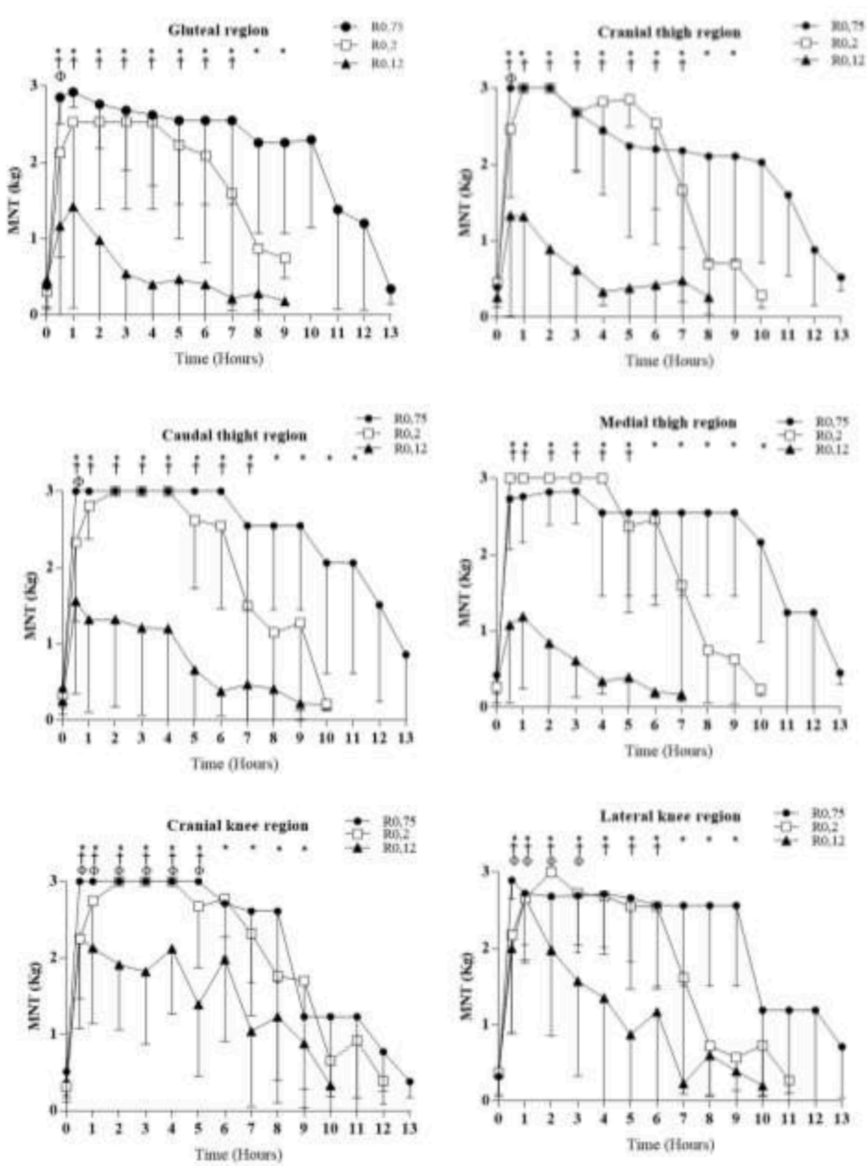

Fig. 1. – Mean and standard deviation values of the mechanical nociceptive threshold (MNT), expressed in kg, of the gluteal region, cranial, caudal and medial thigh region, and cranial and lateral knee region in bovines subjected to combined sciatic and femoral nerve block by a parasacral approach and a ventral to the ilium approach, with 0.75%, 0.2% and 0.12% ropivacaine.
*significant difference in the 0 h moment, for treatment R0.75
†significant difference in the 0 h moment, for treatment R0.2
Φ significant difference in the 0 h moment, for treatment R0.12.

MNT increase, in each respective area, are shown in Figs. 1 and 2.

In at least one of the evaluations, severe ataxia was seen in 100% (6/6), 66.7% (4/6) and 16.7% (1/6) of the animals of group R0.75, R0.2, and R0.12, respectively. Ataxia scores of the R0.75 treatment were significantly higher from 0.5 to 7 h compared to R0.12, and only in the 9- and 10- h moments when compared to R.02 (Fig. 3). Significant differences between R0.2 and R0.12 were only seen in the 4 to 6 h moments (Table 1).

The mean values of MNT elevation times in comparison to the mean ataxia duration time were not significantly different for R0.75 (p = 0.287), R0.2 (p = 0.520) or R0.12 (p = 0.697) (Table 1).

Discussion

Pressure algometry is one of the methods used for testing and quantifying the MNT, considered a robust and practical tool to be used in calves (Millman, 2013). Higginson et al. (2010) showed significant reduction of the nociceptive thresholds with pressure algometry, applied to the surgical area of calves subjected to dehorning, when compared to the preoperative period, indicating lower animal tolerance when there is injury and peripheral sensitizing. When evaluating a similar situation where bovine calves were dehorned, the post-operative nociceptive thresholds were significantly higher in the animals that received analgesia with anti-inflammatory drugs in comparison to the placebo treatment (Tapper, 2011). The use of pressure algometry for evaluating the mechanical nociceptive threshold after perineural block has already been tested in equines (Paz et al., 2016), but there is no description of its use for this purpose in the bovine species. Its use can be promising in the assessment of the extension and duration time of local blocks, since currently the methodology applied to evaluate the response to noxious stimulus in dogs (Trein et al., 2017; Portela et al., 2010) uses techniques such as clamping or cutaneous puncture, which evaluate subjective responses to the generated stimulus.

Human medicine data shows post-operative analgesia, with 0.75% ropivacaine, of 11.9 ± 1.5 h (Erlacher et al., 2000) and of 13 ± 2 h (Greengrass et al., 1998) in brachial plexus block, close to the 9.5 ± 0.7 h found in the present study. However, this disagrees with the findings of Trein et al. (2017), where with the use of 0.75% ropivacaine for sciatic and femoral nerve block in dogs, the mean time of desensitizing, assessed by clamping dermatomes, was 3.36 h.

A study by Erlacher et al. (2000), using 0.75% ropivacaine for brachial plexus block, had a mean period of 11.9 ± 1.5 h until the first

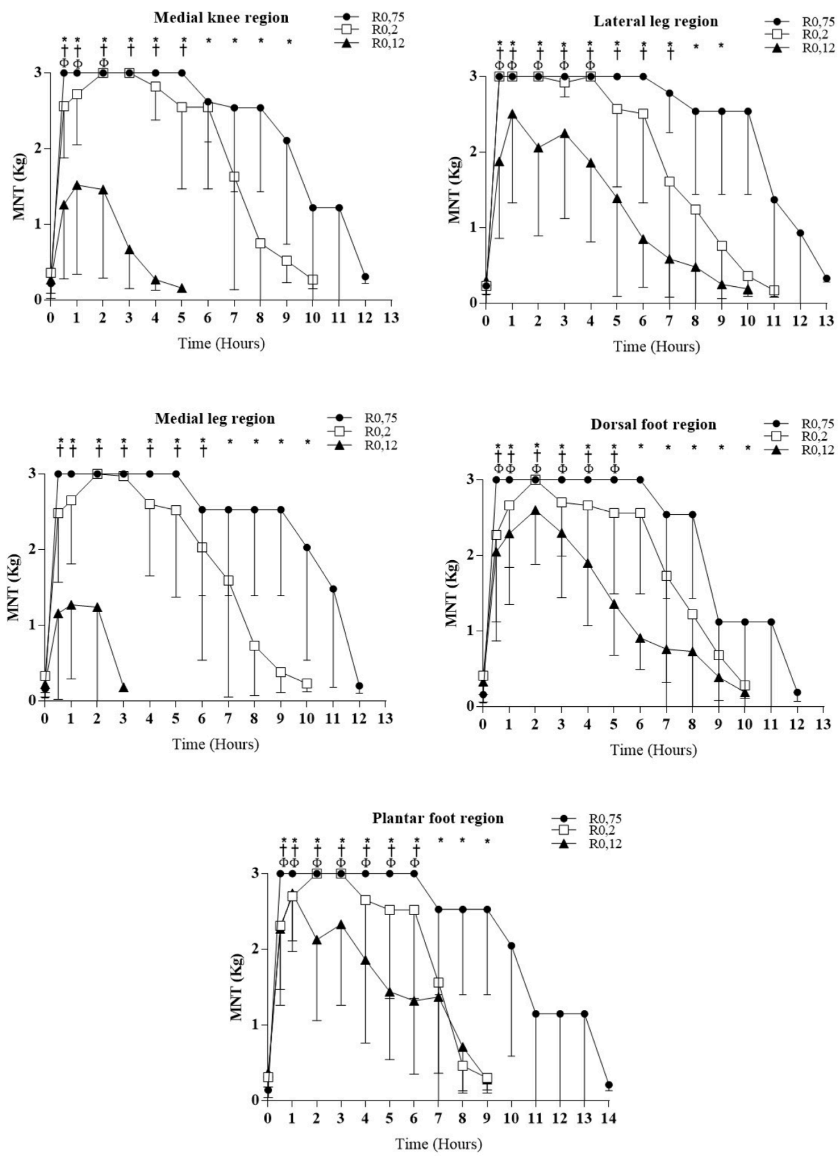

Fig. 2. - Mean and standard deviation values of the mechanical nociceptive threshold (MNT), expressed in kg, of the medial knee region, lateral and medial leg region, and dorsal and plantar foot region in bovines subjected to combined sciatic and femoral nerve block, by a parasacral approach and a ventral to the ilium approach, with 0.75%, 0.2% and 0.12% ropivacaine.
* significant difference in the 0 h moment, for treatment R0.75
†significant difference in the 0 h moment, for treatment R0.2
Φ significant difference in the 0 h moment, for treatment R0.12.

sign of pain and analgesic requirement by the patient, after undergoing wrist and forearm surgery. These same authors did not report significant differences between analgesia and motor block times. On the other hand, in a study performed with dogs subjected to brachial plexus block with 0.75% ropivacaine, also guided by neurostimulation, the mean duration time of desensitizing the thoracic limb dermatomes was 7.6 ± 1.4 h (Sakonju et al., 2009), although the authors used clamping of the dermatomes for the evaluation. It was also noted, in a second experimental group, that the desensitizing times with the use of 0.5% ropivacaine did not differ significantly from the ones that used 0.75%, which suggests that there is no benefit in using a higher concentration (Sakonju et al., 2009).

It is observed that the search for the minimal effective concentration is investigated in human medicine, as well as the benefits from sufficient doses, such as motor block, anesthesia and analgesia during surgery (Simpson et al., 2005). Bertini et al. (1999) obtained, as a result of 0.5% ropivacaine use on brachial plexus block by neurostimulation in patients that underwent surgical procedures in the hand region, an analgesic period of up to 11 h, with motor block lasting for 8 h, besides the high satisfaction reported by the patients. Another study conducted in humans that underwent total knee arthroplasty and received 0.5% ropivacaine for sciatic and femoral nerve block, showed that the thermal sensitivity time reduction of the patients was maintained for up to 24 h, and the time for motor recovery of the limb was close to 12 h. Simpson et al. (2005) observed that as the anesthetic concentration is lowered, its motor block times decreases in a correlate manner. The minimal effective concentration, for analgesic effect, of ropivacaine seems to be close to 0.1%, as shown by Paauwe et al. (2008) while assessing the analgesic comfort and degree of motor impairment of patients in the postoperative period of total knee arthroplasty. They found no benefits from the use of 0.025% and 0.05% ropivacaine, when compared to a concentration of 0.1% that promoted better quality analgesia and higher patient satisfaction, allowing the beginning of physiotherapy treatment on the first day after surgery.

Pharmacodynamic knowledge of the different concentrations used in locoregional anesthesia affects directly in its application in distinct scenarios of anesthetic and analgesic management of patients. For ideal conditions during the surgical act, adequate level of muscle relaxation and analgesia should be obtained, which demands higher concentrations of the local anesthetics used. These concentrations allow faster and facilitated penetration into the nervous fibers and promote greater effect homogeneity and block consistency (Kuthiala & Chaudhary, 2011). However, due to differences in block sensitivity between the different nervous fibers, it is known that sensory fibers are more easily

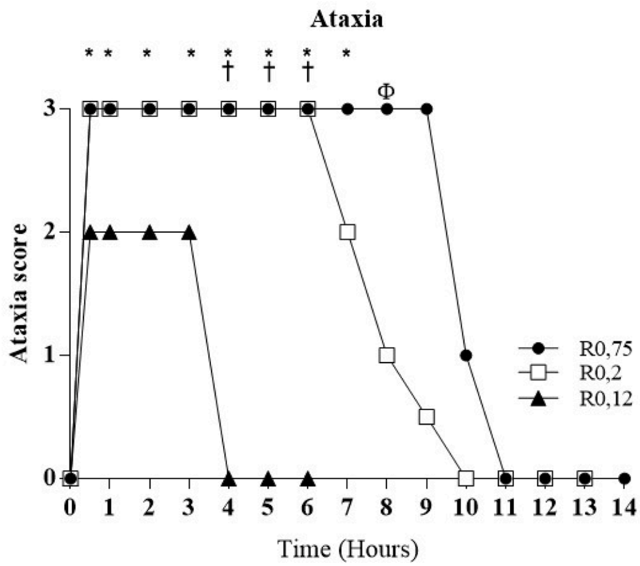

Fig. 3. – Ataxia score values in calves subjected to combined sciatic and femoral nerve block by a parasacral approach and a ventral do the ilium approach, with 0.75%, 0.2% and 0.12% ropivacaine.
* significant difference in the 0 h moment, for treatment R0.75
†significant difference in the 0 h moment, for treatment R0.2
Φ significant difference in the 0 h moment, for treatment R0.12.

Table 1
– Mean values of MNT elevation time and ataxia duration in calves subjected to combined sciatic and femoral nerve block by a parasacral approach and a ventral do the ilium approach, with 0.75%, 0.2% and 0.12% ropivacaine.

	Treatments		
	R0.75	**R0.2**	**R.012**
MNT elevation time (h)	9.5 ± 0.7a	6 ± 0.9b	2.9 ± 2.1c
Ataxia time (h)	10.5 ± 3.1a	6.5 ± 2.2b	2.8 ± 1.7b

Different lower-case letters, shown in the same line, indicate significant differences between treatments.

penetrated, allowing the use of anesthetic solutions with a lower concentration while guaranteeing analgesia and minimal motor impairment (Simpson et al., 2005; Casati et al., 2004; Zaric et al., 1996). Such claims are evidenced by the indication of different ropivacaine concentrations in human literature. Consistent nerve blocks, with adequate levels of muscle relaxation and satisfaction from the surgeon and patient during surgery, are obtained 0.5% and 0.75% concentrations (Simpson et al., 2005). On the other hand, 0.2% and 0.15% concentrations are indicated for neuroaxis anesthesia (epidural or subarachnoid), or for post-operative continuous analgesia (Kuthiala & Chaudhary, 2011).

It is worth mentioning that different methods of local anesthetic perineural administration and volumes used can influence the sensory motor effects. Techniques of single administration (single shot), even with lower concentrations of local anesthetics, due to the large initial volume used, can result in more intense sensory motor changes than the techniques that use continuous infusion via perineural catheter (Simpson et al., 2005). Studies evaluating different volumes and administration methods of lower ropivacaine concentrations should be performed to address the possible gaps mentioned here, and to indicate the best combination of volume, concentration, and administration method.

Conclusion

Based on the proposed methodology, it was concluded that sensory and motor block duration is concentration-dependent, but the use of higher concentrations promoted more homogeneous effects in the unsensitized areas. The use of lower ropivacaine concentrations did not allow the differentiation of sensory and motor block.

CRediT authorship contribution statement

Marcos Paulo Antunes de Lima: Conceptualization, Data curation, Formal analysis, Investigation, Writing – review & editing. **Renata Andrade Silva:** Investigation, Methodology, Writing – review & editing. **Patrícia de Castro Duarte:** Investigation, Methodology. **Pablo Ezequiel Otero:** Conceptualization, Formal analysis, Supervision, Writing – review & editing. **Rafael Resende Faleiros:** Conceptualization, Methodology, Formal analysis, Writing – review & editing. **Suzane Lilian Beier:** Conceptualization, Funding acquisition, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by a CNPq grant. Preliminary results were presented as an abstract at the XIII Brazilian Congress of Veterinary Anaesthesiology.

References

Bertini, L., Tagariello, V., Mancini, S., Ciaschi, A., Posteraro, C. M., Di Benedetto, P., & Martini, O. (1999). 0.75% and 0.5% ropivacaine for axillary brachial plexus block: A clinical comparison with 0.5% bupivacaine. *Regional Anesthesia and Pain Medicine, 24*, 514–518.
Bigham, A. S., Habibian, S., Chamsemina, F., et al. (2010). Caudal epidural injection of lidocaine, tramadol, and lidocaine-tramadol for epidural anesthesia in cattle. *Journal of Veterinary Pharmacology and Therapeutics, 33*, 439–443.
Campoy, L., & Schoroeder, K. (2013). General Considerations. *Small animal regional anesthesia and analgesia* (pp. 11–18). Oxford: Wiley-Blackwell.
Casati, A., Vinciguerra, F., Cappelleri, G., Aldegheri, G., Grispigni, C., Putzu, M., & Rivoltini, P. (2004). Levobupivacaine 0,2% or 0,125% for continuous sciatic nerve block: A prospective, randomized, double-blind comparison with 0,2% ropivacaine. *Anesthesia and Analgesia, 99*, 919–923.
DeVlamynck, C., Pille, F., Hauspie, S., et al. (2013). Evaluation of three approaches for performing ultrasonography-guided anesthetic bloackade of the femoral nerve in calves. *American Journal of Veterinary Research, 74*, 750–756.
Erlacher, W., Schuschnig, C., Orlicek, F., Marhofer, P., Koinig, H., & Kapral, S. (2000). The effects of clonidine on ropivacaine 0,75% in axillary perivascular brachial plexus block. *Acta Anaesthesiologica Scandinavica, 44*, 53–57.
Greengrass, R. A., Klein, S. M., DErcole, F. J., et al. (1998). Lumbar plexus and sciatic nerve block for knee arthroplasty: Comparison of ropivacaine and bupivacaine. *Canadina Journal of Anaesthesia, 45*, 1094–1906.
Higginson, J. H., Millman, S. T., Leslie, K. E., & Kelton, D. F. (2010). Validation of the new pedometry system for use in behavioural research and lameness detection in dairy cattle. In *Proceedings of the 1st North American conference on precision dairy management* (pp. 132–133).
Hogan, Q. H. (2008). Pathopysiology of peripheral nerve injury during regional anesthesia. *Regional Anesthesia and Pain Medicine, 33*, 435–441.
Kuthiala, G., & Chaudhary, G. (2011). Ropivacaine: A review of its pharmacology and clinical use. *Indian Journal of Anaesthesia, 55*, 104–110.
Millman, S. T. (2013). Behavioral responses of cattle to pain and implications for diagnosis, management, and animal welfare. *Veterinary Clinics Animals, 29*, 47–58.
Paauwe, J. J., Thomassen, B. J., & Weterings, J. (2008). Femoral nerve block using ropivacaine 0.025%, 0.05% and 0.1%: Effects on the rehabilitation programme following total knee arthroplasty: A pilot study. *Anaesthesia, 63*, 948–953.
Paz, C. F. R., Magalhães, J. F., Mendes, H. M. F., Rocha Junior, S. S., Belknap, J. K., Alves, G. E. S., & Faleiros, R. R. (2016). Mechanical nociceptive thresholds of dorsal laminae in horses after local anaesthesia of the palmar digital nerves or dorsal branches of the digital nerve. *The Veterinary Journal, 214*, 102–108.
Portela, D. A., Otero, P. E., Tarragona, L., Briganti, A., Breghi, G., & Melaine, P. (2010). Combined paravertebral plexus block and parasacral sciatic block in healthy dogs. *Veterinary Anaesthesia and Analgesia, 37*, 531–541.
Re, M., Blanco-Murcia, J., Fernández, A. V., et al. (2014). Ultrasound-guided anaesthetic blockade of the pelvic limb in calves. *The Veterinary Journal, 200*, 434–439.
Sakonju, I., Maeda, K., Maekawa, R., Maebashi, R., Kakuta, T., & Takase, K. (2009). Relative nerve blocking properties of bupivacaine and ropivacaine in dogs undergoing brachial plexus block using a nerve stimulator. *The Journal of Veterinary Medical Science, 71*, 1279–1284.

Simpson, D., Curran, M. P., Oldfield, V., & Keating, G. M. (2005). Ropivacaine. *Drugs, 65*, 2675–2717.

Taha, A. M., & Abd-Elmaksoud, A. M. (2014). Ropivacaine in ultrasound-guided femoral nerve block: What is the minimal effective anaesthetic concentration (EC90)? *Anaesthesia, 69*, 678–682.

Tapper, T. R. (2011). *An investigation of pressure algometry and thermal sensitivity tests for assessing pain associated with a sow lameness model and calf disbudding*. USA: Thesis, Master of Science, University of Iowa.

Trein, T. A., Floriano, B. P., Wagatsuma, J. T., Ferreira, J. Z., Silva, G. L., Dos Santos, P. S. P., Perri, S. H. V., & Oliva, V. N. L. S. (2017). Effects of dexmedetomidine combined with ropivacaine on sciatic and femoral nerve blockade in dogs. *Veterinary Anaesthesia and Analgesia, 44*, 144–153.

Waag, S., Stoffel, M. H., Spadavecchia, C., et al. (2014). Ultrasound-guided sciatic and femoral nerves: An anatomical study. *Laboratory Animal, 48*, 97–104.

Yang, S., Abrahams, M. S., & Hurn, P. D. (2011). Local anesthetic schwann cell toxicity is time and concentration dependent. *Regional Anesthesia and Pain Medicine, 36*, 444–451.

Zaric, D., Nydahl, P. A., Philipson, L., Samuelsson, L., Heierson, A., & Axelsson, K. (1996). The effect of continuous lumbar epidural infusion of ropivacaine (0.1%, 0.2%, 0.3%) and 0.25% bupivacaine on sensory and motor block in volunteers: A double-blind study. *Regional Anesthesia, 21*, 14–25.