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Liposomal bupivacaine provides longer duration analgesia than bupivacaine hydrochloride in an adjustable sole-pressure model of equine lameness

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OBJECTIVE
To compare the efficacy and duration of action for perineural analgesia with liposomal bupivacaine (LB) versus bupivacaine hydrochloride (BHCl) in a sole-pressure induced model of forelimb lameness in horses.

ANIMALS
6 healthy adult research horses.

PROCEDURES
In 1 randomly assigned forelimb, grade 3/5 lameness was induced by use of a sole-pressure lameness model. Objective lameness (vector sum [VS]) was determined with an inertial sensor system at 0, 1, 6, and 24 hours after lameness induction to evaluate the model. Mechanical nociceptive thresholds (MNTs) and objective lameness (VS and force platform kinetics) were recorded prior to and at 1, 6, 24, 48, and 72 hours after perineural anesthesia of the palmar nerves at the level of the proximal sesamoid bones with LB or BHCl in random order, with a 1-week washout period between crossover treatments. Data analysis was performed with mixed-model ANOVA.

RESULTS
When evaluating the lameness model, there was a decrease in lameness at 24 hours in at least 1 limb of each horse (7/12 limbs); thus, screw length was increased by 1 to 2 mm at each 24-hour interval to maintain lameness. Compared with results at baseline, horses treated with BHCl had significant improvements in median MNT and VS identified at only 1 hour after injection, whereas treatment with LB yielded significantly improved median MNT, VS score, and peak vertical force for up to 24 hours.

DISCUSSION
In this experimental model of forelimb lameness, LB provided longer analgesia when compared with BHCl and should be further investigated for treatment of pain in horses.

Severe pain in the distal limb of horses is challenging to manage and is an important issue of equine welfare. Horses with fractures or laminitis or horses in the immediate postoperative period would benefit in many cases from more intensive pain management for several days' duration. Untreated or poorly treated pain leads to activation of the sympathetic nervous system, causing elevations in cortisol, norepinephrine, and epinephrine.[1] Prolonged elevation of these "fight-or-flight" hormones can cause vasoconstriction, increased myocardial workload, and decreased blood flow to the gut and other vital organs. Untreated pain increases stress and decreases quality of life, which in some cases results in euthanasia of the patient.[2]

The most commonly used therapeutic options for pain control in horses include NSAIDs, opioids, and α_2-adrenergic receptor agonists.[3] Opioid use in horses has been questioned due to concerns regarding adverse effects on gastrointestinal motility, and opioids have become increasingly expensive and difficult to obtain for veterinary patients.[4] In addition, NSAIDs may also have clinically important side effects, including nephrotoxicity and right dorsal colitis.[5] Drugs that target neuropathic pain (eg, gabapentin) and multimodal treatments that use constant-rate infusions of lidocaine, ketamine, butorphanol, or morphine, alone or in combination, may be used as alternatives to treatment with NSAIDs for hospitalized cases.[3] Although combinations of therapies are available to mitigate severe pain associated with chronic conditions, such as laminitis or postoperative pain, there remains a critical need to improve pain management in the equine patient while minimizing systemic side effects.

Local anesthetics are commonly administered by perineural injection to provide regional analge-

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sia for both diagnostic and therapeutic procedures in horses.[6] This class of drugs is ideal for pain management due to minimal systemic side effects when administered locally. One of the main drawbacks of currently available local anesthetics is their duration of action, which generally is no longer than 8 to 12 hours.[6] However, a long-acting formulation of bupivacaine (liposomal encapsulated bupivacaine) has been shown to provide analgesia of up to 72 hours in other species.[7]

The objective of the study reported here was to compare the efficacy and duration of action for perineural analgesia obtained with administration of liposomal bupivacaine (LB) versus bupivacaine hydrochloride (BHCl) in an adjustable sole-pressure–induced model of forelimb lameness in horses. As this model was not used for a duration of > 3 to 4 hours, we had a subobjective to determine whether the model resulted in similar lameness for a 24-hour period. We hypothesized that LB would provide a significantly longer duration regional analgesia than BHCl following perineural injection of the palmar nerves at the level of the proximal sesamoid bones (abaxial sesamoid nerve block), the incidence of systemic or local side effects for both treatments would be low, and the sole-pressure model would produce a similar level of lameness for 24 hours.

Materials and Methods

Animals

Six clinically normal, research American Quarter Horses with no evidence of forelimb lameness at the trot (grade 0/5 according to the American Association of Equine Practitioners Lameness Scale) were used. The study was completed between January 22, 2019, and February 23, 2019. Study protocol approval was obtained by Institutional Animal Care and Use Committee of Colorado State University prior to study initiation.

Study design

This study was performed as an incomplete block randomized crossover design by use of 2 blocks of 3 horses. Each block of horses was treated with either BHCl (Marcaine; Hospira Inc; 7.5 mg of BHC1/mL) or LB (Nocita; Aratana Therapetics Inc; 13.3 mg of BL/mL), and following a 7-day washout period, the opposite treatment was performed on the contralateral limb. Horses were randomized to both initial treatment (BHCl or LB) and initial forelimb (right or left). The day before starting the BHCl or LB perineural anesthesia trials, each horse underwent lameness model assessment trials **(Figure 1)**.

Sole-pressure lameness model

Horses' feet were trimmed and balanced by a farrier prior to initiating the study. Steel keg shoes were placed on both forelimb hooves, with nuts welded to the inner web of the medial and lateral branches of the shoes between the third and fourth nail holes **(Figure 2)**.[8,9] Lameness was induced by insertion of a 6-mm-diameter threaded screw with a 2-mm-diameter tapered end into the medial and lateral nuts of the shoe in a single forelimb to induce grade 3 lameness (American Association of Equine Practitioners),[10] and screw length was recorded. Screws were inserted into the shoes at each time point and were removed between time points to prevent acclimation.

Perineural anesthesia

After collection of baseline data (immediately following lameness induction), 3 mL of either BHCl or LB was injected SC around both medial and lateral palmar nerves at the level of the proximal sesamoid bones (abaxial sesamoid block) by the same investigator (LP). The skin was first aseptically prepared with betadine and alcohol. To perform the injection, a 25-gauge, 1.6-cm hypodermic needle was placed along the axial aspect of the neurovascular bundle with the needle directed distally. Loss of skin sensation was evaluated subjectively at 15 minutes postinjection by use of increasing pressure with a blunt-ended pen cap at the medial and lateral heels, with loss of sensation indicating a successful block.

Horse clinical assessments

All assessments were performed prior to perineural anesthesia (baseline) and at 1, 6, 24, 48, and 72 hours postinjection. For each horse, rectal temperature, pulse, and respiratory rate were recorded. Limb circumference of the treated forelimb was measured in triplicate at the level of the metacarpophalangeal joint with a flexible fabric tape measure, and the 3 measurements were averaged and then

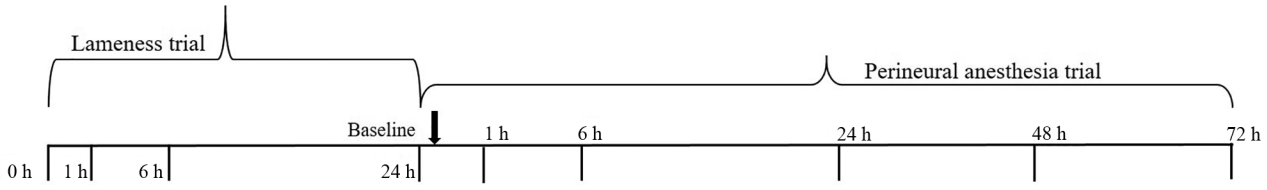

Figure 1—Timeline for the lameness model assessment trials (assessed at hours 1, 6, and 24) after a unilateral forelimb sole-pressure lameness grade 3/5 was induced at hour 0 in 6 healthy research horses without preexisting signs of lameness, subsequent establishment of baseline lameness (arrow), and then the perineural anesthesia trial (liposomal bupivacaine vs bupivacaine hydrochloride), during which lameness assessments with the use of inertial sensor and stationary force platform systems were performed at 1, 6, 24, 48, and 72 hours following administration of perineural anesthesia immediately after baseline measurements. The study was conducted as a crossover design with a 1-week washout period between treatments conducted between January 22, 2019, and February 23, 2019.

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Figure 2—Picture of the steel keg shoe with nuts welded onto the inner aspect of the medial and lateral branches of the shoe for insertion of screws, which were used to induce unilateral forelimb lameness grade 3/5 in the horses described in Figure 1.

recorded. Horses were also monitored when stalled for any abnormal behavior, appetite, and fecal and urinary production.

Mechanical nociceptive threshold testing

An approximate 1-cm circular dot of acrylic paint was placed over the dorsolateral aspect of the limb, approximately 1.5 cm proximal to the coronary band of the treated limb. A pressure algometer (Wagner Force Dial FDK/FDN series force gauge; Wagner Instruments) with a 1-cm^2 rubber tip was used to apply pressure at 5 to 10 $kg/cm^2/s$ until the horse demonstrated avoidance behavior (started to move the limb). The mechanical nociceptive threshold (MNT) measurement was then recorded. Measurements were taken after lameness induction (baseline) and at 1, 6, 14, 48, and 72 hours after perineural anesthesia, and the average of the 3 measurements at each time point was recorded.

Lameness trials

Horses were examined for lameness at the trot in a straight line over an asphalt surface covered by a rubber mat (1.2 X 24.8 m). In the center of the long axis of the runway, 2 stationary force platforms (FPs; Model FP6090; Bertec Corp; 60 X 90-cm each) were embedded and covered with the same rubber mat. Lameness was induced at times 0, 1, 6, and 24 hours, then inertial sensor (IS; Lameness Locator; Equiosis LLC) data were collected to determine the repeatability of the model; the 24-hour time served as baseline lameness for the perineural anesthesia trials. Following perineural anesthesia, lameness was again induced at 1, 6, 24, 48, and 72 hours, and objective lameness data (IS and stationary FP kinetics) were recorded.

IS instrumentation and analysis—Each horse was instrumented with the IS system, as has been previously described.[11] Briefly, a uniaxial accelerometer was attached to the halter with a commercial bonnet, a second uniaxial accelerometer was placed between the tuber sacrale by use of a hook-and-loop fastener and reinforced with nonelastic tape (Duck Tape; Shurtape Technologies LLC), and a uniaxial gyroscope was placed on the dorsal aspect of the right pastern region with a neoprene wrap. A single trot trial was collected at each time point, consisting of 25 to 35 strides, and vector sum (VS) measurements were extracted via automated system analysis within the IS software.

Stationary FP kinetics—Ground reaction measurements were determined after lameness induction (baseline) and at 1, 6, 24, 48, and 72 hours after perineural anesthesia with BHCL or LB. Each horse was led in hand at the trot at a consistent velocity across the stationary FPs, and at least 5 successful trials were collected for each condition. A trial was defined as successful if the ipsilateral fore- and hind limb both landed completely within the same platform and the opposite fore- and hind limb both landed completely within the opposite platform. Kinetic data were collected at 3,000 Hz by use of commercial motion capture software (Qualisys Track Manager version 2016; Qualisys North America Inc). Data were then exported and analyzed by use of commercial software (Visual 3D version 5; Tracklab by Freedspace). Variables analyzed included peak vertical, braking, and propulsive forces and vertical, braking, and propulsive impulses. These variables were collected for both the lame and nonlame forelimbs and were normalized to each horse's body weight.

Statistical analysis

Data were analyzed by use of commercial software (STATA version 13.1; StataCorp LLC). Data were assessed for normality with Shapiro-Wilk tests and visual evaluation of histograms and QQ plots. Data did not meet the criteria for normality, so all variables were ranked prior to analysis and medians and ranges were calculated. Data were analyzed across each treatment by use of a repeated-measures mixed-model ANOVA, with horse as random effect and time as a fixed effect; results for each time point were compared with baseline results. Significance was set at $P < 0.05$.

RESULTS

Animals

There were 5 mares and 1 gelding, age 5 to 10 years (median, 8 years), that had a median body weight of 473 kg (range, 441 to 609 kg). All horses had loss of skin sensation at 15 minutes after injection with BHCl or LB. Rectal temperatures, pulses,

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and respiratory rates were within reference limits for all horses at all time points evaluated. All horses also displayed typical stalled behavior, including feed and water consumption and fecal and urinary output. When horses were treated with BHCl, the median rectal temperature, pulse, and respiratory rate were 37.3 °C (range, 36.2 to 37.9 °C; reference range, 37 to 38.6 °C), 40 beats/min (range, 26 to 52 beats/min; reference range, 24 to 52 beats/min), and 20 breaths/min (range, 12 to 32 breaths/min; reference range, 8 to 20 breaths/min), respectively. When horses were treated with LB, the median rectal temperature, pulse, and respiratory rate were 37.3 °C (range, 36.5 to 37.8 °C), 40 beats/min (range, 24 to 52 beats/min), and 20 breaths/min (8 to 48, breaths/min), respectively. No horse had clinically meaningful inflammation (eg, heat, major swelling, or signs of pain on palpation) around the injection sites for either the BHCl or LB treatment. Median metacarpophalangeal joint circumference was significantly larger at 48 hours after perineural anesthesia with BHCl (23.9 cm), compared with baseline (23.8 cm; $P = 0.009$), and at 24 hours after perineural anesthesia with LB (24.3 cm), compared with baseline (23.9 cm; $P = 0.003$; **Table 1**).

MNT testing

MNTs were significantly ($P < 0.001$) increased after perineural anesthesia with BHCl at 1 hour after injection, compared with baseline. When treated with LB, horses had significantly ($P \leq 0.001$) increased MNTs at 1, 6, and 24 hours, compared with baseline (Table 1).

Lameness model assessment trials

Following induction of lameness, subjectively a similar degree of lameness was induced at the 0-, 1-, and 6-hour time points with the same screw length. However, for 7 of 12 forelimbs, the screw length had to be increased by 1 to 2 mm (depending on the horse) to create a similar degree of lameness at the 24-hour time point. Thus, for successive 24-hour time points following administration of perineural analgesia (24, 48, and 72 hours), screw length was advanced by the same amount (1 to 2 mm) that preserved the degree of lameness for the 24-hour time point before perineural analgesia.

Perineural anesthesia trials

When results for VS scores were compared at time 0 of the lameness model trials at the end of

Table 1—Median and range metacarpophalangeal joint (MCPJ) circumferences and mechanical nociceptive thresholds (MNTs) in 6 healthy research horses after unilateral forelimb sole-pressure lameness induction 24 hours earlier to establish grade 3/5 baseline lameness, and then at 1, 6, 24, 48, and 72 hours after perineural anesthesia of the medial and lateral palmar nerves, grouped by treatment (bupivacaine hydrochloride [BHCl] or liposomal bupivacaine [LB]) in a crossover study conducted between January 22, 2019, and February 23, 2019.

Variable	Treatment	Baseline	1 hour	6 hours	24 hours	48 hours	72 hours	*P* value
MCPJ circumference (cm)	BHCl	23.8 (23.5–26.4)	23.9 (23.3–26.1)	23.8 (23.4–26.5)	24.0 (23.1–26.4)	23.9 (23.6–26.5)*	23.9 (23.5–26.0)	0.050
	LB	23.9 (23.5–26.1)	24.1 (23.6–26.0)	24.2 (23.5–26.2)	24.3 (23.7–6.8)*	24.3 (23.7–26.7)	24.3 (23.7–26.3)	0.032
MNT (kg/cm^2)	BHCl	10.5 (7.0–12.6)	20.8 (19.5–24.5)*	11.3 (7.7–12.6)	9.5 (6.7–12.5)	9.6 (7.9–13.5)	8.5 (7.4–10)	< 0.001
	LB	8.9 (6.7–10.3)	21.9 (20.4–24.9)*	20.1 (12.4–22.5)*	12.8 (8.5–20.0)*	9.1 (7.4–9.9)	8.4 (7.1–14.2)	< 0.001

*Indicates a significant difference ($P < 0.05$) between that time point and the baseline measurement for that specific treatment.

Table 2—Median and range vector sum (VS) scores obtained with the use of an inertial sensor system for the horses described in Table 1 during the 24-hour lameness model assessment to establish a baseline before (time 0 hours) and after (1, 6, 14, 48, and 72 hours) perineural anesthesia with BHCl or LB, grouped by treatment. The 0-hour time point for the perineural anesthesia trial was the baseline and the same as the 24 hours lameness model assessment time point.

Treatment	Time (h)	Lameness model assessment vs (mm)	Perineural anesthesia vs (mm)	*P* value
BHCl	0	55.0 (13.1–75.9)	26.3 (5.2–57.1)	0.066
	1	82.8 (14.0–257.9)	12 (3.9–48.2)	< 0.001
	6	51.1 (11.9–152.3)	40.3 (6.9–116.7)	0.156
	24	26.3 (5.2–57.1)	18.2 (4.0–50.8)	0.612
	48	—	22.3 (3.9–51.9)	—
	72	—	68.8 (30.9–102.7)	—
LB	0	34.0 (19.0–69.7)	58.4 (14.2–180.2)	0.306
	1	91.0 (35.4–207.3)	13.6 (1.4–47.9)	< 0.001
	6	57.6 (27.5–125.1)	12.7 (4.9–29.3)	< 0.001
	24	58.3 (14.2–180.2)	14.0 (0.7–100.4)	0.003
	48	—	20.1 (5.3–191.2)	—
	72	—	18.55 (7.1–108.9)	—

*Indicates a significant difference ($P < 0.05$) between that time point and the 0 h measurement for that specific treatment.

— = Not applicable.

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Table 3—Median and range peak vertical, braking, and propulsive forces and vertical, braking, and propulsive impulses, normalized to horse body weight, for the treated forelimbs of the horses described in Table 1 at baseline, then at 1, 6, 24, 48, and 72 hours after perineural anesthesia with BHCl or LB.

Treatment	Variable	Baseline	1 hour	6 hours	24 hours	48 hours	72 hours	*P* value
BHCl	Peak vertical force (N/kg)	8.82 (8.2–10.2)	9.3 (8.83–10.13)	9.03 (7.04–10.34)	9.08 (6.31–10.06)	8.96 (7.23–10.10)	8.07 (7.44–8.42)*	0.003
	Vertical impulse (N·s/kg)	1.82 (1.61–1.92)	1.86 (1.80–1.94)	1.77 (1.56–2.04)	1.86 (1.38–2.00)	1.71 (1.48–2.04)	1.60 (1.40–1.80)*	0.001
	Peak braking force (N/kg)	−0.90 (−1.12 to −0.63)	−0.94 (−1.03 to −0.82)	−0.82 (−1.07 to −0.73)	−0.88 (−1.05 to −0.69)	−0.79 (−1.16 to −0.76)	−0.69 (−0.96 to −0.55)	0.132
	Braking impulse (N·s/kg)	−0.12 (−0.14 to −0.07)	−0.11 (−0.14 to −0.10)	−0.11 (−0.13 to −0.08)	−0.11 (−0.14 to −0.07)	−0.10 (−0.14 to −0.08)	−0.08 (−0.12 to −0.06)	0.269
	Peak propulsive force (N/kg)	0.60 (0.42–0.90)	0.64 (0.47–0.93)	0.68 (0.45–0.90)*	0.64 (0.41–0.85)	0.59 (0.47–0.84)	0.53 (0.49–0.80)	0.004
	Propulsive impulse (N·s/kg)	0.05 (0.03–0.09)	0.05 (0.04–0.09)*	0.06 (0.04–0.09)*	0.05 (0.03–0.09)	0.05 (0.04–0.09)	0.04 (0.04–0.08)	0.007
LB	Peak vertical force (N/kg)	8.04 (5.29–9.07)	9.58 (8.79–9.97)*	9.50 (9.02–10.25)*	9.28 (7.94–10.43)*	9.31 (7.53–9.98)*	8.86 (7.79–9.74)*	< 0.001
	Vertical impulse (N·s/kg)	1.55 (1.28–1.75)	1.83 (1.71–2.02)*	1.89 (1.78–2.03)*	1.82 (1.66–1.96)*	1.78 (1.60–1.92)*	1.74 (1.61–1.99)*	< 0.001
	Peak braking force (N/kg)	−0.68 (−1.02 to −0.47)	−1.03 (−1.14 to −0.73)*	−0.93 (−1.18 to −0.78)*	−0.91 (−1.06 to −0.66)	−0.84 (−0.98 to −0.66)	−0.84 (−0.96 to −0.74)	< 0.001
	Braking impulse (N·s/kg)	−0.07 (−0.12 to −0.06)	−0.13 (−0.15 to −0.08)*	−0.12 (−0.15 to −0.09)*	−0.11 (−0.13 to −0.07)*	−0.11 (−0.11 to −0.07)	−0.10 (−0.13 to −0.08)	< 0.001
	Peak propulsive force (N/kg)	0.56 (0.37 to 0.75)	0.64* (0.47 to 0.84)	0.70 (0.49 to 0.91)*	0.76 (0.48 to 0.91)*	0.76 (0.54 to 0.94)*	0.69 (0.55 to 0.91)	< 0.001
	Propulsive impulse (N·s/kg)	0.05 (0.03 to 0.07)	0.05 (0.04 to 0.08)	0.06 (0.04 to 0.08)	0.06 (0.04 to 0.09)*	0.06 (0.04 to 0.10)*	0.06 (0.05 to 0.10)	< 0.005

Table 4—Median and range peak vertical, braking, and propulsive forces and vertical, braking, and propulsive impulses, normalized to horse body weight, for the nontreated, nonlame forelimbs of the horses described in Table 1 at baseline, then at 1, 6, 24, 48, and 72 hours after perineural anesthesia with BHCl or LB was administered to the contralateral forelimb in which lameness had been experimentally induced.

Treatment	Variable	Baseline	1 hour	6 hour	24 hours	48 hours	72 hours	*P* value
BHCl	Peak vertical force (N/kg)	9.62 (9.04 to 10.51)	9.64 (8.66 to 9.95)	9.53 (8.97 to 10.66)	9.53 (8.99 to 10.13)	9.60 (9.12 to 10.30)	9.67 (9.60 to 10.57)*	< 0.001
	Vertical impulse (N·s/kg)	1.87 (1.82 to 2.05)	1.87 (1.75 to 1.95)	1.89 (1.78 to 2.06)	1.88 (1.80 to 2.12)	1.92 (1.75 to 2.13)	1.99 (1.70 to 2.23)	0.348
	Peak braking force (N/kg)	−0.95 (−1.16 to −0.78)	−0.90 (−0.97 to −0.59)	−0.93 (−1.21 to −0.80)	−0.91 (−0.98 to −0.86)	−0.98 (−1.06 to −0.80)	−1.01 (−1.24 to −0.77)	0.058
	Braking impulse (N·s/kg)	−0.12 (−0.14 to −0.09)	−0.11 (−0.12 to −0.06)*	−0.12 (−0.14 to −0.09)	−0.11 (−0.12 to −0.10)	−0.12 (−0.14 to 0.09)	−0.13 (−0.15 to −0.08)	0.025
	Peak propulsive force (N/kg)	0.68 (0.43 to 0.88)	0.74 (0.59 to 0.89)*	0.75 (0.56 to 0.85)*	0.73 (0.53 to 0.84)	0.67 (0.56 to 0.82)	0.78 (0.57 to 0.92)*	< 0.001
	Propulsive impulse (N·s/kg)	0.06 (0.03 to 0.09)	0.06 (0.05 to 0.09)*	0.06 (0.04 to 0.09)	0.06 (0.05 to 0.09)	0.06 (0.05 to 0.09)	0.06 (0.05 to 0.09)*	0.037
LB	Peak vertical force (N/kg)	9.87 (9.31 to 11.45)	9.65 (8.56 to 10.83)*	9.51 (8.77 to 10.79)*	9.55 (9.04 to 10.61)*	9.62 (9.00 to 10.78)*	9.70 (9.01 to 10.57)	0.021
	Vertical impulse (N·s/kg)	1.94 (1.72 to 2.30)	1.89 (1.68 to 2.01)*	1.87 (1.78 to 1.92)*	1.88 (1.69 to 2.08)	1.86 (1.72 to 2.11)*	1.97 (1.68 to 2.09)	0.011
	Peak braking force (N/kg)	−1.14 (−1.24 to −0.70)	−0.95 (−1.04 to −0.68)*	−0.98 (−1.02 to −0.69)*	−0.99 (−1.32 to −0.62)*	−0.96 (−1.14 to −0.75)*	−1.06 (−1.39 to −0.67)	0.005
	Braking impulse (N·s/kg)	−0.14 (−0.19 to −0.07)	−0.11 (−0.14 to −0.07)*	−0.12 (−0.13 to −0.07)*	−0.12 (−0.18 to −0.06)	−0.12 (−0.14 to −0.08)	−0.13 (−0.18 to −0.07)	0.01
	Peak propulsive force (N/kg)	0.65 (0.56 to 0.82)	0.71 (0.53 to 0.87)	0.68 (0.51 to 0.90)	0.70 (0.36 to 0.93)	0.64 (0.57 to 0.93)	0.65 (0.49 to 0.90)	0.949
	Propulsive impulse (N·s/kg)	0.05 (0.04 to 0.07)	0.06 (0.04 to 0.09)	0.06 (0.05 to 0.09)	0.06 (0.03 to 0.09)	0.05 (0.04 to 0.09)	0.05 (0.04 to 0.09)	0.619

*Indicates a significant difference ($P < 0.05$) between that time point and the baseline measurement for that specific treatment.

the 24-hour lameness model, which served as the baseline lameness for the treatment trials, median VS scores at baseline within a treatment group (BHCl group or LB group) did not differ substantially **(Table 2)**. However, the median VS score was significantly ($P < 0.001$) lower at 1 hour after treatment with BHCl, compared with baseline, and at 1, 6, and 24 hours after treatment with LB). When results for baseline versus treatment were compared, the median VS score was significantly ($P = 0.007$) higher for horses 72 hours after treatment with BHCl, whereas the median VS score was significantly ($P = 0.012$, 0.002, 0.01, and 0.041, respectively) lower at 1, 6, 24, and 48 hours after treatment with LB.

When kinetics across time (results at baseline vs results at 1, 6, 24, 48, and 72 hours after perineural anesthesia with BHCl or LB), results for the BHCl treated limbs had significantly ($P < 0.005$) higher median peak propulsive force 6 hours after treatment (0.68 N/kg) and median propulsive impulse 1 and 6 hours after treatment (0.054 N•s/kg and 0.06 N•s/kg, respectively; $P = 0.01$) and significantly ($P < 0.005$) lower median peak vertical force (8.07 N/kg) and vertical impulse (1.60 N•s/kg) 72 hours after treatment, compared with baseline. For the contralateral nonlame forelimbs, the median peak vertical force was significantly ($P < 0.001$) higher at 72 hours (9.67 N/kg) versus baseline (9.62 N/kg), the median braking impulse was significantly ($P = 0.03$) decreased at 1 hour (−0.11 N•s/kg) versus baseline (−0.12 N•s/kg), the median peak propulsive force was significantly ($P < 0.001$) higher at 1 (0.74 N/kg), 6 (0.75 N/kg), and 72 (0.78 N/kg) hours after treatment versus at baseline (0.68 N/kg), and the median propulsive impulse was significantly higher at 1 (0.06 N•s/kg) and 72 (0.06 N•s/kg) hours after treatment with BHCl versus at baseline (0.06 N•s/kg)

When horses were treated with LB, there were significant increases in the median peak vertical ($P < 0.001$), braking ($P < 0.001$), and propulsive ($P = 0.001$) forces and vertical ($P < 0.001$), braking ($P < 0.001$), and propulsive ($P < 0.005$) impulses in the lame forelimb up to 72 hours after perineural anesthesia **(Table 3)**. For the contralateral nonlame limb, there were significant decreases in peak vertical ($P = 0.02$) and braking ($P = 0.005$) force as well as vertical ($P = 0.01$) and braking ($P = 0.005$) impulse up to 48 hours after perineural anesthesia **(Table 4)**.

Discussion

From this investigation, our results supported the main hypothesis that LB as injected around the palmar nerves at the level of the proximal sesamoid bones would have longer analgesic effect, compared with the use of BHCl. Additionally, the horses did not demonstrate negative systemic side effects following perineural injection of LB, as they maintained normal physical examination parameters and normal stalled behavior during the 72-hour study period.

We also did not identify substantial inflammation around the LB injection sites. None of the horses demonstrated heat, major swelling, or pain on palpation of the injection sites. In a previous study[12] in which LB was injected over the palmar digital nerves (2 mL/site), 1 horse showed small, raised areas over

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both injection sites at 48 hours, which resolved in 24 hours. In the present study, we measured the metacarpophalangeal joint circumference as an objective method to assess swelling around the injection sites. There was a very small but significant increase in circumference at 24 hours after treatment with LB and at 48 hours after treatment with BHCl. However, as the horses did not show any other local signs of inflammation (eg, heat or sensitivity to palpation), the clinical importance of this was unknown.

We found that response to pressure, as determined by an increase in MNTs, was significantly higher in the LB-treated forelimbs for at least 24 hours, but they were not substantially different between measurements before injection and at 48 or 72 hours after injection. A recent equine study[12] of nonlame horses showed that MNTs in LB-treated limbs were not substantially different from the saline control at 6 hours when a total of 53.2 mg was used (26.6 mg/nerve). In the present study, a slightly larger amount of LB was used (a total of 79.8 mg: 39.9 mg/nerve). It was possible that the larger volume of LB used in the present study resulted in a longer duration of action, compared with the previous study.[12]

In the present study, we also found that the objective VS lameness scores returned toward baseline levels within 72 hours after treatment. This effect was not unexpected, as in a previous study in which a 5.3-mg/kg dose of LB for postoperative analgesia was given to dogs after stifle joint surgery, only 42% and 38% of dogs did not require rescue analgesia at 48 and 72 hours, respectively.[7] This indicated that not all animals would be expected to have sufficient analgesia at 72 hours after treatment with LB.

One potential reason for the lack of analgesia observed at 72 hours was that we did not provide a sufficient volume or dose of LB to provide the necessary depot to last a full 72 hours. We chose to use a consistent volume (6 mL/limb) of LB or BHCl instead of dosing based on milligrams of drug per kilogram of body weight because the volume of drug administered per limb has been a common method used when performing perineural anesthesia for diagnostic or treatment purposes. The total dose used in this group of horses ranged from 0.13 to 0.18 mg/kg, similar to the dose used in a recent study[13] of intra-articular use of LB (0.12 mg/kg) in horses.[13] In a study[7] of postoperative pain following stifle joint surgery in dogs, a dose of 5.3 mg/kg was used as an SC infiltration. We anticipated that a perineural block would require a smaller amount of bupivacaine, compared with local infiltration because of increased specificity; thus, we anticipated that a lower dose of LB would be appropriate. In a previous study[14] of rabbits and dogs, perineural use of LB resulted in mild granulomatous inflammation within perineural fat in approximately one-third of animals following the administration of each of the 3 tested doses (9 to 30 mg/kg), which was suspected to be a normal effect following the introduction of a foreign material (ie, liposomes). Although we used a much lower dose, it was unknown whether a lower dose would be expected to decrease the long-term complications. Two recent equine studies[12,15] have demonstrated that LB had a shorter duration of analgesic effect, but both studies used smaller volumes of LB (0.75 to 2 mL/nerve site). However, because of limited research on the use of LB in horses, the optimal dose for perineural use and whether a larger dose may result in longer-duration analgesia were unknown.

We did find differences between the timing of the return of skin sensation (MNTs) at 48 hours, compared with when objective lameness (72 hours) returned to baseline in the LB group. This was not unexpected, as in 1 previous study, the time point at which skin sensitivity returned was not the same as the return of lameness when lidocaine was used for perineural anesthesia.[16] It has been postulated that differences in the effectiveness and time to onset of local anesthetics with different nerve fiber types (ie, C-fiber vs A-delta) may be the reason for the discrepancy of lameness resolution/reappearance and skin sensitivity.[16,17]

One limitation of the present study was the change in lameness with this model that occurred over time. We chose to only insert screws at data collection times points, as we felt that leaving the screws in place for the entire 72-hour study duration had the potential to induce longer-term lameness. Several of the horses had a decrease in lameness intensity at the 24-hour time point when we performed the initial lameness induction trials. Another previous study[16] had identified changes within a defined lameness grade when a similar model of lameness was used, with 2 of 6 horses showing some decreasing level of lameness over time. To maintain a similar degree of lameness throughout the study duration, the length of the screw was increased by 1 to 2 mm every 24 hours following initial perineural anesthesia. Because the length of screws during both the preblocking and postblocking changed, this could affect the objective lameness (VS) scores, so the longitudinal assessments of VS scores past 24 hours may be misleading. To account for the intrahorse changes in lameness, results for both the BHCl and LB groups were compared with results before and up to 24 hours after treatment. Because of time and budgetary limitations, we did not collect 48 and 72-hour preblocking lameness data, which would have been necessary to better understand the lameness model and to make more accurate assessments of the LB group beyond 24 hours.

From this investigation, we concluded that the use of LB at the dose used in the present study (approx 40 mg/nerve site) had analgesic effects of longer duration than did BHCl: up to 24 hours of analgesia. Further work is needed to determine whether a higher dose may result in a longer duration of effects while still resulting in low levels of systemic side effects. Investigating the use of LB for the treatment of perioperative surgical pain is also warranted to determine additional clinical benefits of this formulation of bupivacaine.

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References

1. Lerche P, Muir WW. Perioperative pain management. In: Muir WW, Hubbell AE, eds. *Equine Anesthesia: Monitoring and Emergency Therapy.* 2nd ed. Elsevier; 2009:369–380.
2. Matthews NS, Carroll GL. Review of equine analgesics and pain management. In: *Proceedings of the 53rd Annual Convention of the American Association of Equine Practitioners.* American Association of Equine Practitioners; 2007;53:240–244.
3. Sanchez LC, Robertson SA. Pain control in horses: what do we really know? *Equine Vet J*. 2014;46(4):517–523.
4. Mackenzie C. Do opioids cause colic? *Equine Vet Educ*. 2017;29:401–302.
5. Clutton RE. Opioid analgesia in horses. *Vet Clin North Am Equine Pract*. 2010;26(3):493–514.
6. Skarda RT, Muir WW, Hubbell JAE. Local anesthetic drugs and techniques. In: Muir WW, Hubbell AE, eds. *Equine Anesthesia: Monitoring and Emergency Therapy.* 2nd ed. Elsevier Inc; 2009:210–242.
7. Lascelles BDX, Rausch-Derra LC, Wofford JA, Huebner M. Pilot, randomized, placebo-controlled clinical field study to evaluate the effectiveness of bupivacaine liposome injectable suspension for the provision of post-surgical analgesia in dogs undergoing stifle surgery. *BMC Vet Res*. 2016;12(1):168. doi:10.1186/s12917-016-0798-1
8. Moorman VJ, Reiser RF II, Peterson ML, McIlwraith CW, Kawcak CE. Effect of equine forelimb lameness on hoof kinematics at the trot. *Am J Vet Res*. 2013;74(9):1183–1191.
9. Moorman VJ, Reiser RF II, Mahaffey CA, Peterson ML, McIlwraith CW, Kawcak CE. Use of an inertial measurement unit to assess the effect of forelimb lameness on three-dimensional hoof orientation in horses at a walk and trot. *Am J Vet Res*. 2014;75(9):800–808.
10. American Association of Equine Practitioners. *Guide for Veterinary Service and Judging of Equestrian Events.* 4th ed. American Association of Equine Practitioners; 1991:19.
11. Keegan KG, Kramer J, Yonezawa Y, et al. Assessment of repeatability of a wireless, inertial sensor-based lameness evaluation system for horses. *Am J Vet Res*. 2011;72(9):1156–1163.
12. McCracken MJ, Schumacher J, Doherty TJ, Sun X, Nichols CL, Olivarez J. Efficacy and duration of effect for liposomal bupivacaine when administered perineurally to the palmar digital nerves of horses. *Am J Vet Res*. 2020;81(5):400–405.
13. Knych HK, Mama KR, Moore CE, Hill AE, McKemie DS. Plasma and synovial fluid concentrations and cartilage toxicity of bupivacaine following intra-articular administration of a liposomal formulation to horses. *Equine Vet J*. 2019;51(3):408–414.
14. Richard BM, Newton P, Ott LR, et al. The safety of EXPAREL (bupivacaine liposome injectable suspension) administered by peripheral nerve block in rabbits and dogs. *J Drug Deliv*. 2012;2012:962101. doi:10.1155/2012/962101
15. Le KM, Caston SS, Hossetter JM, Hay Kraus BL. Comparison of analgesic and tissue effects of subcutaneous perineural injection of liposomal bupivacaine and bupivacaine hydrochloride in horses with forelimb lameness induced via circumferential clamp. *Am J Vet Res*. 2020;81(7):551–556.
16. Hoerdemann M, Smith RL, Hosgood G. Duration of action of mepivacaine and lidocaine in equine palmar perineural blocks in an experimental lameness model. *Vet Surg*. 2017;46(97):986–993.
17. Palmer SK, Bosnjak ZJ, Hopp FA, von Colditz JH, Kampine JP. Lidocaine and bupivacaine differential blockade of isolated canine nerves. *Anesth Analg*. 1983;62(8):754–757.

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