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Effect of Needle and Extracorporeal Shockwave Stimulation of Acupuncture Points on Equine Chronic Multilimb Lameness Using a Single-Formula Approach

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Abstract

Objective The aim of this study was to compare effects with conventional (needle and electroacupuncture, NAP) and shockwave stimulation of acupuncture points (SAP) on chronic multilimb lameness in horses.

Study Design Randomized crossover block design; ten mature Standardbred mares with multilimb lameness (< 4/10) underwent 3-weekly point stimulations (NAP/SAP) selected on the basis of their uniform applicability. Groups were reversed following a washout period (9 weeks). Lameness at the trot was video recorded and quantified objectively using an inertial sensor-based system during a 4-week pre- and post-treatment period. Blinded expert review of recordings resulted in subjective qualitative (better, same, or worse) and quantitative outcome measures (0–10 lameness grade). Mixed effect repeated measures analyses were performed on objective quantitative gait parameters specific to fore (Vector sum [VS_{Head}]) and hindlimb lameness (average differences in minimum [$DIFFMIN_{Pelvis}$] and maximum pelvic height [$DIFFMAX_{Pelvis}$]) Qualitative data were assessed in non-parametric tests.

Results SAP had no effect on forelimb but improved hindlimb lameness ($DIFFMIN_{Pelvis}$; $p < 0.001$). NAP was associated with deterioration of forelimb lameness (VS_{Head}, $p < 0.001$) and had no effect on hindlimb lameness. VS_{Head} data differed between modalities when accounting for the time of observation (interaction effect; $p = 0.002$). For other quantitative gait parameters, a difference between modalities was not observed. SAP was associated with greater animal comfort post-treatment compared with pre-treatment assessments ($p = 0.036$). Typically, improvement occurred by one and deterioration by two lameness grades.

Conclusion SAP and NAP were not associated with the same treatment outcome. SAP slightly improved but did not alleviate all lameness. Given the non-invasive nature of SAP, this method may have potential in the management of chronic multilimb lameness.

Keywords
- ► acupuncture
- ► horse
- ► shockwave
- ► lameness
- ► Lameness Locator

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Introduction

Successful management of multilimb lameness incorporates multimodal pain control integrating different therapeutic and supportive principles often including methods of Traditional Chinese Veterinary Medicine (TCVM).[1–4] Conventional equine lameness therapy is reliant on precise diagnoses formed on the basis of the localization of pain and identification of the underlying pathology. In TCVM, the same detailed pathoanatomical understanding is not required. Treatment principles are based on the recognition of various specific disease patterns and the promotion of free energy flow and the body systems' balance. This is achieved utilizing acupuncture points with general local or remote therapeutic effects along their connecting pathways or via special points attributed to a specific function—for example influential, master (foundational), or classical points. The latter unlike any other points are animal-specific and not derived from human transposition.[5] In horses, selection of locoregional acupuncture points is further aided by palpation of sentinel points which alerts the practitioner to areas of interest and augments treatment strategies based on remote targeting.[6–8] The body of evidence proposing the utility of veterinary acupuncture in musculoskeletal pain control, including the effect on equine lameness is growing; however, robust scientific evidence for its efficacy is limited.[9–13] Acupuncture-induced analgesia is largely attributed to group A nerve fibre excitation and spinal microglial neuromodulation. Release of opioid peptides following acupuncture constitutes one of the known effector pathways[14] for which evidence has also been found in horses.[15] With needle acupuncture relying on patient compliance to introduce and maintain needles for an adequate duration, alternate methods for point stimulation avoiding skin penetration are of interest. Potentially fulfilling this purpose are optical low level lasers[16,17] and shockwave systems,[18,19] but clinical evidence to support these approaches is limited or absent for horses. Shockwave systems produce a focal or radial wave based on piezoelectric, electrohydraulic or electromagnetic mechanisms. Therapeutic effects have been attributed to effects of cavitation resulting in local analgesia and microtrauma, invigorating reparative processes in chronic disease.[20] Considering the availability of veterinary shockwave systems and their adaptability to requirements of penetration depth and patient comfort, this modality may offer advantages over conventional methods of acupuncture point stimulation (needle acupuncture with or without electrostimulation).

Therefore, it was hypothesized that focused shockwave stimulation of acupuncture points (SAP) in horses with chronic multilimb lameness would result in a quantifiable improvement and that this response would be on par with that following needle acupuncture ($\pm$ electrostimulation; NAP).

Materials and Methods

Animals

With approval of the animal ethics committee at Charles Sturt University (19256), 10 female Standardbred trotters (11.3 ± 4 years; 516 ± 56.3 kg body weight) were recruited from the teaching and research herd. Horses had to be multilimb lame (subjective lameness grade $\leq$ 4/10) and could not participate in other research or teaching activities for the duration of the study. This assessment was performed by the first author (specialist in equine surgery and sports medicine, certified veterinary acupuncturist). Normal husbandry was maintained throughout including regular foot trimming intervals.

Experimental Schedule

A prospective randomized crossover design was adopted. Trial 1 consisted of a 4-week long pre-treatment, 3-week long treatment and 4-week long post-treatment period. This protocol was repeated after a 9-week long washout period forming trial 2. In trial 1, five horses were randomly allocated to each treatment group (NAP or SAP) and groups were crossed over for trial 2. In both trials during pre- and post-treatment periods, lameness was recorded three times at a minimum weekly interval using a portable wireless inertial sensor-based system (PISBS; EquinosisQ, Lameness Locator, Columbia, Missouri, United States) and a digital video camera (Sony FDR-AX100E 4K, Tokyo, Japan). Data recording intervals were kept the same in both trials.

Needle Stimulation of Acupuncture Points

Classical, influential and master points were prioritized and selected based on their ascribed function and uniform applicability to this population; ►**Table 1**). Acupuncture point locations were clipped and cleaned using chlorhexidine followed by alcohol. Depending on the point, either dry needling or electrostimulation of inserted needles was performed (►**Table 1**). Dry needles were manually stimulated every 5 minutes by gentle agitation. For electroacupuncture, needles were connected in specific pairs to an electrostimulator (JM-2A, Jing Mei; Jiajian Medical Instruments Co., Ltd, Wuxi, China) using a continuous wave (30 minutes; 30 Hz) of variable maximum intensity depending on the animals' tolerance threshold. This meant that whenever possible, the intensity of the electrostimulation was gradually increased to result in subtle muscle fasciculations and slight 'needle tremor' while not eliciting avoidance reactions (intensity range 1–3/10). Based on subjective pre-trial lameness examinations, acupuncture point selections included 'LI-1' in horses with predominant fore and 'Yan-Chi' in those with predominant hind limb lameness; otherwise, all points in ►**Table 1** were stimulated in every horse.

Shockwave Stimulation of Acupuncture Points

With the animal unsedated and restrained in stocks, 100 impulses (0.22 mJ/mm^2; 5 Hz) were applied using a portable, focused shockwave system (Duolith, SD1 T-Top VET, 'F-SW ultra', Storz Medical AG, Tägerwilen, Switzerland). The probe maintained surface contact throughout treatment, but its angle to the skin was continuously varied. Depending on the acupuncture point treated, shocks were either applied with or without stand-off to adjust the focal zone appropriately (►**Table 1**). Skin contact was optimized by clipping hair,

Table 1 Treatment protocol details. All acupuncture points listed were treated in every horse except for LI-1 and Yan Chi which were included in horses with perceived dominant fore or hindlimb lameness respectively. Point localization was performed in accordance with a core textbook.[5]

Acupuncture point	Location	TCVM justification	Needle size	Connections for electrostimulation	Focal zone of shockwave probe
Bai-hui	On dorsal midline at the lumbosacral space	Hind quarter pain; calming/permission	0.3×75 mm[1]	Dry needle only	35–65 mm
Shen-shu	2 cun[3] lateral to Bai-hui	Hind quarter pain; bone/ Joint support via kidney element	0.3×75 mm[1]	Dry needle only	35–65 mm
Shen-peng	2 cun cranial to Shen-shu	Hind quarter pain; bone/ Joint support via kidney element	0.3×75 mm[1]	BL-54 (ipsilateral)	35–65 mm
Shen-jiao	2 cun caudal to Shen-shu	Hind quarter pain; bone/ Joint support via kidney element	0.3×75 mm[1]	BL-11 (ipsilateral)	35–65 mm
Yan-Chi (dominant hindlimb lx group)	Midpoint between top of tuber coxa and Shen-peng	Hind quarter pain (arthritis); poor performance	0.3×75 mm[1]	Yan-Chi (contralateral)	35–65 mm
Qi-hai-shu Sea of Chi	In the groove between the longissimus dorsi and the iliocostalis mm at the level of the 16th intercostal space	Qi deficiency; poor performance; general weakness	0.3×50 mm[1]	Qi-hai-shu (contralateral)	35–65 mm
BL-11	Cranial to the withers, at 2nd thoracic vertebral space, 1 cun lateral to dorsal midline	Forelimb lameness; influential point for bone (arthritis)	0.3×75 mm[1]	Shen-Jiao (ipsilateral)	35–65 mm
BL-54	Midway online connecting Bai-hui and the greater trochanter	Hindlimb lx; masterpoint hindlimb	0.3×75 mm[1]	Shen-peng (ipsilateral)	35–65 mm
SI-9	Large depression along the caudal border of the deltoid muscle at its juncture with the lateral and the long heads of the triceps brachii	Forelimb lx; general pain relief; masterpoint forelimb	0.3×50 mm[1]	LI-1 (ipsilateral, *dominant forelimb lx group*) SI-9 (contralateral, *dominant hindlimb lx group*)	15–45 mm
GB-34	Craniodistal to the head of the fibula, in the interosseous space between the tibia and fibula, between the long and lateral digital extensors	Influential point for tendon and ligaments	0.35×25 mm[2]	Dry needle only	15–45 mm
LI-1 (dominant forelimb lx group)	Craniomedial aspect of the front hoof, proximal to coronary band	Forelimb lx, foot pain	0.35×15 mm[2]	SI-9 (ipsilateral)	0–30 mm

Abbreviations: BL, bladder channel; GB, gallbladder channel; LI, large intestine channel; Lx, lameness; SI, small intestine channel.
[1]Hwato, Suzhou Medical Instruments, China; [2]Jing Mei, Jiajian Medical Instrument Co., Ltd. Wuxi, China. [3]Cun is a proportional measurement unit; 1 cun is the width of two fingers of an average sized hand in an average sized horse; relative to a horse's size it corresponds to the width of its last rib.

wiping with alcohol and applying contact gel at administration sites.

Gait Assessment

Prior to horses being assessed, feet were cleaned and hoof testers applied to confirm the absence of acute subsolar pain and exclude the potential for scheduled foot trims or accidental subsolar bruising to affect outcomes. The same handler then trotted horses in hand and in a straight line on a 40-m packed dirt track while continuously recording data using the PISBS to standard specifications and the digital camera set-up.

Quantitative Gait Analysis

Vertical displacement data (mm) of accelerometers located on the horses' head and pelvis were automatically processed within the PISBS's software program and summary statistics for each trot-up episode retrieved:

Average (Δ) and standard deviation (SD) of the differences between minimal head (pelvic) heights during the stance of the right forelimb (hindlimb) and the stance of the left forelimb (hindlimb); differences in minimum head height ($DIFFMIN_{Head}$) (differences in minimum pelvic height [$DIFFMIN_{Pelvis}$])

Average and SD of the differences between the maximal head (pelvic) heights after the stance of the left forelimb (hindlimb) and the stance of the right forelimb (hindlimb); $DIFFMAX_{Head}$ ($DIFFMAX_{Pelvis}$)
Vector sum of $DIFFMIN_{Head}$ and $DIFFMAX_{Head}$; Vector sum (VS_{Head})

Per industry standards,[21,22] horses were considered forelimb lame when $\Delta DIFFMIN_{Head}$ or $\Delta DIFFMAX_{Head}$ exceeded 6 mm or when VS_{Head} was greater than 8.5mm. Values exceeding 3 mm for $\Delta DIFFMIN_{Pelvis}$ and $\Delta DIFFMAX_{Pelvis}$ were considered the threshold for hindlimb lameness. Specific to the PISBS and associated computations, negative $\Delta DIFFMIN_{Pelvis}$ and $\Delta DIFFMAX_{Pelvis}$ attribute lameness to the left hindlimb and characterize the lameness pattern as impact or push-off respectively. Lameness of the right hindlimb is implicated by positive values. As $\Delta DIFFMIN_{Pelvis}$ and $\Delta DIFFMAX_{Pelvis}$ values are independent from each other, simultaneous push-off and impact hindlimb lameness patterns can be detected with this system. Due to the interdependence of maximum and minimum vertical head positions, positive $\Delta DIFFMIN_{Head}$ and $\Delta DIFFMAX_{Head}$ signify right fore impact lameness and positive $\Delta DIFFMIN_{Head}$ and negative $\Delta DIFFMAX_{Head}$ characterize a right fore push-off lameness. The opposite sign applies to identification of left forelimb lameness. For the purpose of this investigation, the influence and relevance of compensatory limb lameness were not considered.

Qualitative Gait Analysis

First pre- and last post-treatment video observations from each trial were presented to an equine specialist surgeon and sports medicine clinician with more than 30 years of clinical experience (second author) for blind scoring. First, horses in which treatment resulted in a more comfortable presentation were given a score of '1'; those that appeared worse post-treatment were scored '-1' and any horse showing no change received a score of '0'. The horse's lameness was then given a grade on a subjective 0 to 10 lameness scale with 0 being sound and 10 being non-weight bearing lame.[23] This process was completed by presenting paired videos on individual digital slides in random order (Keynote V11.0.1, Apple Inc., Cupertino, California, United States) and displaying them on a high definition widescreen TV for comparative review.

Statistical Analyses

Quantitative gait analysis: Mixed repeated measures analysis of variance was performed for each treatment modality and gait parameter independently. Model terms were 'Horse' (Horse 1–10; subject and random effect), 'Time' (Pre- and post-treatment; repeated effect), 'Observation' (Observation 1, 2 and 3; repeated effect), 'Trial' (Trial 1 and 2) and 'Classification' (Fore and hindlimb treatment protocol). When modelling VS_{Head} 'Lameness side' (Left and right forelimb) was also included. To allow comparison between treatment modalities while also accommodating repeated effects, VS_{Head}, $\Delta DIFFMIN_{Pelvis}$ and $\Delta DIFFMAX_{Pelvis}$ were averaged across pre- and post-treatment observations and modelled using a mixed repeated measures analysis of variance. Model terms were: 'Horse' (Horse 1–10; subject and random effect), 'Classification' (Fore and hindlimb protocol), 'Modality' (SAP and NAP; repeated effect), 'Time' (pre- and post-treatment; repeated effect) and the interaction term 'Modality*Time' (repeated effect). When modelling $VS_{Head,}$ the term 'Lameness side' was also included. Assumptions of normally distributed residuals and homogeneity of variances were assessed using Kolmogorov–Smirnov and Levene's test respectively. Effect estimates were expressed using least squares means (LSM) and their differences (DLSM $\pm$ standard error, SEM). Post-hoc multiple comparisons were adjusted using the Bonferroni correction. Descriptive statistics (mean $\pm$ SD for parametric; median and interquartile range, IQR, for non-parametric data) summarized absolute head and pelvic height differences and VS_{Head}. Coefficients of variation (CV) for $DIFFMIN_{Head}$, $DIFFMAX_{Head}$, $DIFFMIN_{Pelvis}$ and $DIFFMAX_{Pelvis}$ illustrated within subject data variability at each observation point. Coefficients of variation were further compared grouped by 'Modality' and 'Time' using Kruskal–Wallis tests to assess if treatment affected inter-stride variability rather than the magnitude of gait asymmetries.

Qualitative gait analysis: SAP and NAP associated outcome scores (-1, 0, 1) were compared using a Wilcoxon signed rank test. Within treatment groups, the likelihood for median scores to be greater than 0 was assessed using a one-tailed Wilcoxon signed rank test. Statistical tests (Cary, North Carolina, United States) were performed with significance set at *p*-value of 0.05 or lower unless multiple comparisons included the Bonferroni adjustment.

Results

On initial examination, four multilimb lame horses were interpreted to be predominantly fore and six predominantly hindlimb lame. Based on PISBS analyses, eight horses were simultaneously fore- and hindlimb lame, while two horses only showed concurrent hindlimb lameness during the first pre-treatment observation. At the horses' final assessment with the PISBS, none were considered sound following either of the two treatment protocols (**►Table 2**).

Quantitative Gait Analysis

Effect of Needle Stimulation of Acupuncture Points

VS_{Head} showed independence from model terms except for 'Time' ($p < 0.001$; $DLSM_{Pre\text{-}Post} = -5.54 \pm 1.38$ mm) and 'Classification' ($p = 0.001$; $DLSM_{FL\text{-}HL} = 10.8 \pm 3.1$ mm). Models fitted for $\Delta DIFFMAX_{Pelvis}$ and $\Delta DIFFMin_{Pelvis}$ did not show significant effects. Data from one post-treatment observation (3rd Observation, Horse 7) were lost and not included.

Effect of Shockwave Stimulation of Acupuncture Points

Modelling of VS_{Head} and $\Delta DIFFMAX_{Pelvis}$ showed no significant effects. In the analysis of $\Delta DIFFMIN_{Pelvis}$, 'Time' reached significance levels ($p < 0.001$; $LSM_{Pre} = -2.13$ mm; $LSM_{Post} = 1.19$ mm; $DLSM_{Pre\text{-}Post} = -3.3288 \pm 0.85$ mm).

Comparison of Treatment Modalities

For VS_{Head} significant model terms were 'Time*Modality' ($p = 0.014$) and 'Classification' ($p = 0.002$; $DLSM_{FL\text{-}HL} = 9.055 \pm 2.61$ mm). The former was due to a significant difference between pre- and post-treatment NAP data ($P_{adjusted} = 0.002$; $DLSM_{Pre\text{-}Post}$: -5.86 ± 1.47 mm) and due to different post-treatment effects with NAP as opposed to SAP ($p = 0.045$; $P_{adjusted} = 0.27$; $DLSM_{NAP\text{-}SAP}$: 5.7 ± 2.58 mm). Models fitted to $\Delta DIFFMIN_{Pelvis}$ and $\Delta DIFFMAX_{Pelvis}$ did show no significance of terms included.

Within Subject Variability

Indicative of overall within subject data variability, the median CV (IQR) for $\Delta DIFFMIN_{Head}$, $\Delta DIFFMAX_{Head}$, $\Delta DIFFMIN_{Pelvis}$ and for $\Delta DIFFMAX_{Pelvis}$ was 147 (220%), 92 (69%), 109 (101%), and 119 (145%) respectively. No significant difference between each observation's CV was observed when assessed by 'Time' or 'Modality' separately. Summary statistics for the absolute values of quantitative gait parameters are shown in ►**Table 3.**

Qualitative Outcome Scoring

When comparing first with the horses' last trot up following NAP treatment, five of ten horses were scored to be less comfortable, three were thought to show no change and two horses' lameness was interpreted to be better. Regarding SAP outcomes, adequate video data were available for review in nine of ten horses. One of the nine horses was thought to be less comfortable, two were interpreted to be the same and six horses were thought to trot up more comfortably. Using this overall outcome measure, there was no difference between the two modalities ($p = 0.066$; Wilcoxon signed rank test). As opposed to horses post-NAP treatment, horses post-SAP treatments were likely to be more comfortable (> 0; one-tailed Wilcoxon signed rank test; $P_{SAP} = 0.036$; $P_{NAP} = 0.825$). All lameness that did improve following NAP did so by one out of 10 grades, while any deterioration following treatment was typically by 2 grades (median and mode 2; range 1–2). Following SAP, lameness typically improved by 1 lameness grade (median and mode 1: range 1–2) and on one occasion deteriorated by one grade (►**Table 2**).

Discussion

In support of the authors' first hypothesis, SAP improved one of three equine gait parameters. The biological magnitude of this effect is considered small, specific to impact lameness and explained by pelvic asymmetry resolving in one but appearing during the trial in the contralateral hindlimb, thereby not resolving lameness in an animal. Unexpectedly, data were further suggestive of a negative effect of NAP contrasting effects of SAP in forelimbs. Consequently, the second hypothesis that results with SAP would be on par with outcomes following conventional acupuncture methods is rejected. In support of quantitative results, qualitative analyses confirmed an improvement with SAP.

Gait asymmetries and lameness are not constant and naturally vary between different strides and at different time points, illustrating the need for appropriate temporal controls when assessing treatment outcomes.[24–26] Consequently, study outcomes deduced from one single pre- and post-treatment observation may lack validity. Quantitative lameness evaluations were based on multiple pre- and post-treatment assessments, recording of a horse's gait for a minimum of 25 strides and consistency in horse handling to ensure validity of the data. Qualitative evaluations were justified in support of these robust quantitative methods and with the specific primary objective to assess a higher-level treatment outcome in horses with multilimb lameness, that is, overall animal comfort. Nevertheless, the observed inter-stride variability and CVs speak to 'unstable' lameness and highly variable gait parameters in particular regarding forelimbs. From the authors' clinical experience, inter-stride variability may increase when horses are not accustomed to being trotted in hand, when trotting at varying speeds with inconsistent handler interactions, when distracted, when running over uneven surfaces or when pain perception is variable. Others have questioned the role of breed and indeed Standardbred race horses showing particular inter-stride variability of gait parameters.[27] Considering that inter-stride CV did not differ between observations, it is plausible that the horses' temperament rather than biological variability of chronic conditions was responsible for the encountered data spread. Consequently, conditioning Standardbred race horses which may be unfamiliar to trotting in hand to experimental procedures may have improved data quality and ultimately aided the detection of treatment effects. In the context of 'unstable' lameness and variable pain perception, lunging horses for 15 to 20 minutes in both directions has been suggested to render lameness more consistent by way of 'warming-up'.[28] Recent evidence showing less measurement variability with increasing repetition supports this approach.[26] However, this was not adopted here but could have been accomplished at the same time as conditioning efforts prior to final data collection.

Performing this study in Standardbred horses, re-homed after an active racing career, meant that chronic musculoskeletal conditions such as osteoarthritis or previous tendon/ligament injuries were prevalent. Contrary to an individually customized treatment approach, in this investigation acupuncture points were selected considering the population and treatment of prevalent conditions thereby prioritizing influential, classical and master (foundational) points and a limb rather than regional approach. Consequently, it is conceivable that a personalized TCVM treatment protocol maximizing the use of local acupuncture points when lameness has been localized could have demonstrated greater treatment efficacy. Conversely given known analgesic effects of locally applied shockwave therapy,[29,30] outcomes would then be indistinguishable from effects attributable to TCVM principles targeting local acupuncture points. In a previous report using an acute model of foot lameness, NAP resulted in a noticeable improvement in lameness grade and stride length. This was achieved with electrostimulation of local and distant acupuncture points of longer duration (45 minutes) and greater frequency (80–120 Hz) than was

Table 2 Lameness details for horses' first and last observation following treatment

Horse ID	Treatment	Observation	Forelimb	Hindlimb	Verbal summary (computer system)	Expert		
			Side/Vector Sum (mm)/Timing *VS threshold 8.5 mm*	Side/ΔDIFF(mm)/Timing *ΔDIFF threshold 3 mm*		Lameness grade		Qualitative outcome
Horse 1 (FL protocol)	NAP	First pre-treatment	R/29.6/impact	L/3.6/push-off L/3.8/impact	Moderate RF impact lx mild LH impact and push-off lx	2/10 RF	3/10 LH	−1
		Last post-treatment	R/37.3/impact	R/2.5/push-off L/5.1/impact	Moderate/severe RF impact lx mild LH impact lx	4/10 RF	3/10 LH	
	SAP	First pre-treatment	R/20.8/impact	R/3.4/push-off L/7.3/impact	Mild/moderate RF impact lx mild/moderate LH impact lx mild RH push-off lx	Lost	Lost	Lost
		Last post-treatment	L/12.4/push-off	R/2.4/push-off L/2.4/impact	Mild LF push-off Lx	Lost	Lost	
Horse 2 (FL protocol)	NAP	First pre-treatment	L/14.9/impact	L/10.5/push-off L/1.1/impact	Mild LF impact lx moderate LH push-off	2/10 LF	2/10 LH	−1
		Last post-treatment	L/27.8/impact	L/4.1/push-off L/4.8/impact	Moderate LF impact lx mild LH impact mild LH push-off	3/10 LF	3/10 LH	
	SAP	First pre-treatment	L/40.4/impact	L/9.6/push-off L/8.0/impact	Moderate/severe LF impact lx mild/moderate LH impact Lx moderate LH push-off lx	3/10 LF	4/10 LH	1
		Last post-treatment	L/26.8/impact	L/5.4/push-off L/4.0/impact	Moderate LF impact lx mild LH impact lx mild LH push-off lx	3/10 LF	3/10 LH	
Horse 3 (HL protocol)	NAP	First pre-treatment	R/3.1/push-off	R/8.6/push-off R/0.2/impact	Mild moderate RH push-off lx	2/10 RF	3/10 RH	1
		Last post-treatment	L/16.6/impact	R/9.6/push-off L/3.5/impact	Mild LF impact lx mild LH impact lx moderate RH push-off lx	1/10 RF	2/10 RH	
	SAP	First pre-treatment	R/10.8/impact	R/1.9/push-off R/1.4/impact	Mild RF impact lx	3/10 RF		1
		Last post-treatment	R/12.7/push-off	R/7/push-off L /0.8/impact	Mild RF push-off lx mild moderate RH push-off lx	2/10 RF		
Horse 4 (HL protocol)	NAP	First pre-treatment	R/16.0/mid stance	L/2.8/push-off R/6.8/impact	Mild RF midstance lx Mild-moderate RH impact lx	2/10 RF		−1
		Last post-treatment	R/51.0/impact	R/3.5/push-off R/9.2/impact	Moderate-severe RF impact lx Moderate RH impact lx Mild RH push-off lx	4/10 RF		
	SAP	First pre-treatment	R/28.0/impact	R/6.3/push-off L/1.3/impact	Moderate RF impact lx mild-to-moderate RH push-off lx	4/10 RF	2/10 RH	1
		Last post-treatment	R/16.8/impact	R/3.2/push-off R/7.4/impact	Mild RF impact lx lx Mild-to-moderate RH impact lx Mild RH push-off lx	2/10 RF	2/10 RH	

Table 2 (*Continued*)

Horse ID	Treatment	Observation	Forelimb	Hindlimb	Verbal summary (computer system)	Expert		
			Side/Vector Sum (mm)/Timing *VS threshold 8.5 mm*	Side/*ΔDIFF(mm)*/Timing *ΔDIFF threshold 3 mm*		Lameness grade		Qualitative outcome
Horse 5 (HL protocol)	NAP	First pre-treatment	R/23.5/push-off	R/2.3/push-off R/6.0/impact	Mild-to-moderate RF push-off lx Mild RH impact lx		3/10 RH	0
		Last post-treatment	R/25.1/push-off	R/1.6/push-off R/9.2/impact	Mild-to-moderate RF push-off lx moderate RH impact lx		3/10 RH	
	SAP	First pre-treatment	L/9.4/impact	R/1.5/push-off R/2.8/impact	Mild LF impact lx		2/10 RH	−1
		Last post-treatment	L/17.9/impact	L/1.2/push-off R/7.5/impact	Mild-to-moderate LF impact lx Mild-to-moderate RH impact lx		3/10 RH	
Horse 6 (HL protocol)	NAP	First pre-treatment	L/12.4/mid-stance	R/1.3/push-off R/3.6/impact	Mild LF midstance lx Mild RH impact lx	3/10 LF	2/10 RH	0
		Last post-treatment	R/13.2/impact	R/5.3/push-off R/5.8/impact	Mild RF impact lx mild RH push-off and impact lx	2/10 LF	3/10 RH	
	SAP	First pre-treatment	R/26.7/impact	R/3.2/push-off R/5.0/impact	Moderate RF impact lx Mild RH impact lx Mild RH push-off lx	4/10 LF	3/10 RH	0
		Last post-treatment	L/13.6/push-off	R/2.8/push-off R/9.4/impact	Mild LF push-off moderate RH impact lx	4/10 LF	3/10 RH	
Horse 7 (HL protocol)	NAP	First pre-treatment	L/16.5/push-off	R/4.1/push-off R/5.5/impact	Mild LF push-off lx Mild RH impact lx Mild RH push-off lx	2/10 LF	2/10 RH	−1
		Last post-treatment	R/29.1/impact	R/7.4/push-off R/13.9/impact	Head variability prohibits lateralization moderate severe RH impact Mild-to-moderate RH push-off lx	2/10 LF	4/10 RH	
	SAP	First pre-treatment	R/16.8/impact	R/4.5/push-off R/4.0/impact	Mild RF impact lx Mild RH impact and push-off lx	4/10 RF	3/10 RH	0
		Last post-treatment	L/21.1/push-off	R/6.6/push-off R/9.5/impact	Mild-to-moderate LF push-off lx moderate RH impact lx Mild-to-moderate RH push-off lx	2/10 RF	2/10 RH	
Horse 8 (FL protocol)	NAP	First pre-treatment	L/7.9/impact	L/1.7/push-off L/10.1/impact	Moderate LH impact lx	3/10 LH		0
		Last post-treatment	L/35.9/impact	L/1.5/push-off L/12.6/impact	Moderate-severe LF impact lx moderate-severe LH impact lx	3/10 LH	2/10 LF	
	SAP	First pre-treatment	L/37.2/impact	R/1.9/push-off L/14.1/impact	Moderate-to-severe LF impact lx Moderate-to-severe LH impact lx	2/10 LF		1
		Last post-treatment	L/33/impact	R/1.3/push-off L/8.8/impact	Moderate LF impact lx Mild-to-moderate LH impact lx	0/10 LF		

(*Continued*)

Table 2 (*Continued*)

Horse ID	Treatment	Observation	Forelimb	Hindlimb	Verbal summary (computer system)	Expert		
			Side/Vector Sum (mm)/Timing *VS threshold 8.5 mm*	Side/Δ*DIFF(mm)*/Timing Δ*DIFF threshold 3 mm*		Lameness grade		Qualitative outcome
Horse 9 (HL protocol)	NAP	First pre-treatment	L/10.9/impact	L/0.7/push-off L/9.6/impact	Mild LF impact lx Moderate LH impact lx		1/10 RH	1
		Last post-treatment	L/15.8/impact	L/2.3/push-off L/5.4/impact	Mild LF impact lx Mild LH impact lx		0/10 RH	
	SAP	First pre-treatment	L/20.2/impact	L/5.4/push-off L/8.3/impact	Mild-to-moderate LF impact lx Mild-to-moderate LH impact lx	1/10 LF	2/10 LH	0
		Last post-treatment	L/17.1/impact	R/3.0/push-off L/1.9/impact	Mild-to-moderate LF impact lx	1/10 LF	2/10 LH	
Horse 10 (FL protocol)	NAP	First pre-treatment	L/29.3/push-off	R/4.4/push-off R/4.2/impact	Moderate LF push-off lx Mild RH impact and push-off lx	3/10 LF	1/10 RH	−1
		Last post-treatment	L/25.6/push-off	R/6.4/push-off R/4.9/impact	Moderate LF push-off lx Mild RH impact lx Mild-to-moderate RH push-off lx	3/10 LF	3/10 RH	
	SAP	First pre-treatment	L/22.6/push-off	R/5.2/push-off R/4.6/impact	Mild-to-moderate LF push-off lx Mild RH push-off and impact lx		3/10 RH	1
		Last post-treatment	R/18.7/impact	R/4.9/push-off R/3.6/impact	Head variability prohibits lateralization Mild RH push-off and impact lx		2/10 RH	

Abbreviations: FL, forelimb; HL, hindlimb; L, left; LF, left fore; LH, left hind; NAP, needle stimulation of acupuncture points; R, right; RF, right fore; RH, right hind; SAP, shockwave stimulation of acupuncture points. 0 = the same / 1 = better / −1 = worse after treatment; Lameness grades from 0 (sound) to 10 (non-weightbearing).

Table 3 Summary of quantitative lameness data collected at three pre-and post-treatment observations

Treatment modality	Time	VS_{Head}	Abs Δ $DIFFMIN_{Head}$	Abs Δ $DIFFMAX_{Head}$	Abs Δ $DIFFMIN_{Pelvis}$	Abs Δ $DIFFMAX_{Pelvis}$
Needle stimulation of acupuncture points	Pre-treatment	16.71 (10.24) mm	8.99 (8.313) mm	13.36 ± 8.86 mm	5.78 ± 3.59 mm	3.87 (2.9) mm
	Post-treatment	24.51 ± 10.94 mm	11.07 (15.15) mm	18.9 ± 8.76 mm	5.15 (5.03) mm	4.72 ± 3.13 mm
Shockwave stimulation of acupuncture points	Pre-treatment	21.97 ± 9.72 mm	11.48 (11.87) mm	15.76 ± 8.34 mm	6.1 (6.16) mm	3.64 (3.39) mm
	Post-treatment	19.04 ± 7.3 mm	10.95 ± 5.8 mm	14.45 ± 7.4 mm	6.44 ± 3.44 mm	3.32 (4.15) mm

Abbreviations: Abs $\Delta DIFFMAX_{Head}$, absolute value for differences in maximum head height; Abs $\Delta DIFFMAX_{Pelvis}$, absolute value for differences in maximum pelvic height; Abs $\Delta DIFFMIN_{Head}$, absolute value for differences in minimum head height; Abs $\Delta DIFFMIN_{Pelvis}$, absolute value for differences in minimum pelvic height; $VS_{Head,}$ Vector sum.
Depending on data distribution the median (interquartile range) or mean ± standard deviation is listed.

used in this work.[11] Conversely, a lack of NAP treatment response using electrostimulation (2–5 Hz; 8 treatments for 20 minutes) was reported in horses with chronic, naturally-occurring foot lameness.[31] So while a lack of treatment response may not be unusual, particularly when treating chronic multilimb lameness, deterioration in forelimb lameness following NAP would definitely be unusual, given the very low prevalence of acupuncture induced pain/inflammation in people.[32] Given this response was not observed following SAP and the absence of a temporal bias ('Trial' had no effect), needling could be considered responsible for the deterioration. While operator-experience has not been universally linked to treatment success,[33,34] it is conceivable that inaccuracies in point localization could have resulted in poor treatment responses and, given the mild exercise levels to which horses were exposed, allowed deterioration of lameness to occur. Considering that a broader target area is being stimulated with SAP, inaccuracies in point identification may become less relevant and still facilitate a positive treatment effect. This may have effectively remediated any lameness deterioration and thereby contrasted outcomes with NAP. Based on the lack of true controls (horses were not treated in non-acupuncture points), the counter argument would be that SAP effects could have been unrelated to TCVM principles but rather related to non-specific analgesic effects of shockwaves on local soft tissues. However, given that SAP improved hindlimb lameness and considering typical causes of hindlimb lameness in Standardbreds,[35,36] it is less plausible that local effects of applying SAP in the proximal limb, close to the axial skeleton could, have facilitated this outcome.

To the authors' knowledge, only one other study with a similar quantitative methodology is currently available for comparison of NAP results.[37] In this work, horses that were sound or only minimally lame underwent three acupuncture sessions in 1 week. With this treatment, an improvement in gait symmetry was not universally detected but more consistently observed in hindlimbs mirroring our findings with SAP. Authors suggested that the limited improvement was likely explained by horses not having been lamer at the outset, given the potential impact of data variability on the detection of subtle differences. That being said, in the presented work the magnitude of improvement in horses with more noticeable lameness was comparable to that of the previous report. While a comparison between two different modalities may be flawed, it would suggest that there is a finite improvement potential with acupuncture point stimulation which is not proportional with lameness severity.

As stated above, in light of the availability of objective quantitative data and the presence of multilimb lameness, analysis of qualitative outcome measures focused on summary assessments of animal comfort. While in this investigation subjective and objective outcome measures largely agreed with each other, the PISBS highlighted involvement of other limbs when the expert did not. A relevant body of evidence has established that lameness identification and grading of lameness can be unreliable and that a PISBS system is typically better at identifying subtle asymmetries.[38–40] Consequently, in our opinion sole use of lameness grades for outcome assessment is generally undesirable and considered inferior to the use of quantitative gait parameters with/without simple qualitative comparisons, that is, better, worse or the same. Concerning the validity of 'retrospective' gait assessments using video recordings, this has recently been established and justifies our use of this methodology.[41]

In summary, a conclusive cause for the unexpected deterioration in lameness with NAP was not apparent; however, the fact that under the same circumstances SAP did have a positive effect is in support to consider investigating this treatment approach further. Speed of treatment delivery, patient compliance and a multilimb effect would render SAP an attractive modality but additional work is needed to conclusively assess its value.

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Conflict of Interest
Authors declare no conflict of interest.

Authors' Contributions
RL and ARTN were involved in conceptualization, methodology, formal analysis, and data curation. RL, MS, JH and ARTN contributed to investigation, draft preparation, and review and editing of the manuscript.

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