Effect of acupuncture on heart rate variability at rest and on stride length and frequency at gallop in thoroughbred racehorses

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Submitted in fulfilment of the requirements for the degree.

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In the subject

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Dedication

For my parents.

Thank you for your unconditional love.

Acknowledgements

Completing a master’s in research cannot be done alone. There were many people involved in making this endeavour a success and I would like to offer my sincerest gratitude for your contribution towards this piece of work.

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Abstract

Background

Acupuncture has been practised on humans and animals as a healing modality for thousands of years, the effectiveness and validity of the treatment is still controversial and debated within the academic and clinical settings today. Clinical studies have shown that acupuncture speeds up and enhances the healing of injuries such as tendon and ligament ruptures, fractures, muscle injuries and respiratory problems. However, the question remains, if acupuncture has any performance enhancement effects in racehorses. And also, how to use this treatment modality ethically in the racehorse industry. There have been no papers published on withdrawal periods or any other safety concerns regarding the use of acupuncture in sport horses.

The running performance of a horse can be measured by using stride parameters, measured during training, and heart rate variability as a non-invasive method of measuring physiological health. Stride parameters are normally measured during the gallop on the “straight” 600 m of the track as the jockeys normally increase the riding speed of the horses during this part of the training session. Also, the trainer uses this as a subjective indicator of the running performance. These parameters may be used to measure the effect of acupuncture on the horse and may give indication of any benefit or adverse effects after treatments.

Aim of the study

The aim of this study was to determine if acupuncture treatments had a positive effect on performance in the healthy thoroughbred racehorse. The effect of three dry needle acupuncture treatments for a three-week period during training, on changes in heart rate variability (HRV) at rest, and stride parameters (SP) during a 600 m gallop were assessed.

Methodology

Ethical approval (REC185-19) was obtained from the Animal Ethics Committee of the University of Pretoria. This study was a quantitative randomised control trial during field conditions. The racing trainer selected 30 horses that qualified for the study. All participants were kept in uniform conditions within one stable yard, received identical nutrition i.e., racing concentrates, roughage and training. The

researcher randomly allocated these horses into an acupuncture treatment group (A) and a control or non-treatment group (B), with 15 horses in each group. In Group A there were four fillies, one mare, three colts and eight geldings (mean age ± SD; 4 ± 0.8 years) and in Group B seven geldings and eight colts with no fillies / mares (mean age ± SD; 3 ± 0 years). Female horses under the age of five years were classified as fillies and intact male horses under the age of five years as colts respectively.

The HRV and SP were measured pre-treatment and post-treatment, i.e., after the treatment group received three dry needle acupuncture treatments spaced evenly over a three-week period.

Acupuncture

The dry needle acupuncture technique was performed by inserting acupuncture needles into pre-selected acupuncture points (GB21, ST36, LI10, Bai Hui, Shen Shu, Shen Peng, Shen Jiao, Yan Chi, BL40, GB27, LU1, BL54 and SI9). Needles were inserted for 20 minutes and stimulated in a clockwise direction every five minutes. The non-treatment group was haltered and kept in the stable for the same period.

Heart Rate Variability

The Televet100 echocardiogram (ECG) device was used to record, digitally on a laptop 15 minutes of echocardiogram data. The data were recorded pre- and post-treatment at the stables midmorning on a Sunday when the horses were rested, one hour after feeding.

The data was downloaded from the recording monitor using the Televet100 software, then transferred to the HRV Analysis Software 3.3.1 for Windows or Kubios.

The following HRV indicators (SDNN, RMSSD, LF, HF, LF/HF, LF nu, HF, SD2) and heart rate measures (mean RR, mean HR) were determined and statistically analysed.

Stride parameters

A GPS girth-mounted device EquinITy™ was used to measure stride parameters and running performance in a gallop over 600 m. The following was recorded by the EquinITy™ software: maximum stride length (SL Max), average stride length (SL Avg), maximum stride frequency (SF Max), average stride count (SC Avg), maximum stride count (SC Max), maximum speed (Max Speed), stride count at maximum speed (SC Max Speed), stride length at maximum speed (SL Max Speed) and average speed (Avg Speed). Once the EquinITy™ data was downloaded from the device at the end of the training session it was processed by the EquinITy™ Technology website per horse and work session.

Statistical analysis

The difference between pre- and post-treatments within each group, for each indicator, was used to compare the treatment group and non-treatment group. Not all the data were normally distributed, hence the independent Mann Whitney U test (non-parametric test) was used to investigate if a significant difference existed between the differences calculated in the treatment group and non-treatment group, tested at a 5% level of significance.

Results

HRV indicators

There were no significant changes in any of the HRV indicators when comparing the delta values (post-treatment minus pre-treatment) of the two groups. In addition, the indicators associated with the parasympathetic nervous system (PNS) (RMSSD, SD2 and HF) did not show a clear pattern, i.e., the delta median for the non-treatment group showed a shift towards the PNS (RMSSD = 1.47; HF = 19.42; SD1 = 1.04), while the treatment group showed mixed results (RMSSD = 1.54; HF = - 20.19; SD1 = 1.10).

During the randomised allocation of the treatment and control groups all the fillies / mares ended up in the same treatment group (A). This created an unequal distribution of gender in the groups influencing the results. The recalculation of the data i.e., excluding the fillies/mares, allowed a coherent and valid representation of the results. The new statistics completed indicated that, although still not significant

($p < 0.05$), all the pure vagal (parasympathetic) indicators in the treatment group showed a shift towards inhibition of the vagal system after the intervention (RMSSD = - 2.19; HF = - 37.19; SD1 = - 1.56). Thus, there was less parasympathetic nervous system (PNS) cardiac influence present when measured post-treatment in the acupuncture group compared with increased PNS cardiac influence after three weeks measured in the non-treatment group.

The delta median of the HRV indicators, associated with the combined effect of the PNS and SNS, showed a decrease in cardiac influence after the three weeks in the non-treatment group (SDNN = - 4.00; LF = - 397.26; SD2 = - 7.48) and in the treatment group a shift in the opposite direction, namely an increase in cardiac influence after the acupuncture treatments (fillies / mares included: SDNN = 3.61; LF = 22.95; SD2 = 5.85 and fillies / mares excluded: SDNN = 4.39; LF = 179.48; SD2 = 8.55) In view of the fact that the pure parasympathetic indicators decreased, these results imply that the sympathetic cardiac component increased.

The HRV indicators associated with cardiac autonomic balance also showed a shift in the opposite direction when comparing the delta median for the non-treatment group (LF/HF = - 0.32; LF norm = - 0.80; HF norm = 3.54) and the treatment group (fillies / mares included: LF/HF = 0.18; LF norm = 3.28; HF norm = - 3.07 and fillies / mares excluded: LF/HF = 0.26; LF norm = 14.23; HF norm = - 4.78).

Finally, the heart measures also showed opposite patterns when comparing the delta median of the non-treatment group, which consisted of only colts and geldings, (Mean HR = - 3.00; Mean RR = 107.54) and the treatment group especially, when the fillies / mares were excluded from the comparison (fillies / mares included: Mean HR = - 3.01; Mean RR = - 84.37 and fillies / mares excluded: Mean HR = 3.96; Mean RR = - 169.72). It is important to note that although there were no significant differences, the results of the two groups (treatment and non-treatment) moved in opposite directions (both consisting of only colts and geldings) after a training period of three weeks.

Stride parameters

There were no significant changes in any of the stride parameters when comparing the delta (post-treatment minus pre-treatment) of the two groups. However, it is important to note that the delta median of the following parameters responded in different directions: SL Avg, SC Avg, SC Max, Max Speed, SL Max Speed and Avg Speed when comparing the treatment and non-treatment group. As the randomisation of the groups caused an over-representation of fillies / mares in Group A, the fillies / mares were removed from the data set and the stride parameters were again statistically analysed. The median delta of the following parameters consistently moved in different directions for the two groups, with or without the removal of the fillies / mares; with median delta results reported for the non-treatment group first and the treatment group second (fillies / mares included and then fillies / mares excluded): SL Avg (- 0.06; 0.07; 0.05), SC Avg, SC Max (0.84; - 2.44; - 5.70), Max Speed (1.30; - 1.40; - 0.80) and SL Max Speed (0.13; - 0.03; - 0.01). Of importance is that SC Max was marginally significant with the fillies / mares included ($p = 0.067$) and significant ($p = 0.004$) with the fillies / mares excluded. Thus, the non-treatment group significantly increased their stride count during a 600 m gallop after three weeks compared to the acupuncture treatment group.

Conclusions

In this study the effects of acupuncture on the stride parameters and physiological stress response (HRV) in thoroughbred racehorses were investigated. There was no statistical difference in the HRV indicators between the treatment and non-treatment group when comparing the delta (difference in the changes) of pre-and post-treatment. However, there was a decrease in the PNS cardiac influence present when measured post-treatment in the acupuncture group compared with increased PNS cardiac influence after three weeks measured in the non-treatment group.

Although there were no significant changes in stride parameters when comparing the delta (difference in the changes) of pre-and post-treatment of the acupuncture treatment group with the non-treatment group, there was a significant difference regarding the max stride count when data from only colts and geldings were analysed.

To summarize, there were overall no significant differences found between the treatment and non-treatment group, but the changes observed may be of clinical value especially when recommendations for withdrawal period are considered.

These findings suggest that dry needle acupuncture may have a negative effect on the performance of a horse for five days post-treatment.

In addition, 20% (three horses) from the non-treatment group and 6.7% (one horse) of the treatment group were injured and had to be withdrawn from the study. Thus, these acupuncture treatment sessions may have an injury protective and fatigue reducing effect.

Key Terms:

Stride parameters, Stride count, Racing performance Parasympathetic nervous system, Sympathetic nervous system, Autonomic nervous system, Dry needle, GPS gallop monitor.

Language editor

12 June 2021

TO WHOM IT MAY CONCERN

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I proofread and language edited the above MSc Dissertation for Dr Hartwigsen using track changes. No references or appendices were checked and no formatting was done. Dr Hartwigsen had to accept or reject the suggested changes and I did not see the final product. Dr Hartwigsen paid me for this service.

Ellen Joubert

Proofreading/Editing
Professional Editors Guild JOU001

List of abbreviations

ANS	Autonomic nervous system
AP	Acupuncture
Avg Speed	Average speed during the gallop
bpm	Heart beats per minute
CF	Correction factor(s)
DN	Dry needle acupuncture
EA	Electro-acupuncture
ECG	Electrocardiogram
FEI	International Federation for Equestrian Sports
HF	High frequency
HF norm	High frequency power normalised units
HRV	Heart rate variability
LF	Low frequency
LF norm	Low frequency power normalised units
LF/HF	Low frequency to high frequency ratio
LnHF	Natural logarithm of low frequency spectral analysis
Max HR	Maximum heart rate in the gallop
Max Speed	Maximum speed achieved during gallop
Mean HR	Mean heart rate
ms	milliseconds
OIE	World Organisation for Animal Health
OTS	Over Training Syndrome
PNS	Parasympathetic nervous system
RMSSD/RMDD	Root mean square of successive differences
RR	Beat-to-beat (consecutive heart beats)
SC Avg	Average stride count during the gallop
SC Max	Maximum stride count in the gallop
SC Max Speed	Stride count at maximum speed in the gallop
SD	Standard deviation

SD1	Standard deviation 1 (derived from poincaré plot)
SD2	Standard deviation 2 (derived from poincaré plot)
SDNN	Standard deviation of normal-to-normal intervals
SL Max	Maximum stride length achieved during gallop
SL Avg	Average stride length during the gallop
SL Max Speed	Stride length at maximum speed during gallop
SNS	Sympathetic nervous system
SP	Stride parameters
TCVM	Traditional Chinese veterinary medicine
VHRmax	Velocity at maximal heart rate

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Chapter 1: General orientation

1.1 Introduction

The OIE (World Organisation for Animal Health) defines animal welfare as the 'physical and mental state of an animal as it relates to the conditions in which it lives and dies'. Thus, 'an animal experiences good welfare if the animal is healthy, comfortable, well nourished, safe, is not suffering from unpleasant states such as pain, fear and distress, and is able to express behaviours that are important for its physical and mental state' (OIE, 2019).

Equine athletes often face high psychological and physical demands in preparing for and during competitions leading to possible injuries and overtraining syndrome (McGowan and Whitworth, 2008), which are associated with poor performance and threatens these horses' well-being. The most common injuries recorded in equine athletes include exercise-induced myopathies (Chiaradia et al., 1998), tendon injuries (Spargo et al., 2019; Hill et al., 2001), fractures, stress related fractures (Davidson and Ross, 2003; Verheyen et al., 2006), and cardiomyopathy (Young, 2003). In addition, catastrophic injuries are frequently associated with previously minor musculoskeletal injuries at the same site especially in the third metacarpal bone (Stover, 2003). Treating these injured horses can be problematic as certain pharmaceuticals are often prohibited during training and competition (NH Authority, 2020; FEI, 2012).

For this reason, acupuncture may be a viable alternative to treat and prevent injuries (Angeli et al., 2007). 'Acupuncture involves the insertion of acupuncture needles into acupuncture points' that are specifically described loci in the superficial tissues of the body (Mittleman and Gaynor, 2000). A recent survey showed that 33% of the respondents, associated with equine veterinary practices use rehabilitation modalities in their practices and that 68% of them, used acupuncture in sports horses (Wilson et al., 2018). The FEI (International Federation for Equestrian Sports) Veterinary Regulations allows acupuncture dry needling at competitions, but only the FEI primary treating veterinarian may give the treatment and must be present for the entire treatment session.

Several studies and review articles have been published on the use and effectiveness of acupuncture in equines (Shmalberg and Xie, 2011; Tangjitjaroen et al., 2009). Of particular importance to the equine athlete is the influence of acupuncture on stress (Rizzo et al., 2017), lameness and pain (Schoen, 2000) and gait changes (Dunkel et al., 2017).

The effect of acupuncture on horses has been measured using endocrine parameters (cortisol measurements) behavioural indicators (reactivity) and cardiac autonomic variation (HRV) in response to startling (Villas-Boas et al., 2015). HRV is a method used to quantify the variations in the inter-beat interval in a non-invasive way (van Vollenhoven et al., 2016). These changes are due to autonomic cardiac regulation, for example, the heart-beat increases when the sympathetic nervous system (SNS) is activated and slows down in response to parasympathetic nervous system (PNS) influence (Stucke et al., 2015). The changes in inter-beat length can be measured and used to determine the HRV indicators in the horse giving insight into the responses of the autonomic nervous system (ANS) in the body, without the need to perform invasive tests (von Borell et al., 2007). HRV can therefore be used to measure the physiological adaptability of the animal to a stressful or a painful stimuli (van Vollenhoven et al., 2017). HRV has also been used to measure the effect of acupuncture on horses during restraint (le Jeune et al., 2014), when being startled (Villas-Boas et al., 2015) and when experiencing stress during a clinical examination (Slabber, 2021).

According to Barrey (2013) the outcome of equine athlete treatment can be measured using a performance test battery such as stride length, stride frequency, stride count, speed, as well as heart rate and respiration rate during exercise and in recovery (Barrey, 2001). Barrey (2013) defines stride frequency (SF) as the number of strides taken per unit of time and expressed in strides or Hertz (Barrey, 2013), while the stride length (SL) is the measured distance between two hoof placements of the same limb and is measured in metres (Barrey, 1999). Ishii et al., (1989) showed that the SL increases significantly at maximum velocity and is a bigger contributor to increase in velocity when compared to SF. A recent study conducted by Morrice-West et al. (2020) showed that sex of the horse, age, the track surface, and the distance raced all significantly influence the stride parameters (SP). They found that males (geldings and colts) were able to achieve a longer stride duration and had fewer strides per sectional (SF) when compared to females (fillies and mares). Older horses gave longer SL in the beginning of the race while finishing with shorter SL and shorter stride durations towards the end of the race. Horses that have had several races as well as horses with a good performance record had a significantly higher stride count (SC) from the middle to the end of the race sectionals. Morris-West et al. (Morrice-West et al., 2020) concluded that there is significant inter-individual variation when it comes to SP in the thoroughbred racehorse as individuals use different strategies to increase and maintain high speeds during the races.

1.2 Problem statement

There is a substantial number of studies on acupuncture treatments used in human athletes (Wadsworth, 2006; Akimoto et al., 2003; Lin Shihang, 2017; Lin et al., 2011; Gentil, 2018). Yet, there is limited research on the use of acupuncture in equine sports medicine to guide the effective and ethical use of this treatment modality. Research within acupuncture on equines in general is available, but not in abundance (Angeli et al., 2007; Dunkel et al., 2017; le Jeune et al., 2014). Limitations within acupuncture studies include the lack of a proper standardised control group or sham protocols, low numbers of participants within the groups, inadequate diagnostics, poor execution of standardised acupuncture treatment and no proper “washout” period in cross-over studies (Habacher et al., 2006).

In addition, the effectiveness and validity of acupuncture treatment is still controversial and debated within the academic and clinical settings today, although acupuncture has been practised on humans and animals as a healing modality for thousands of years (Xie and Preast, 2013). Clinical studies have shown that acupuncture speeds up and enhances the healing of injuries such as tendon and ligament ruptures, fractures, muscle injuries and respiratory problems (Tangjitjaroen et al., 2009). However, the question remains, whether acupuncture has any performance enhancement effects in racehorses and how to use this treatment modality ethically in the racehorse industry. There have been no papers published on withdrawal periods or any other safety concerns regarding the use of acupuncture in sport horses.

1.3 Aims of the research.

The aim of this study was to determine if acupuncture treatments have an effect on welfare and performance in the healthy thoroughbred racehorse.

The effects of three acupuncture treatments (once every seven days) during a three-week training period were assessed to determine if these treatments could potentially improve the welfare and performance of racehorses. Measurements included changes in resting HRV as well as the SP during the gallop of the racehorses.

1.4 Objectives

- Measure resting / baseline HRV of horses before acupuncture treatment in the treatment group (HRV1) and in the control group (HRV2).
- Measure HRV after three acupuncture treatments over a three-week period in the treatment group (HRV3) and measure the HRV in the control group after three weeks (HRV4).
- Determine the difference between the pre- and post-treatment HRV measurements in the treatment group (HRV3-HRV1) and the control group (HRV4-HRV2) and compare statistically.
- Determine the SP during gallop before acupuncture treatment in the treatment group (SP1) and the control group (SP2)
- Determine the SP during gallop after three acupuncture treatments over a three-week period in the treatment groups (SP3) and control group (SP4)
- Determine the difference between the pre- and post-treatment SP measurements in the treatment group (SP3-SP1) and the control group (SP4-SP2) and compare statistically.

1.5 Hypotheses

Ho: Acupuncture treatments over a three-week period have no effect on HRV and SP measured before and after treatment in thoroughbred racehorses.

H1: Acupuncture treatments over a three-week period cause a change in HRV and SP in thoroughbred racehorses.

1.6 Benefits of research

Although, acupuncture is a minimally invasive treatment technique with few side effects reported in humans (Gentil, 2018) or animals (Xie and Ortiz-Umpierre, 2006), acupuncture in equine athletes is not used as part of the standard treatment protocol during training and racing mainly because of the limited research being published on the ethical and safe use of acupuncture in equines. Currently the FEI (FEI, 2012) does allow the use of acupuncture pre- and post-competition without any data on the performance enhancements or side effects that may be present for a prolonged period after treatment. This study may assist equine sports regulatory bodies to draft rules regarding the safe and ethical use of acupuncture in equine sport. When animals are used as commodities or in activities for financial gain, the welfare of the animals is usually a concern especially when the best available treatments are not given or are postponed in order to increase profit margins. With minimal side effects seen with acupuncture treatments, and relatively low costs for treatments, the possibility of performance enhancement needs to be studied and understood in order to use it as an alternative or complementary treatment to pharmaceuticals and invasive procedures.

Chapter 2: Literature review

2.1 The equine athlete

In an observational retrospective investigation into the incidence of catastrophic musculoskeletal injuries (injuries resulting in euthanasia or death) on the racetracks in Gauteng, it was calculated that 0.6 out of 1000 race starts have a fatal catastrophic musculoskeletal injury (Spargo et al., 2019). The equine athlete is subjected to extreme physical demands often resulting in fatigue syndrome or overtraining being some of the main limiting factors in performance (McGowan and Whitworth, 2008). Fatigue and overtraining are often unnoticed until injury occurs (Fairburn et al., 2017; Davidson and Ross, 2003).

When athletes (animal and human) physically train and / or compete, the body is continuously subjected to micro trauma in connective tissue (Kannus, 1997; Rich, 2014), impact fatigue and trauma on the bones and joints (Nunamaker et al., 1990; Rogers and Firth, 2004; Kasapis and Thompson, 2005). These athletes are often in an inflammatory state following intense training (Kasapis and Thompson, 2005). Musculoskeletal fatigue in athletes, human and animal, is a contributing factor causing injuries often ending the racing career of the equine athletes (Spargo et al., 2019); Whitton et al., 2013; Yoshikawa et al., 1994; Rogers et al., 2012). Competing athletes often have trigger points, i.e., areas of fascia trauma, and areas of sensitivities related to muscle and associated tissue fatigue (Schoen, 2000; Sato, 2020; Bowen et al., 2017). These horses are thus constantly under physical and psychological stress. Very often the strain on the connective tissue is subclinical and the trainer and veterinarian only realise that there is an injury when the horse is visibly lame or performing poorly (Davidson and Ross, 2003; Dyson, 2016). Therefore, by enabling the body to recover successfully from the work during training and racing, subclinical injuries are prevented, and the horse can avoid catastrophic injuries. Proactive management of the equine athlete will increase the body’s tolerance to muscle strain and bone fatigue thus preventing catastrophic injuries (Rogers et al., 2012).

The most common injuries the thoroughbred racehorse is at risk of during racing and training are:

- exercise-induced myopathies (Chiaradia et al., 1998);
- tendon injuries (Spargo et al., 2019; Hill et al., 2001);
- fractures, including stress fractures (Spargo et al., 2019; Verheyen et al., 2006; Davidson and Ross, 2003; Nunamaker et al., 1990); and
- cardiomyopathy (Young, 2003).

These injuries often exhibit multifactorial pathophysiology, as the genetics, training programme, running surface, nutrition, and general care all play a role (Cruz et al., 2007, Rogers et al., 2012). The most fundamental principle of preventing injury in the athlete is to aid recovery from the physical fatigue caused by the strenuous exercise (Rogers and Firth, 2004; Whitton et al., 2013; Yoshikawa et al., 1994).

In competitive sports (human and animal) the athletes are pushed to maximum performance and the use of performance-enhancing substances are a regular occurrence (Knych, 2017). To optimise healing and performance, trainers often turn to alternative therapies such as physiotherapy, equine massage and acupuncture (McGowan et al., 2007). Acupuncture has been used to aid in recovery from sports fatigue in humans (Sun et al., 2009; Wadsworth, 2006). It may thus assist the equine athlete in the recovery process during training and post-racing (Klide and Martin Jr, 1989; Angeli and Luna, 2008).

2.2 The science behind acupuncture

Acupuncture originated in the orient and is an ancient healing form, treating disease by stimulating the body's own healing potential (Wong and Shen, 2010). 'It involves the insertion of acupuncture needles into acupuncture points' that are specifically described loci in the superficial tissues of the body (Mittleman and Gaynor, 2000). Electro-acupuncture (EA) is the stimulation of the acupuncture needles inserted into the acupuncture points using electricity from a specialised EA machine to enhance the stimulation and therapeutic effect of the acupuncture (Salazar et al., 2017).
Acupuncture point selection for a treatment is still controversial (Choi et al., 2012). There are different schools of thought on point selection for a specific condition or disease. It is often based on the practitioner's schooling and experience (Chrisman

et al., 2020). Ideally acupuncture treatment should be based on the specific problems and diagnostics including the acupuncture point scan used by practitioners to identify the areas that are diseased (Antonio Alfaro DVM, 2014, Schoen, 2000). Using a pre-selection of points to acupuncture without considering the individual diagnostics will show poor results. This point was reiterated in a pilot study conducted by Dunkel et al., (2017) when they performed a 'single-blinded controlled crossover study to evaluate the effects of acupuncture treatment on movement parameters in horses'. The treatment group did have an improved symmetrical gait which could indicate that the horses treated with acupuncture were more comfortable. The paper also discussed the fact that there are some key limitations when doing a trial to test the effects of acupuncture. When using a traditional Chinese veterinary medicine (TCVM) philosophy the selection of the acupuncture points is very dependent on the treatment strategy of the practitioner. This makes it challenging in setting a standardised treatment strategy for a trial. Furthermore, the anatomical description of the acupuncture points is often vague and not standardised within different schools of acupuncture training (Dunkel et al., 2017).

Studies on the use of acupuncture in horses are well documented, especially studies on pain relief, lameness and musculoskeletal disorders (Klide and Martin Jr, 1989, Xie and Ortiz-Umpierre, 2006). The reported rate of success in treatment is almost 98% for thoracolumbar pain in the horse (Tangjitjaroen et al., 2009). Furthermore, studies reported positive results on the treatment of back pain in equine athletes with dry needling and dry needle acupuncture (DN) (Klide and Martin Jr, 1989) and EA (Rungsri et al., 2009). Table 1 gives a short summary on the most relevant acupuncture studies in horses.

Table 1: Short summary of the relevant studies of different acupuncture techniques and actions in horses.

Effect of acupuncture	Method used	Relevant studies
Treatment of lameness in horses	EA	(Xie et al., 2001a; Xie et al., 2001b)
Gait improvement in horses	DN	(Dunkel et al., 2017)
Treatment of back pain in horses	EA DN Laser acupuncture Aquapuncture	(Rungsri et al., 2009; Klide and Martin Jr, 1989)
Improving the metabolic activity in horses by measuring lactate levels before and after aquapuncture	Aquapuncture	(Angeli and Luna, 2008)
Regulating heart rate variability by having a coherence effect on parasympathetic nervous system and sympathetic nervous system, this reduces the stress levels and morbidity rates	DN EA	(Anderson et al., 2012) (Slabber, 2021)
Lowering cortisol levels during stressful event	DN	(Rizzo et al., 2017)
Tissue regenerative capabilities by releasing mesenchymal stem cells into circulation	EA	(Salazar et al., 2017)
The effect of acupuncture on HRV in horses	DN	(le Jeune et al., 2014)
Acupuncture affects autonomic and endocrine (cortisol) responses induced by startle in horses	DN	(Villas-Boas et al., 2015)

DN = Dry needle acupuncture, EA = Electro-acupuncture

Image 1: Acupuncture treatment being administered to a thoroughbred racehorse in the treatment group.

Acupuncture has several documented mechanisms of actions in humans and animal models that shows acupuncture enhance the process of healing tissues on a cellular and connective tissue level: for example, recovering from exercise-induced fatigue (Ma et al., 2015); a potential cardioprotective effect (Anderson et al., 2012, de Lima Pimentel et al., 2018); and the reduction of cortisol and increases immune function in an athlete (Mittleman and Gaynor, 2000; Rizzo et al., 2017; Akimoto et al., 2003). As well as the ability to reduce free radicals, decrease lipid peroxidation, acupuncture stops dysfunction of the motor hypothalamus-pituitary-gonadal axis, reduces the creatine kinase content, and lowers lactic acid concentration, so as to reduce sports fatigue and enhancing athletic performance (Sun et al., 2009).

Although acupuncture may influence performance of the athlete positively, it is still an invasive procedure and needs to be regulated. Acupuncture is classified as a surgical procedure in South Africa and may only be performed by a veterinarian that has completed specialised training and certification from registered TCVM or Western-acupuncture educational institutions (SAVC, 2003). For this reason, the risk of lay people abusing acupuncture as a performance enhancement therapy is limited.

2.3 The racehorse and performance parameters

It is a complex procedure to estimate the athletic performance of a horse as the interplay between musculoskeletal, nervous-, cardiac systems and psychological factors are complex and will contribute to the physical performance of a horse (Angeli et al., 2007). The individual racehorse should be assessed on its racing ability / potential based on a test battery that includes variables such as stride length (SL), stride frequency(SF), speed (Barrey, 2001), as well as heart rate during exercise and recovery (Erickson et al., 1991). By monitoring these parameters closely the potential of the horse to win and earn an income for the owner and trainer can be estimated and appropriate strategies to improve performance can be implemented (Barrey, 2001).

Performance capacity and fitness indicators are difficult to measure in the racehorse during field conditions. Ideally, these indicators should be measured on the training racetrack to be of most value to the trainer (Erickson et al., 1991). SF and SL are parameters that are often measured to estimate the performance of a racing horse (Barrey, 2013). SF is defined as the "number of strides taken per unit of time and is expressed in strides per second (Hertz)" (Barrey, 2013). The SL is the distance between two hoof placements of the same limb and is measured in metres (Barrey, 1999). In a research study conducted by Seder and Vickery (2003) it was concluded that SF, SL, respiration and the extension range of the distal phalangeal joints influence the velocity. Barrey et al. (Barrey, 2001), determined that SF is linearly correlated to performance i.e., the number of winnings and earnings per racing starts. The results of studies investigating the correlation between SL and SF with running performance are inconsistent, Dusek et al., (1970) showed that horses achieve greater velocity when increasing SF. According to Heglund and Tayor (1988) a doubling in velocity resulted in SL and SF increasing, but SF to a lesser extent. However, Erickson et al., (1991) and Ratzlaf et al., (1985) argue that in the gallop horses both the SL and SF increase and stride time decrease to increase the velocity since there is a decrease in both the swing and support times of the stride. Barrey et al., (2001) determined that SF is linearly correlated to performance i.e., the number of winnings and earnings per racing starts. These top performing racehorses are able to achieve maximal velocity at a gallop by both increasing both SL and SF (Vilar et al., 2010, Barrey, 2001).

A number of factors determine the maximum SF including the length of the limbs the weight distribution of the horse's frame, the range of motion of the limbs and the expansion capacity of the ribcage and the percentage of fast twitch muscle fibres (Barrey, 2001). When the muscles have more power there is a more powerful, faster protraction of the limbs, increasing the SF (Ferrari et al., 2009).

Morris-West et al., (2020) used a GPS device called StrideMasterTM (Thoroughbred Ratings Pty Ltd, Romsey, Victoria, Australia) to determine stride values for thoroughbreds (mean ± SD): SL = 7.08 m ± 0.39 m, number of strides (SC) = 28.32 ± 1.56, strides/200 m, SF = 0.43 ± 0.02 strides/s. This differs slightly from the results obtained by Barrey et al., (2001). They found SL = 6.35 m ± 0.66 m and SF = 2.40 strides/s ± 0.16. It is though important to note that during a race individual horses seem to have different strategies to increase velocity depending on the track conditions and the distance of the race, but in general the SL increases and stride duration decreases with an increase in speed (Morrice-West et al., 2020). In addition, in thoroughbreds and standardbred racehorses there is an increase in SF and SL after a training period of three years (Barrey, 2013). The training age, sex, trainer technique as well as the distances raced influence the individual SL and SF (Morrice-West et al., 2020).

As already mentioned, poor performance in racing horses is usually due to a musculoskeletal pathology (Dyson, 2016). Stiffness in the cervical and thoracolumbar areas is one of the main reasons of pain and discomfort and consequently poor performance (Dyson, 2016). Also, gait fatigue has been described as the reduction in SL and SF towards the end of a race (Morrice-West et al., 2020) and may potentially be used to measure fatigue and over-training in the thoroughbred racehorse (Leach and Sprigings, 1979).

As the respiration system is the primary performance limitation factor of the equine athlete, it is expected that a horse at maximal physical exertion will experience hypoxemia (Franklin et al., 2012). Furthermore, in cantering and galloping horses, an increase in velocity is also achieved by an increase in SL and at maximum velocity, the increase in SL is equalled by an increase in the lung's tidal volume (Butler et al., 1993). The running performance of the thoroughbred racehorse is distinctly linked to the velocity at maximal heart rate (VHRmax) (Gramkow and Evans, 2006). Gramkow et al., (2006) found that horses with a higher VHRmax were able to earn significantly higher earnings per race start and horses with a VHRmax lower than 14.5 m/sec had significantly decreased earnings per race start. They also found that there was no link between earnings and either maximum velocity or the heart rate at maximum speed. Consequently the VHRmax in a gallop can be utilised as an indicator for potential racing performance in the thoroughbred racehorse (Gramkow and Evans, 2006).

In conclusion the gallop test can be employed to assist in determining the racing ability of the thoroughbred racehorse by providing mobility parameters such as SF, SL and Max Speed (Barrey, 2013). In addition, the use of GPS devices can further assist the trainer to evaluate the training programme of these racehorses by producing objective and non-invasive measurements of kinetic and physiological variables (Fonseca et al., 2010). Mounted GPS and heart rate monitoring devices have been used successfully and proven reliable in studying racing parameters in galloping racehorses (Kingston et al., 2006; Ferrari et al., 2009; Witte et al., 2006; Barrey, 2001). Of particular importance are three GPS, location speed, stride parameter and heart rate measuring devices commercially available and in active use in thoroughbred race training: the E-Trakka (Equitronics, Buddina Australia) (Gür and Matur, 2013); the EquinITy™(Equinity technology ltd Wellington house, UK) (Williams et al., 2019); and the Stridemaster™ (Thoroughbred Ratings Pty Ltd, Romsey, Victoria, Australia) (Morrice-West et al., 2020).

2.4 Heart rate variability as a measure of autonomic coherence

2.4.1 The Heart-Brain connection and heart rate variability

Up until recently the heart has been understood only as the most efficient pump that has ever been created (Webster, 1987). Amour et al., (2008) published the discovery of an intricate nervous system inside the heart known as the Heart Brain. This 'little brain' of the mammalian heart consists of an intricate network of afferent, interconnecting and motor neurones , which also connect with other neurons in the intra-thoracic ganglia and are influenced by the central nervous system and circulating catecholamine (Armour, 1991, Armour, 2004).

The heart communicates with the brain and body in particularly two ways:

- Nervous system (neurocardiology): Afferent nerves (especially the vagus nerve) provide feedback from the heart to the brain.
- Endocrine: The heart is classified as part of the endocrine system since it produces the hormone Atrial Natriuretic Peptide (Saito, 2010).

However, the heart does not only influence the ANS but the ANS can also influence the heart. HRV parameters are indicators of the ANS regulation of cardiac activity (von Borell et al., 2007). HRV is the change in length of the inter-beat-interval or RR-interval during the ECG (Stucke et al., 2015). The RR-interval in animals is irregular due to the animal's cardiac autonomic response to external and internal stimuli (von Borell et al., 2007). HRV can be quantified by using three analytical categories namely: time-domain analysis (SDNN, RMSSD), frequency domain analysis (LF, HF, LF/HF, LF norm, HF norm) and geometrical analysis (SD1, SD2) (von Borell et al., 2007). The different HRV indicators represent the a) PNS effect on the heart, b) combination of the SNS and PNS or c) the balance of these branches of the ANS as described in Table 2. In addition, HRV can also be used to determine the modes of the ANS during an event, which may include co-activation, co-inhibition or reciprocal action of the PNS and SNS (van Vollenhoven et al., 2017).

The HRV can be used as a non-invasive indicator of autonomic cardiac control when the correct methodology is followed (van Vollenhoven et al., 2016). In horses, HRV has been used to measure especially stress and pain (Stucke et al., 2015, van Vollenhoven et al., 2017).

2.4.2 The value in measuring HRV in the equine athlete

The equine athlete has an innately high parasympathetic control at rest while the SNS is activated during exercise (Lorello et al., 2017). During periods of overtraining or prolonged periods of high intensity training the horse gets physically and psychologically fatigued. This is known as Over Training Syndrome (OTS) (McGowan and Whitworth, 2008, Hamlin et al., 2002) and can be measured in changes in the ANS (Kinnunen et al., 2006). Heart rate variability can be used to non-invasively measure changes in the ANS (von Borell et al., 2007) and can thus be used as an indicator of OTS and the cardiovascular response to training (Kinnunen et al., 2006). Various HRV indicators have been used to evaluate the ANS in horses (Becker-Birck et al., 2013, Clement and Barrey, 1995, Cottin, 2006, Kuwahara et al., 1999). Van Vollenhoven et al., (2017) recommended a battery of indicators including heart rate measures, time-domain indicators, frequency-domain indicators, and non-linear indicators as summarised in Table 2.

HRV studies in thoroughbreds are readily available and have been well documented. Although, thoroughbred horses have a different HRV response to beta-adrenergic blockers than other mammals, the LF and HF power indicators are still accurate reflections of the ANS in these horses (Ohmura and Jones, 2017, Kuwahara et al., 1999). In horses, HRV has been used to investigate stress associated with transport (Ohmura et al., 2012, Ohmura et al., 2006, Schmidt et al., 2010, Munsters et al., 2013), during transrectal palpations (Visser et al., 2002); van Vollenhoven 2017), during a novel object test and acute gastrointestinal disease (McConachie et al., 2016), as well as during the emersion of the horse into warm spring water (Kato et al., 2003), and the injection of atropine (Ohmura et al., 2001). Several studies on HRV and the equine athlete in training are available (Physick-Sheard et al., 2000, Kuwahara et al., 1999a, Kinnunen et al., 2006).

Physick-Sheard et al., (2000) found that at intense levels of exercise in the thoroughbred racehorse the HRV is influenced significantly by the gait-respiration rhythms but did conclude that HRV is useful in assessing exercise response and disease when measured at rest or recovery. Kuwahara et al., (1999) studied HRV changes before and after a training session in thoroughbred racehorses and found that heart rate was decreased following a training programme and the PNS may be activated even before training commences. This could be a function of co-activation due to the change in routine as described by van Vollenhoven (2017). In a study conducted on the HRV changes at different training intensities it was found that race-mares showed greater SNS activation when compared to stallions and this should be taken into consideration when preparing or participating in races (Janczarek et al., 2016).

Age and training duration has a significant effect on the HRV, with three to seven year old horses showing low HR values but significantly higher low frequency (LF) and high frequency (HF) indicators, this is attributed to the intense training during the peak age for racing (Ohmura and Jones, 2017). In a similar study on Arab endurance horses, standard deviation of longer- and long-term changes in RR-intervals (SD2) and the root square deviation of the standard deviation of the RR-interval (RMSSD) were higher in younger horses and RMSSD and SD2 decreased as the exercise duration increased (Younes, 2016). The authors concluded that RMSSD can be an indicator of performance potential and recovery ability in endurance horses and HR and RMSSD (cardiac recovery) can be used in the selection process of endurance horses.

2.4.3 Heart rate variability and acupuncture in horses

Anderson et al., (2012) hypothesise that acupuncture can cause a coherent state, where the branches of the ANS are working in a synchronized manner to create homeostasis in the body. This means that the ANS is functioning in a more harmonised fashion with a shift in autonomic function towards the PNS ("rest and digest"). The use of acupuncture in the regulation of HRV in human athletes has been investigated (Wang et al., 2015). In a study done on human soccer players acupuncture significantly activated the ANS i.e., SDNN and LnHF (natural logarithm of low frequency spectral analysis) were significantly higher in the acupuncture group post-treatment (four weeks) and LF/HF significantly lower compared to the sham acupuncture group (Lin Shihang, 2017). However, the effects on equine HRV have not been proven (le Jeune et al., 2014). Several limitations were identified in this study: the most significant being the restraint of the horses that may have led to SNS activation or a co-activation of the branches of the ANS. The study was done with DN acupuncture only that may not have an acute effect on the HRV as seen in similar studies where EA was used (Slabber, 2021). No studies could be found that measured a prolonged effect of acupuncture on the thoroughbred racehorse using HRV.

Table 2: Short descriptions for the heart rate measurements and heart rate variability indicators as used in the study of the autonomic nervous system of the horse. (Van Vollenhoven, 2017)

Indicators	Description	Autonomic control of the heart
Mean RR (ms)	Heart rate calculated as a mean of beats per minute	'Long-term influence of the SNS and short-term influence by PNS on cardiac control thus overall HRV' (Stucke et al., 2015).
Mean HR (bpm)	Mean calculated between the inter-beat-interval of two consecutive heartbeats	'PNS and SNS influence on cardiac control' (von Borell et al., 2007, Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).
SDNN or SDRR (ms)	Standard deviation of the RR-intervals	'PNS and SNS influence on cardiac control, overall HRV.' (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996, von Borell et al., 2007, Stucke et al., 2015)
RMSSD (ms)	Root Square deviation of the standard deviation of the RR-interval	'Indicator of vagal influence (short-term) cardiac control' (von Borell et al., 2007).
LF/HF	Ratio between the low frequency and high frequency	'Indicator of autonomic balance' (von Borell et al., 2007).
LF norm (nu)	Low frequency power normalised units	'Indicator of autonomic balance in cardiac control' (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996, Burr, 2007).
HF norm (nu)	High frequency power normalised units	'Indicator of cardiac autonomic balance' (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996, Burr, 2007)
SD1 (ms)	Standard deviation of short term inter-beat-interval (RR)	'Indicator of the SD of the immediate, or short-term, RR-variability due to PNS efferent (vagal) influence on the sino-atrial node' (von Borell et al., 2007, Tulppo et al., 1996).
SD2 (ms)	Standard deviation of longer- and long-term changes in RR- intervals	'Indicator of the SD of the long-term or slow variability of the heart rate' (Tulppo et al., 1996). It is accepted that this value is representative of the total variation in HRV i.e., PNS and SNS on the sino-atrial node' (Mourot et al., 2004).
LF (ms^2)	Low frequency	'Indicator of SNS influence including a PNS component' (Stucke et al., 2015)
HF (ms^2)	High frequency	'Indicator of PNS cardiac influence' (Stucke et al., 2015).

ANS = Autonomic nervous system; PNS = Parasympathetic nervous system; SNS = Sympathetic nervous system;

bpm = heart beats per minutes; ms = milliseconds; nu = normalised units; RR = inter-beat-interval; SD = standard deviation

Chapter 3: Material and methods

3.1 Animals

3.1.1 Animal population: Sample size

The sample size was determined by assuming a clinically relevant difference of 10% between the groups with respect to the stride frequency (SF). A mean of 2.4 strides per second and an SD of 0.16 strides per second was used as a point of departure based on a paper by Barrey et al. (2001). It was determined that a sample of 14 horses per group would have 90% power to detect a change in 0.24 strides per second. To provide for an expected dropout rate of 10%, the group size was increased to 15 horses. This calculation assumed a two-ended test at 0.05 level of significance. The values considered in determining the sample size are summarised in Table 3

Table 3: Statistical parameters related to stride frequency used to determine the sample size (n)

Parameter	Value
Test significance level, α	0.020
1 or 2 sided	2
Group 1 Mean, μ_1	2.4
Group 2 Mean, μ_2	2.160
Difference in Means $\mu1$-$\mu2$	0.240
Common standard deviation δ	0.16
Effect size	0.1
Power %	90%
n per group	15

As four additional animals were required for the pilot study, the Animal Ethics Committee of the University of Pretoria approved a total of 34 horses (Ethics number REC 185-19).

Before the commencement of the study, written consent was obtained from the owners and the trainer of the study group horses.

3.1.2 Selection and description of animals and their husbandry

The trainer compiled a list of horses that were eligible for the trial. The horses in the training yard that participated in races during the study period and horses that were under veterinary treatment were immediately eliminated from this list as well as horses adhering to any other non-inclusion criteria summarised in section 3.1.3. From the remaining horses, 34 horses were randomly selected for the study. The 34 horses were then randomly allocated to Group A or Group B, i.e., 15 horses per group and four horses were selected (randomly) for the pilot project. In Group A there were four fillies, one mare, three colts and eight geldings (mean age ± SD; 4 ± 0.8 years) and in Group B seven geldings and eight colts with no fillies / mares (mean age ± SD; 3 ± 0 years). Female horses under the age of five years were classified as fillies and intact male horses under the age of five years as colts respectively. The trainer and staff were blind as to which horses were selected for the treatment group.

The horses were stabled at Northrand Training Complex, Randjiesfontein (refer to Image 2). The horses were stabled in individual stables in a barn with good ventilation. They were fed a balanced diet of concentrates and roughage three times a day and had ad lib water available (Southwood, 1993). All examinations and medication were under the control of the appointed track veterinarian who visited the training yard twice daily.

Image 2: The stables at Northrand Training Complex, Randjiesfontein. The horse in the picture is being washed and groomed after a training session.

For this study it was necessary to use thoroughbred racehorses that are in active training, but not competing due to the increased risk of injury during racing, and of similar fitness levels and age.

A single training yard was selected to ensure that diet, environment, and training schedule were consistent and identical for all horses in the study.

3.1.3 Non-inclusion and exclusion criteria

Horses were excluded or eliminated from the study for the following reasons. Horses that were:

- scheduled to race during the study period;
- exhibiting any signs of clinical illness or lameness during the initial clinical examination pre-study;
- injured or ill during the study period;
- diagnosed with cardiac arrhythmias through auscultation during the clinical examinations or during the ECG recordings pre- or post-treatments;
- receiving any treatments or pharmaceuticals immediately before or during the study period;
- not of racing age i.e., younger than three years or older than seven years of age; and
- receiving acupuncture treatments within the year prior to the study.

3.2 Experimental design

3.2.1 Study design

This study was a quantitative randomised control trial during field conditions.

3.2.2 Preparation of training yard for the study

The researcher ensured that the responsible caretakers (including the veterinarian, training staff, jockeys, stable staff, or any visitors) were informed regarding the requirements of the study including that no treatments may be given and that supplements should be uniform for all the study participants including volume and frequency given. A laminated red sign was glued onto each of the stables involved in the study with the following words (Image 3):

“*Caution! This horse is currently in a UP clinical trial. No Treatments without authorisation from Mr Laird*.”

In addition, a memo was sent to all role-players to remind them that the specific horses were in a clinical trial.

Image 3: Thoroughbred racehorse in his stable. Each stable was clearly marked to indicate that the horse is part of the study and may not receive any treatments not approved by Mr Laird and Dr Hartwigsen

3.2.3 Preparation of study group

The horses included in the study were already in a training programme. Their training schedule was not changed during the study period. There was no change in the daily routine except for acupuncture treatments and data collection before and after the treatment period of three weeks. A clinical examination of all the horses selected was done upon selection and then again before the HRV collection and gallop test as well as before every acupuncture treatment.

3.2.4 Habituation of horses

To habituate the horses to the Televet100 electrocardiogram machine (see detail of the ECG measurements in section 3.5.3) all the horses were shaved at the locations of the ECG pad attachments a day before the first data collection and fitted with the

Televet100 leads and a surcingle. The horses were allowed to sniff the equipment and it was only removed once the horse seemed calm and familiar with all the new items.

3.3 Materials used.

There are three practical components to this study. The acupuncture treatment, the measurement of heart rate measures and HRV indicators as well as the measurement of SP in a 600 m gallop. Below is a list of material that was required for each section.

Acupuncture treatments

The acupuncture technique used is known as dry needle (Xie and Preast 2013) (see description in section 3.5.2).

- Acupuncture needles ϴ 0.30 x 30 mm; ϴ 0.3 x 50 mm (3 boxes of 100 needles)
- Acupuncture needles ϴ 0.3 xx 75 mm (6 boxes of 100 needles)
- One sharps bin.

Heart Rate Variability

- Televet100 (Rösh & Associates) Veterinary telemetric ECG system;
- Televet100 cables;
- ECG pads;
- Surcingle;
- Laptop

Stride parameters

- EquinITy™ (Equinity Technology Limited) GPS equine fitness monitor;
- Laptop

3.4 Pilot study

A pilot study with four hoses was completed to identify any unforeseen problems with the procedure and the equipment. The horses were randomly allocated into a treatment and control group. The treatment group received three acupuncture sessions over three weeks. The control group received no treatments. HRV (ECG data was recorded for 15 minutes) and stride data during a 600 m gallop (Equinity™ GPS equine fitness monitor) were collected pre- and post-treatments.

3.5 Data collection: General

The researcher performed a clinical health examination including taking temperature, determining respiration rate and heart rate as well as auscultation of the heart and lungs on each horse participating in the project. The examination was done on the data collection days, early in the morning before any activities commenced. All horses were examined before each acupuncture treatment, including those horses in the control group. A short discussion with the assistant trainer and trainer was held on the mornings of the trial period to determine if any of the participants had any signs of lameness or performance problems.

3.5.1 Training and treatment schedule

The horses in the training yard were exercised on the training track six days of the week. On a Sunday, the horses were not exercised but walked around the stable complex in the morning after breakfast. Training on the track consisted of “slow” (canter) or “fast” (gallop) training sessions over 600 m or 800 m. The trainer determined on which days the work would be “fast”. During this study it was decided that fast sessions would be done on Tuesday mornings. After training the horses were cooled down, washed and rested in the stables where they received food and were groomed.

To minimise the disruption of the daily routine for the horses and staff, AP treatments were given after training sessions. The HRV measurements were done on a Sunday morning to not coincide with strenuous exercise. SP were collected using the EquinITy™ on a Tuesday morning during the gallop training sessions. Table 4 shows the schedule followed during the study.

Table 4: Study schedule for the data collection and AP treatments of the horses.

	Day 1	Day 2	Day 3	Day 10	Day 17	Day 22	Day 23
All horses	Clinical exam	Clinical exam	Clinical exam	Clinical exam	Clinical exam	Clinical exam	Clinical exam
Treatment group	Stride parameters measured	HRV data collection	Acupuncture treatment	Acupuncture treatment	Acupuncture treatment	Stride parameters measured	HRV data collection
Control group	Stride parameters measured	HRV data collection	Haltered in stable for 20 minutes	Haltered in stable for 20 minutes	Haltered in stable for 20 minutes	Stride parameters measured	HRV data collection

3.5.2 Acupuncture sessions

The standard acupuncture treatment protocol of three treatments over three weeks were followed. The dry needling technique was used during treatments. The acupuncture point was located using the left index finger. The hub of the acupuncture needle was pressed over the acupuncture point and the spacer removed. With the right index finger the needle was tapped into the skin and the hub was removed. The needle was then glided into the body by pressing and twisting gently on the acupuncture needle handle with the right index finger and thumb. The needle was inserted until the acupuncture point was reached.

The acupuncture treatment was done in the stable after morning training. The horses were haltered by the assistant with minimal restraint. The researcher inserted the acupuncture needles in the pre-determined acupuncture points. The acupuncture points used were GB21, LU1, LI10, SI9, BL23, GB29, Bai Hui, Shen Shu, Shen Jiao, Shen Peng, Yan Chi, BL40, ST36 and BL54 (refer to Table 5). The point location is described in Table 5 below. Point selection was done in order to treat the big muscle groups most often affected by injury in the racehorse as well as some points that are thought to have performance enhancement effects as described in literature (Xie and Preast, 2013). The placement of needles was done in the same order for each treatment. The same brand of needles was used during the study. For the DN acupuncture the needles were left in for 20 minutes and rotated clockwise for seven revolutions every five minutes. After 20 minutes the needles were removed in the specific order in which they were inserted and discarded.

The control group was haltered by the assistant in the stable and the researcher stood by the horse for 20 minutes with no action taking place. This was to standardise the disruption of the daily routine of the horses and entering the stable as was done with the treatment group.

In Table 5 the points are listed and described. The location of the point is described using standard anatomical location and an acupuncture measurement cun. Cun is a proportionate measurement. The body is divided into a fixed number of equal lengths. For example, the width of rib 18 is equal to 1 cun and the lateral length of the femur is 16 cun. By using the cun system the difference in sizes of individuals is equalised within a standardised ratio.

Images 4 and 5 shows Dr Hartwigsen inserting acupuncture needles during the trial treatments.

Table 5: Acupuncture point descriptions including name, description of location, method for insertion and needle size used to achieve described function (Xie and Preast, 2013b)

Acupuncture point	Description of point	Needle size	Method
GB21	'In a groove in the Trapezius muscle just cranial to the edge of the scapula halfway between the most cranial point of the scapula and shoulder joint.' Refer to Image 4.	0.3 x 30 mm	Perpendicular or oblique insertion towards the medial aspect of the scapula 1.5 cun depth
ST36	'Three cun distal to ST 35 (Between the distal border of the patella and proximal border of the tibia) 0.5 cun lateral the cranial aspect of the tibial crest over the cranial tibialis muscle.'	0.3 x 30 mm	Oblique insertion 1.5 cun depth
LI10	'2 cun distal to LI11 (in the transverse cubital crease lateral and cranial to later epicondyle of the humerus) in the muscle groove between the Extensor Carpi radialis and Common digital extensor muscles.'	0.3 x 30 mm	Perpendicular insertion 1.5 cun depth
Bai Hui (Hundred Meetings)	'Classical point'* On the dorsal midline at the lumbosacral space, in the depression between the spinous process of the last lumbar vertebrae and the first sacral vertebrae'	0.3 x 50 mm	Oblique or perpendicular 2 cun depth
Shen Shu (Kidney association point)	'Classical point'* Two cun lateral to Bai Hui.'	0.3 x 75 mm	Perpendicular insertion 1.5 cun depth
Shen Peng (Kidney shelf)	'Classical point'* Two cun cranial to Shen Shu.'	0.3 x 75 mm	Perpendicular insertion 1.5 cun depth

Acupuncture point	Description of point	Needle size	Method
Shen Jiao (Kidney corner)	‘Classical point’* Two cun cranial to Shen Shu.’	0.3 x 75 mm	Perpendicular insertion 1.5 cun depth
Yan Chi (Wing of Ilium)	‘Classical point’* Midpoint between the top of the Tube Coxa and Sheng Peng.’	0.3 x 75 mm	Perpendicular insertion 3 cun depth
BL40	‘Midpoint of the transverse crease of the popliteal fossa.’	0.3 x 50 mm	Perpendicular towards the patella, 1.5 cun
GB27	‘0.5 cun craniodorsal to the cranial aspect of the dorsal ilium.’	0.3 x 50 mm	Perpendicular insertion 1.5 cun depth
LU1	‘Depression in the middle of the pectoralis descendens muscle, 1.5 cun lateral to pectoral sulcus in the first intercostal space.’ Refer to Image 5.	0.3 x 75 mm	Perpendicular insertion 1.5 cun depth
BL54	‘Midway on the line between Bai Hui and the greater trochanter the femur.’	0.3 x 50 mm	Perpendicular 2 cun depth
SI9	‘Large depression along the caudal border of the deltoid muscle at its juncture with the lateral and the long heads of the Triceps brachii.’	0.3 x 50 mm	Perpendicular 2 cun depth

**Classical points are not described as part of an “energy Channel” or Meridian but is described in TCVM literature to have a very specific function and is thought to have a powerful action in the body.*

Image 4: Dr Hartwigsen inserting an acupuncture needle into acupuncture point GB21

Image 5: Dr Hartwigsen inserting an acupuncture needle into acupuncture point LU1

3.5.3 Heart rate variability

The HRV was measured in all the horses participating in the trial. The time of day is an important variable when measuring HRV in equines as they show an increase in HF spectral power at night (von Borell et al., 2007), it is therefore recommended to measure HRV during the day (von Borell et al., 2007). The ECG data was recorded in the stable mid-morning on a Sunday when no training was given, i.e., when the horses had a rest day, one hour after feeding when horses were relaxed. There is inter-individual variation in HRV between horses and this might be due to sex, nutritional status, temperament, genotype and behaviour (von Borell et al., 2007). To limit this the nutrition (ration and volume), breed and workload of the horses were kept uniform.

The Televet100 device was used to record the heart ECG data digitally on a laptop and the four ECG device electrodes were attached with sticky gel on the skin surface and secured with a surcingle, to prevent movement between the electrodes and skin causing recording artefacts. The electrodes were placed in the following locations (Trachsel et al., 2010):

- Green. At the level of the girth on the sternum ‘5 cm caudal to the Olecranon’ on the thorax
- Red. On the right about ‘30 cm distal to withers in the 6th intercostal space’.
- Yellow. On the left about ‘30 cm distal to withers in 6th intercostal space’
- Black. On the left about ‘50 cm blow the withers in the 7th intercostal space.’

The ECG data were recorded for 15 minutes. Images 6 and 7 illustrates how the Televet100 was attached and the data recorded.

Image 6: The attachment of the Televet100 for the ECG recording (electrodes secured with a surcingle)

Image 7: ECG recording on a laptop for Kubios analysis

3.5.3.1 Confounding factors for HRV

The following confounding factors were identified from literature:

- Training intensity and fitness level: HRV may be influenced in horses subjected to long periods of training or intense training sessions (Ohmura and Jones, 2017). To limit the influence of these factors a group of horses at a similar fitness level and training period was selected.
- Age: The HRV changes with the age of the horse (Ohmura and Jones, 2017) therefore, it is preferable to have a group of similar or identical age.
- Gender: Fillies are known to have a higher SNS tone than stallions or geldings (von Borell et al., 2007). Oestrus will also influence the HRV (van Vollenhoven et al., 2016) and since fillies / mares are seasonal breeders ideally HRV studies should be conducted in the winter. Due to the Covid lockdown the study was delayed, but completed in early spring, thus the project was restricted to a specific season.
- Circadian rhythms: HRV is influenced by the time of day (Stucke et al., 2015). To compare the change in HRV the measurements should be done at the same time of day. To limit this effect the HRV measurements were done in a specific horse order and started at the exact same time for both measurements.

3.5.3.2 HRV standardisation

To ensure that the ECG recordings were standardised the following was done:

- Attachment areas of ECG electrodes were shaved, and adhesive electrodes used to minimise movement artefacts.
- The same person attached the electrodes and the Televet100 to all the horses.
- The ECG recording was recorded for 15 minutes with the middle five minutes used for HRV analysis.

3.5.4 Stride parameters

On gallop days the horses were all warmed up in a paddock at the training yard by walking in circles. The study participants were listed, and the EquinITy™ device attached just before the gallop. For this study, the researcher had four EquinITy™

devices available. The horses were galloped in pairs. The jockeys and pairing of running partners were kept the same where possible pre- and post-treatment.

Image 8: EquinITy GPS girth sleeve being attached by Dr Hartwigsen to the horse before the gallop training session

An EquinITy™ GPS device was attached to the girth of the saddle (as seen in Image 8 and 10) in a specially designed sleeve. The body of the horse was moistened with lukewarm water to allow for good contact between the girth sleeve and body wall. The device recorded the accelerometric parameters during training to a network cloud on the World Wide Web that could be accessed by the account holder as well as a memory card on the device that could be downloaded. The GPS device recorded multiple readings in a day without the need to turn it off or re-set the device. All the study horses were equipped with the GPS device, warmed up in a circle (Image 9), and galloped on a sand track (called the beach) over an 800 m distance of which 600 m was in a straight line (Image 11). The following was recorded by the EquinITy™ software for the purpose of this study: SL max, SL Avg, SF Max, SC Avg, SC Max, Max Speed, SC Max Speed, SL Max Speed and Avg Speed (See Table 6 for descriptions).

Image 9: Early morning at Northrand Training Complex in Randjiesfontein. Working riders warming up the horses before the gallop

Table 6: Description of stride parameters as measured by the EquinITy™ GPS device during the training session.

Stride parameters	Description
SL Max (m)	Maximum stride length achieved during gallop
SL Avg (m)	Average stride length in the gallop
SF Max (stride/sec)	Maximum stride frequency in the gallop
SC Avg	Average stride count in the gallop
SC Max	Maximum stride count during the gallop
Max Speed (km/h)	Maximum speed achieved during the gallop
SC Max Speed	Stride count at maximum speed
SL Max Speed (m)	Stride length at maximum speed
Avg Speed (km/h)	Average speed during the gallop

Image 10: Apache Trail waiting to be fitted with the EquinITy™ GPS device before the gallop

Image 11: Two thoroughbred horses in a gallop with the EquinITy™ devices fitted for the measurement of the stride parameters.

3.5.4.1 Stride parameters standardisation

- The thoroughbred racehorse tends to be within at racing age of three to six years. We did try to include horses in the trial with similar racing experience and levels of training.
- The gallop was completed on the sand tract, also known as the beach.
- The same jockeys were used during the SP measurements
- The horses were galloped in the same pairs for the measurements.

3.6 Data processing and analysis

3.6.1 Heart Rate Variability processing

The ECG data were recorded for 15 minutes to ensure that a five-minute tachogram could be chosen with the least “background noise”. Processing and analysis of data was done according to van Vollenhoven et al., (2016 and 2017). The data for the HRV were downloaded from the recording monitor (Televet100) using the Televet100 software, then transferred to the HRV Analysis Software 3.3.1 for Windows or Kubios (Kubios HRV Standard, Version 3.3.1, The Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland). The RR-intervals were quantified (determining the various HRV parameters) using the Kubios software. The frequency band widths in Kubios were set at 0.01- 0.06 Hz for LF and 0.07-0.6 Hz for HF (Cottin, 2006).

A five-minute segment (within the 15 minutes recording) with the least number of artefacts (visually) was chosen. This five-minute segment was then analysed using no correction, very low, low correction, medium- and high-correction factors (CF) for each of the horses pre- and post-treatment. The lowest CF, that corrected the data without affecting the variability, was then chosen on an individual basis as recommended on the Kubios website (McDuffee et al., 2019; Tarvainen and Niskanen, 2012). To identify the RR-intervals from artefacts the correction filters were thus set at no correction to strong CF, which differed from the mean RR-interval with 0, 0.45, 0.35, 0.25, and 0.15 sec respectively. Using piecewise cubic spline interpolation, the Kubios software replaced the artefacts with interpolated intervals (Ille et al., 2014; van Vollenhoven et al., 2017). Smoothness priors were all set at 500 ms to calculate the detrending procedure (Tarvainen and Niskanen, 2012). Where the tachogram quality was extremely poor the results were excluded from the analysis.

3.6.2 Stride parameter processing

Once the EquinITy™ data was downloaded from the device at the end of the training session it was processed by the EquinITy™ Technology website (www.equinitytechnology.com). The data was processed per horse and per work session (refer to Figure 1-5). The SP were exported to a Microsoft Excel sheet for statistical analysis.

The figures below are examples of the EquinITy™ work data for each horse. The selected data points were exported to Excel and used for the statistical analysis of the data.

Figure 1: EquinITy™ work data summary for Apache Trail

Figure 2: EquinITy™ speed profile for Apache Trail

Figure 3: Work Data for Apache Trail in the different split zones on the tract as recorded by EquinITy™

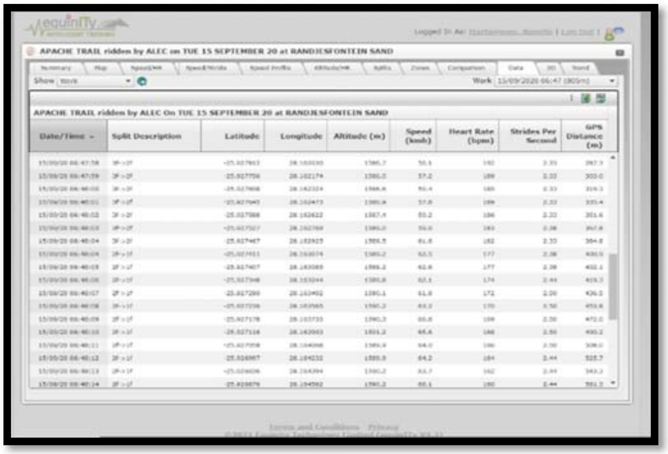

Figure 4: EquinITy™ work data for Apache Trail as exported for analysis

Figure 5: EquinITy™ graph showing the speed/stride profile for Apache Trail

3.7 Statistical data analysis

The data analysis (HRV and SP) consists of descriptive statistics including the median and interquartile range to describe the results and graphical representation were investigated to assist in visualizing aspects of the data. The difference between pre- and post- treatments within each group, for each indicator, was used to compare Group A (treatment group) and Group B (control group or non-treatment group). The Shapiro Wilk test was used to investigate if the data is normally distributed. Since the normality assumption did not hold, and since the groups being compared are independent, the non-parametric Mann Whitney U test was used to investigate if differences exists between the data distributions of the acupuncture treatment groups and the group not treated. All tests were performed at a 5% level of significance.

Chapter 4: Results

In this study a total of 34 healthy horses participated. Four in the pilot trial and 30 in the study. A total of five horses from Group B were eliminated from the study due to injury.

4.1 Pilot study results

Due to the small sample size in the pilot trial a significance test per metric was not possible. The motivation for a pilot trial was to test the procedures and equipment of the study and not necessarily the possible or expected outcome based on the results. The results from the pilot study were not included in the data analysis.

The following observations were made during the pilot study:

- The horses adapted quickly to the equipment and did not seem to be stressed during the attachment of the Televet100 or the EquinITy™.
- All the horses in the pilot trial were comfortable with the acupuncture points and allowed insertion of the acupuncture needles into all the selected points.
- The attachment of the EquinITy™ girth on the training track was challenging with the exited horses. The grooms and assistant trainer were able to adopt a routine to put on the girth quickly.

No changes to the protocol were made following the pilot trial. The grooms and assistants were educated on all the procedures during the pilot trial.

4.2 Heart Rate Variability Results

In Table 7 the descriptive statistics for the HR and HRV indicators are given for both Group A and Group B for the pre-acupuncture treatment measurements. In Table 8 the values for Group A and Group B post-acupuncture treatments are described and Table 9 displays the statistical difference between the pre- and post-treatment results (delta) of the HRV indicators and heart rate measures to determine if there was a statistical difference between the treatment and the control group.

After each Table, the boxplots (refer to Figure 6-14) showing a visual representation of each indicator, are given.

Table 7: Descriptive statistics for heart rate measures and heart rate variability indicators in both Group A and Group B as measures of pre-acupuncture treatments.

	Combined data (n = 25)	Group A (n = 15)	Group B (n = 10)
	Median (iqr)	**Median (iqr)**	**Median (iqr)**
Heart Rate Measures			
Mean HR (beats per minute)	40.92 (35.34, 47.76)	37.91 (35.28, 47.72)	41.25 (36.72, 49.10)
Mean RR (ms)	1466.22 (1256.36,1697.69)	1582.69 (1257.32, 1700.61)	1454.61 (1224.95, 1638.30)
HRV Indicators			
SDNN (ms)	50.80 (44.72, 56.20)	47.89 (39.65, 52.68)	58.40 (50.18, 65.75)
RMSSD (ms)	42.62 (33.17, 48.13)	36.8 (32.96, 46.96)	45.59 (42.91, 48.81)
LF (ms^2)	1260.46 (854.40, 1678.10)	1182.56 (634.56,1365.51)	1827.64 (1249.10, 2659.16)
HF (ms^2)	853.32 (610.71, 1197.12)	821.72 (509.06, 1180.36)	1022.83 (658.27, 1304.18)
LF norm (nu)	81.89 (60.70, 94.12)	78.59 (58.42, 85.73)	90.54 (71.08, 108.48)
HF norm (nu)	62.32 (46.47, 72.47)	64.95 (57.22, 73.07)	51.10 (37.86, 63.40)
LF/HF	1.25 (0.91, 1.89)	1.10 (0.84, 1.50)	1.78 (1.12, 2.87)
SD1 (ms)	30.19 (23.52, 34.13)	26.12 (23.38, 33.29)	32.33 (30.40, 34.61)
SD2 (ms)	64.81 (58.41, 71.53)	63.44 (48.28, 67.88)	75.66 (62.40, 87.82)

Treated Group A = dry needling for 20 minutes at points GB21, ST36, LI10, Bai Hui, Shen Shu, Shen Peng, Shen Jiao, Yan Chi, BL40, GB27, LU1, BL54, SI9; Group B = haltered in stable for 20 minutes.; iqr = interquartile range; HRV = heart rate variability; RR = beat-to-beat interval; HR = heart rate; SDNN = standard deviation of RR-interval; RMSSD = root mean square of successive differences in RR intervals; HF = high frequency components; LF = low frequency components; LF/HF = autonomic balance; LF norm = low frequency power normalised units; HF norm = high frequency power normalised units; SD1 = standard deviation of short-term variability; SD2 = standard deviation of long-term variability

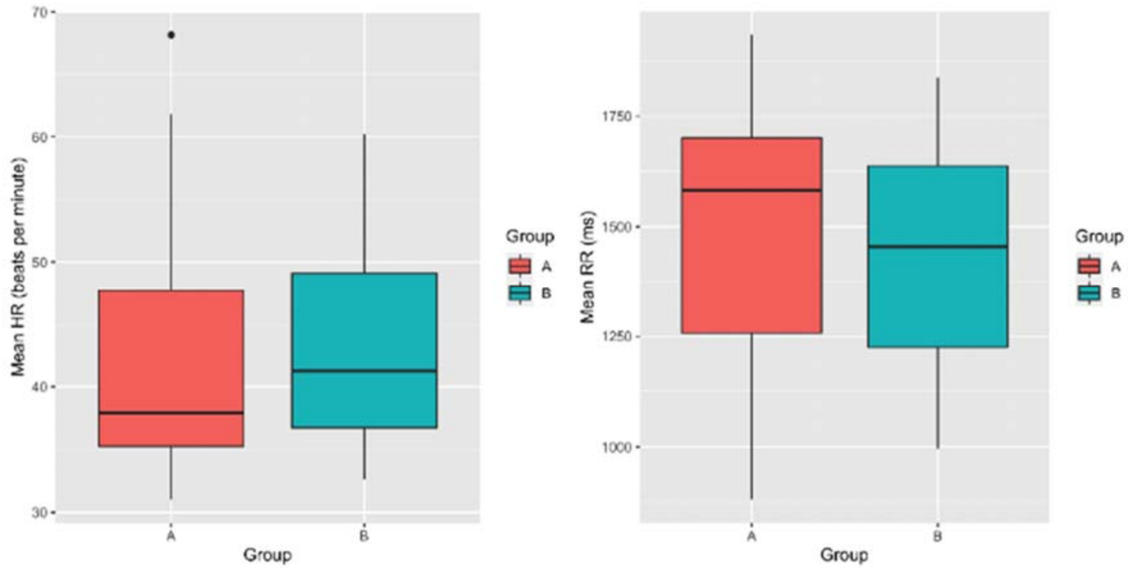

Figure 6*:* Boxplots of the heart rate measures for *G*roups A and B pre-acupuncture treatments

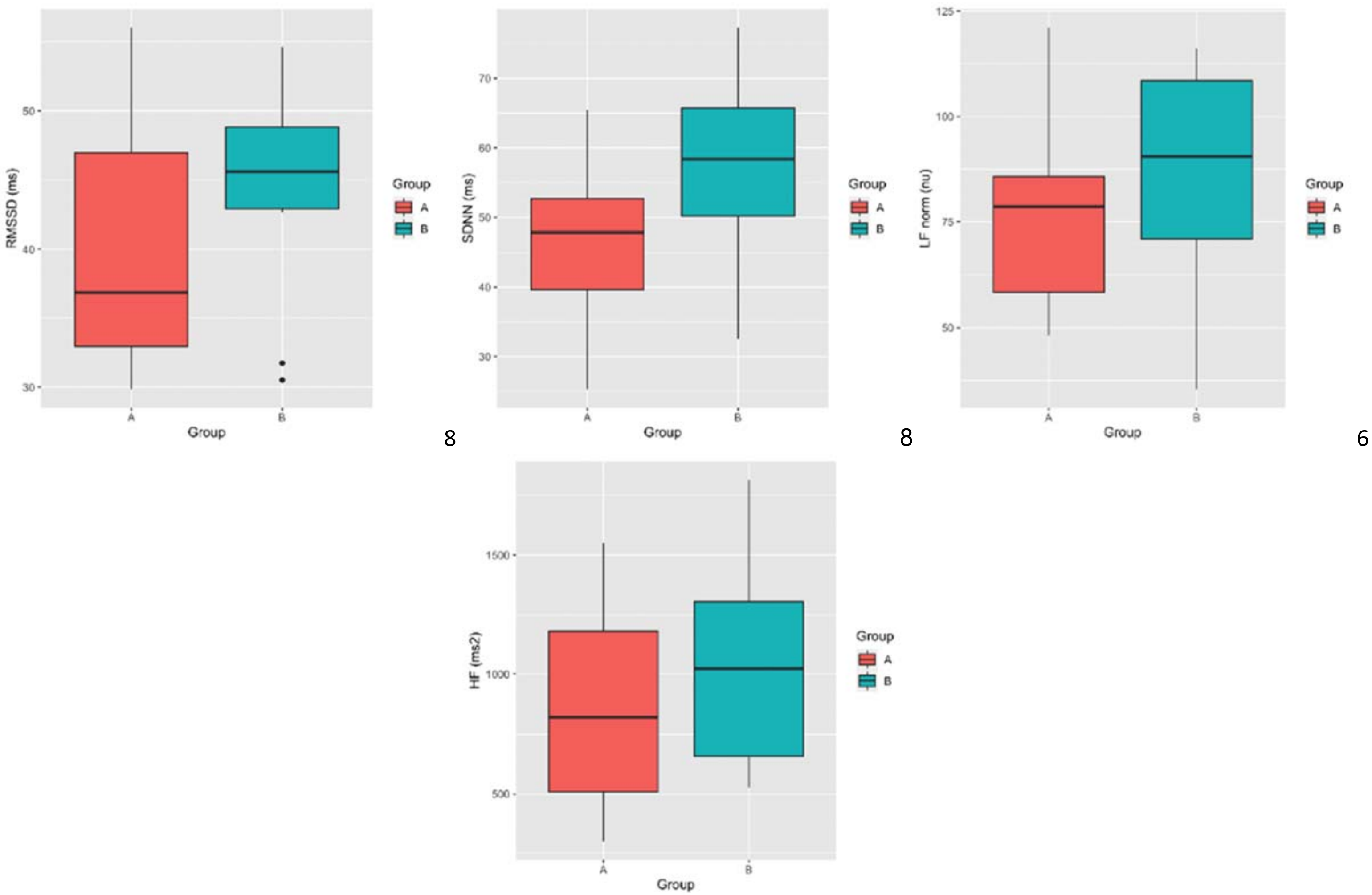

Figure 7 Boxplots of RMSSD, SDNN, LF norm and HF indicators for Groups A and B pre-acupuncture treatments

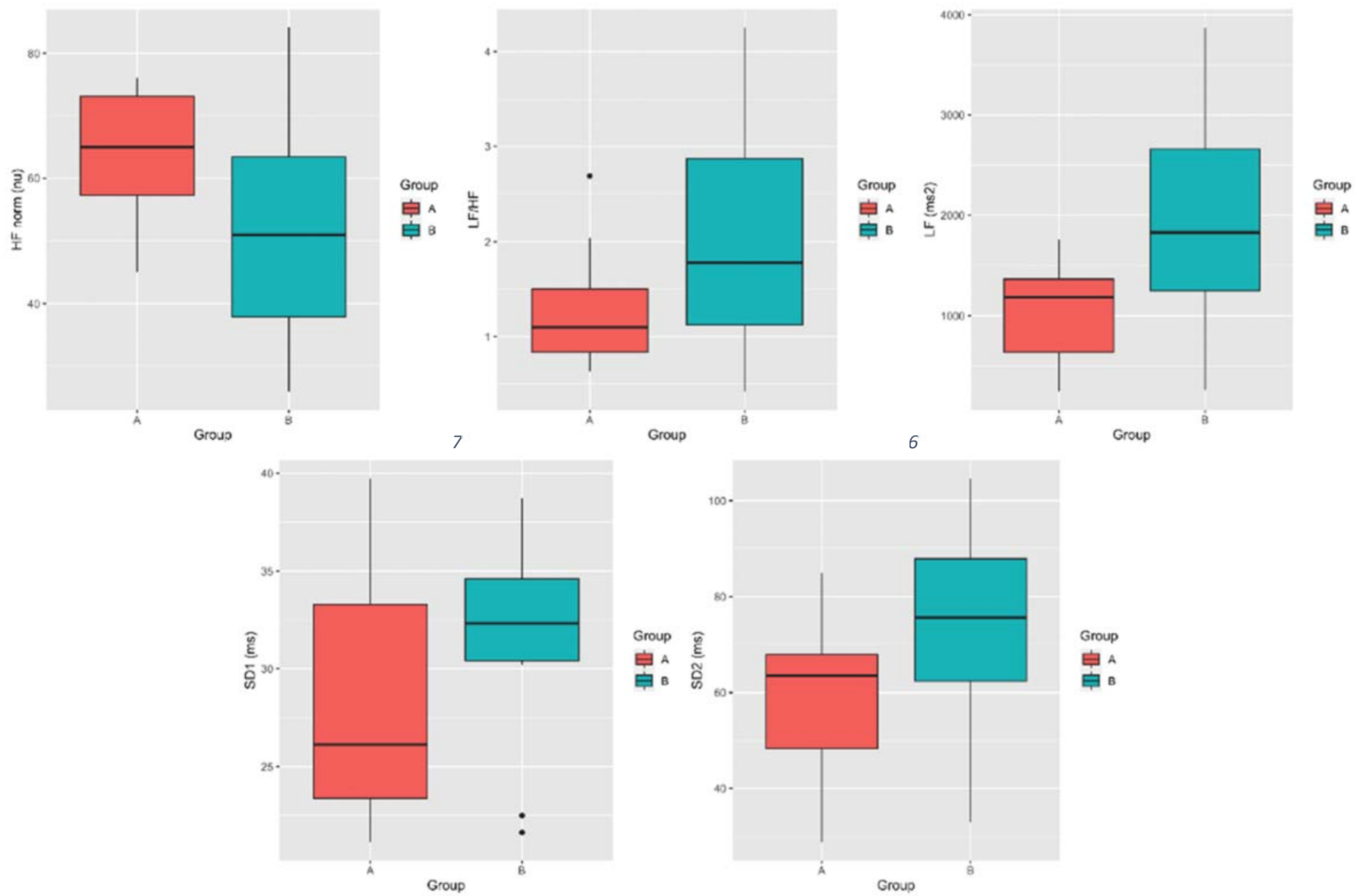

Figure 8: Boxplots of the HF norm, LF/HF, LF, SD1, SD2 indicators for Groups A and B pre-acupuncture treatments

Table 8: Descriptive statistics for heart rate measures and heart rate variability indicators in Groups A and B post-acupuncture treatments

	Combined data (n = 25)	Group A (n = 15)	Group B (n = 10)
	Median (iqr)	**Median (iqr)**	**Median (iqr)**
Heart Rate Measures			
Mean HR (beats per minute)	40.32 (36.59, 44.75)	40.32 (36.76, 45.75)	39.58 (36.63, 44.00)
Mean RR (ms)	1487.97 (1340.73, 1639.91)	1487.97 (1312.09, 1632.27)	1521.38 (1364.50, 1637.91)
HRV Indicators			
SDNN (ms)	49.78 (44.98, 55.62)	47.05 (43.82 54.27)	53.68 (49.48, 59.71)
RMSSD (ms)	42.85 (36.82, 50.10)	42.85 (35.35, 45.95)	41.64 (37.54, 52.06)
LF (ms^2)	1195.03 (816.75, 1757.92)	1048.56 (814.47, 1388.89)	1638.25 (1007.25, 1813.36)
HF (ms^2)	829.08 (763.09, 1129.17)	853.82 (763.72, 1070.32)	808.05 (734.36, 1430.91)
LF norm (nu)	86.33 (58.31, 102.62)	84.85 (64.78, 95.24)	89.75 (60.12, 106.05)
HF norm (nu)	57.27 (49.59, 70.69)	62.56 (51.57, 70.69)	54.91 (47.10, 72.92)
LF/HF	1.57 (0.79, 2.07)	1.37 (0.90, 1.85)	1.63 (0.85, 2.31)
SD1 (ms)	30.35 (26.09, 35.53)	30.35 (25.05, 32.57)	29.52 (26.60, 36.87)
SD2 (ms)	63.24 (53.56, 73.36)	61.38 (52.87, 71.13)	67.69 (63.42, 74.43)

Treated Group A = dry needling for 20 minutes at points GB21, ST36, LI10, Bai Hui, Shen Shu, Shen Peng, Shen Jiao, Yan Chi, BL40, GB27, LU1, BL54, SI9; Group B = haltered in stable for 20 minutes.; iqr = interquartile range; HRV = heart rate variability; RR = beat-to-beat interval; HR = heart rate; SDNN = standard deviation of RR-interval; RMSSD = root mean square of successive differences in RR intervals; HF = high frequency components; LF = low frequency components; LF/HF = autonomic balance; LF norm = low frequency power normalised units; HF norm = high frequency power normalised units; SD1 = standard deviation of short-term variability; SD2 = standard deviation of long-term variability

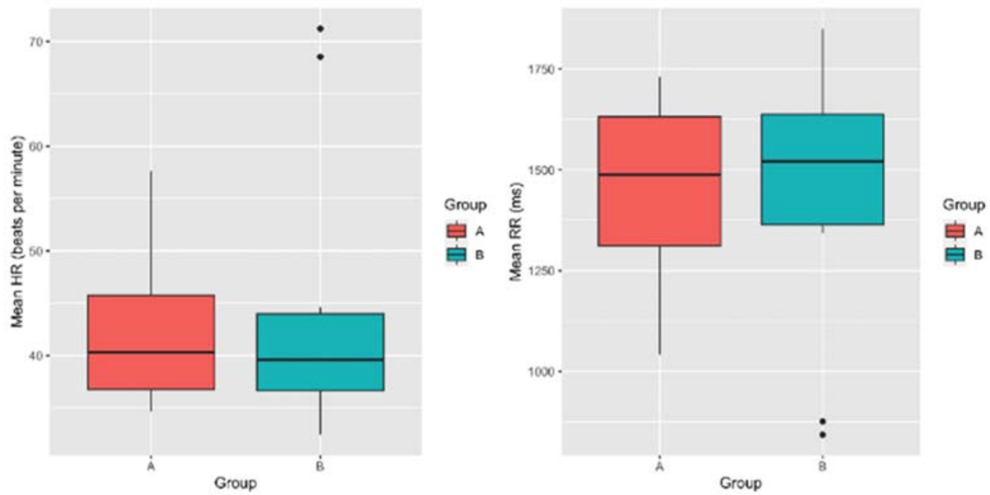

Figure 9: Boxplots of heart rate measures for Groups A and B post-acupuncture treatments

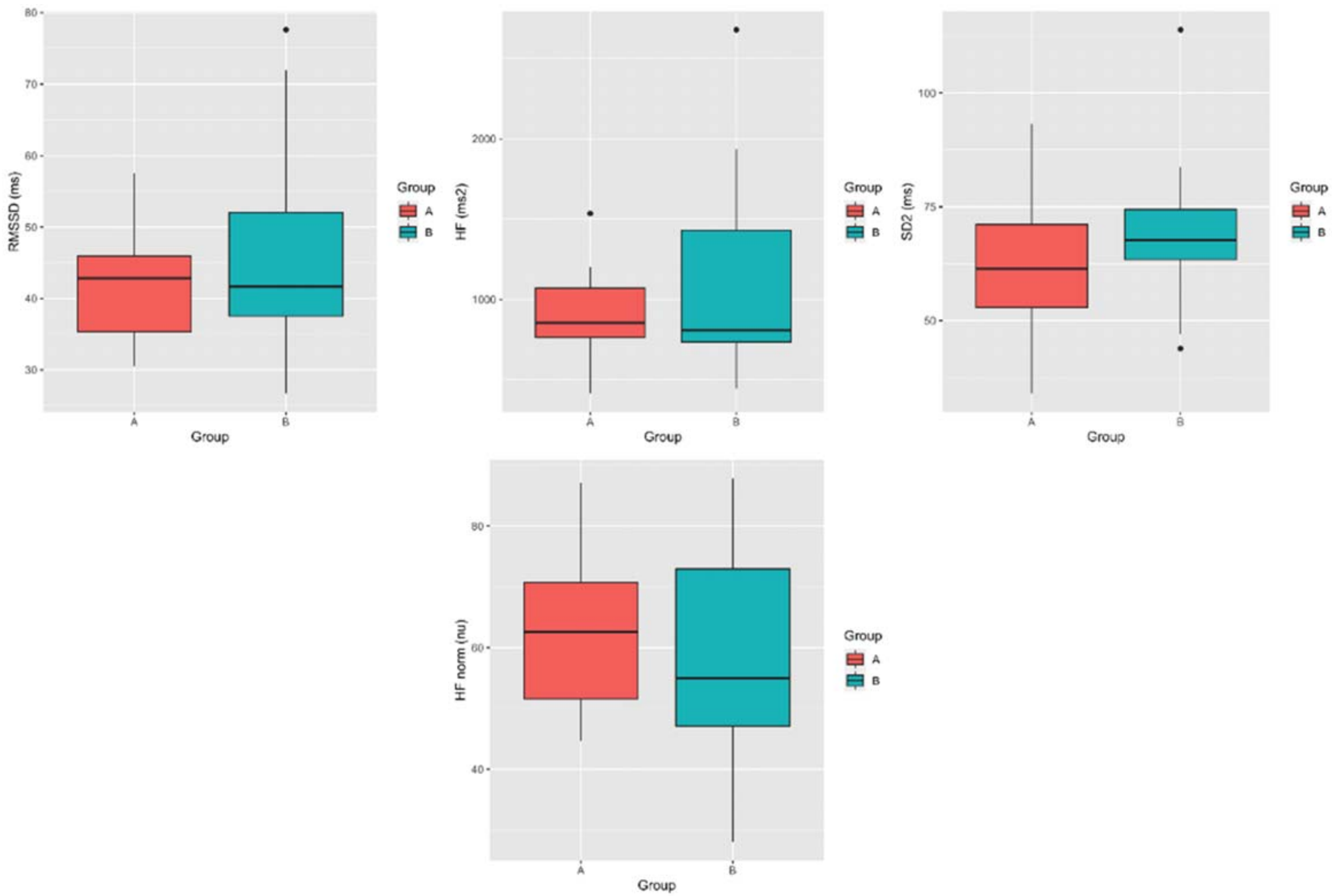

Figure 10: Boxplots of the RMSSD, HF, SD2, HF norm measures for Groups A and B post-acupuncture treatments

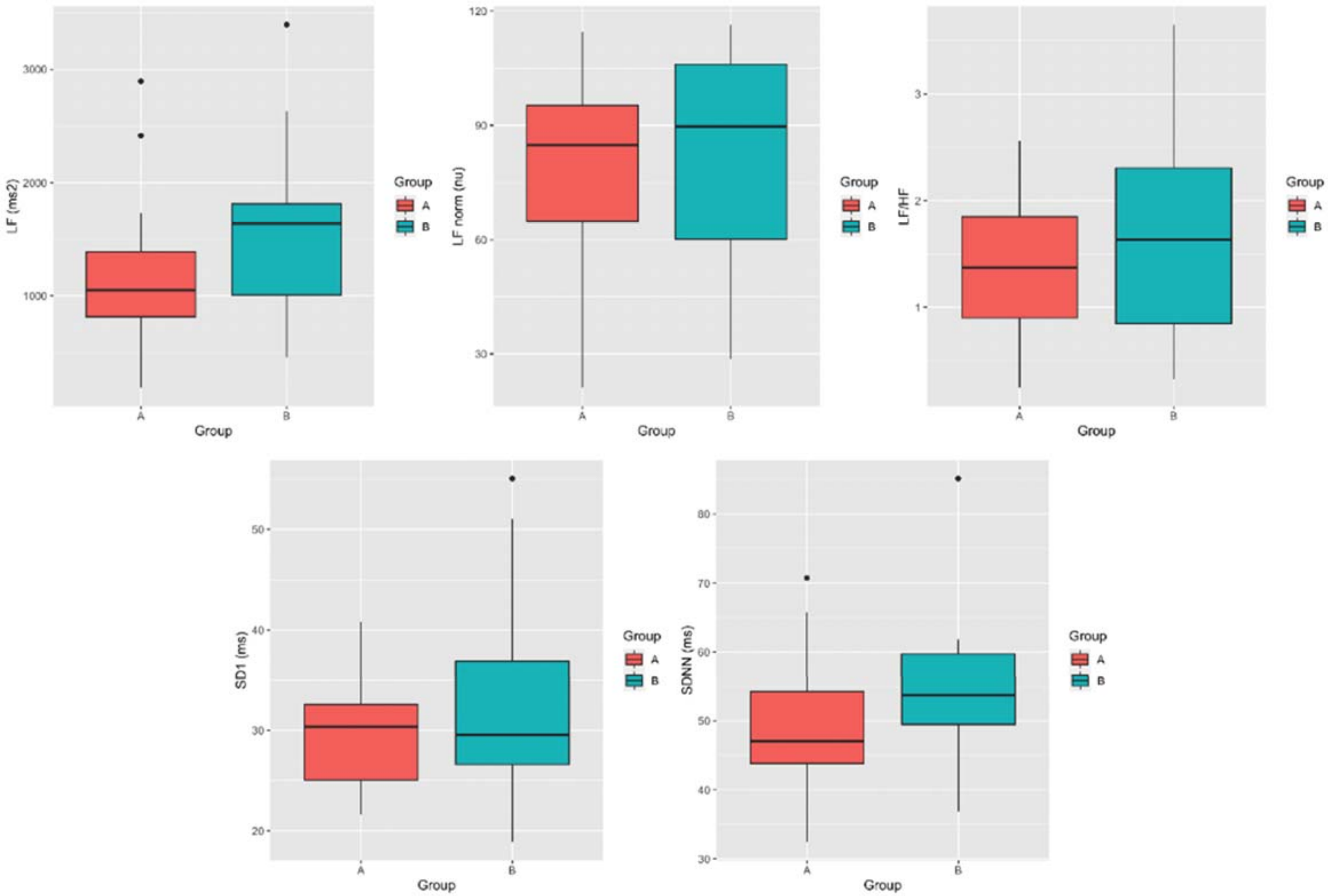

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Figure 11: Boxplots of LF, LF norm, LF/HF, SD1, SDNN for Groups A and B post-acupuncture treatments

Table 9: Descriptive statistics of heart rate measures and heart rate variability indicators determined by comparing the delta (difference of pre- and post-treatment measurements) of a treatment group of racehorses subjected to three acupuncture sessions and a control group

	Combined data (n = 25)	**Group A (n = 15)**	**Group B (n = 10)**	
	Median (iqr)	**Median (iqr)**	**Median (iqr)**	**Independent Mann Whitney U test P-value**
Heart Rate Measures				
Mean HR (beats per minute)	-3.01 (-5.79, 7.52)	-3.01 (-9.05, 6.52)	-3.00 (-4.13, 6.77)	0.76
Mean RR (ms)	98.20 (-223.53, 178.86)	84.37 (-297.97, 350.78)	107.54 (-197.88, 162.73)	0.89
HRV Indicators				
SDNN (ms)	-0.30 (-8.65, 8.54)	3.61 (-4.52, 7.73)	-4.00 (-14.28, 6.72)	0.37
RMSSD (ms)	1.54 (-2.60, 7.48)	1.54 (-2.43, 8.83)	1.47 (-4.73, 6.88)	1
LF (ms^2)	-178.94 (-496.52, 467.95)	22.95 (-251.13, 521.66)	-397.26 (-754.78, 41.82)	0.18
HF (ms^2)	-5.44 (-137.58, 371.59)	-20.19 (-129.37, 278.19)	19.42 (-123.72, 360.50)	0.81
LF norm (nu)	3.28 (-22.31, 19.94)	3.28 (-1.14, 20.55)	-0.80 (-28.46, 10.96)	0.57
HF norm (nu)	2.13 (-8.55, 14.59)	-3.07 (-13.99, 8.82)	3.54 (-8.19, 17.46)	0.60
LF/HF	0.08 (-0.60, 0.80)	0.18 (-0.20, 0.65)	-0.32 (-1.04, 0.68)	0.50
SD1 (ms)	1.10 (-1.85, 5.34)	1.10 (-1.72, 6.28)	1.04 (-3.37, 4.90)	1
SD2 (ms)	-1.08 (-14.17, 8.74)	5.85 (-7.43, 12.40)	-7.48 (-17.26, 1.74)	0.26

Independent Mann Whitney U test = Significance set at $p < 0.05$. Treated group A = dry needling for 20 minutes at points GB21, ST36, LI10, Bai Hui, Shen Shu, Shen Peng, Shen Jiao, Yan Chi, BL40, GB27, LU1, BL54, SI9; Group B = haltered in stable for 20 minutes. ; iqr = interquartile range; HRV = heart rate variability; RR = beat-to-beat interval; HR = heart rate; SDNN = standard deviation of RR-interval; RMSSD = root mean square of successive differences in RR intervals; HF = high frequency components; LF = low frequency components; LF/HF = autonomic balance; LF norm = low frequency power normalised units; HF norm = high frequency power normalised units; SD1 = standard deviation of short-term variability; SD2 = standard deviation of long-term variability

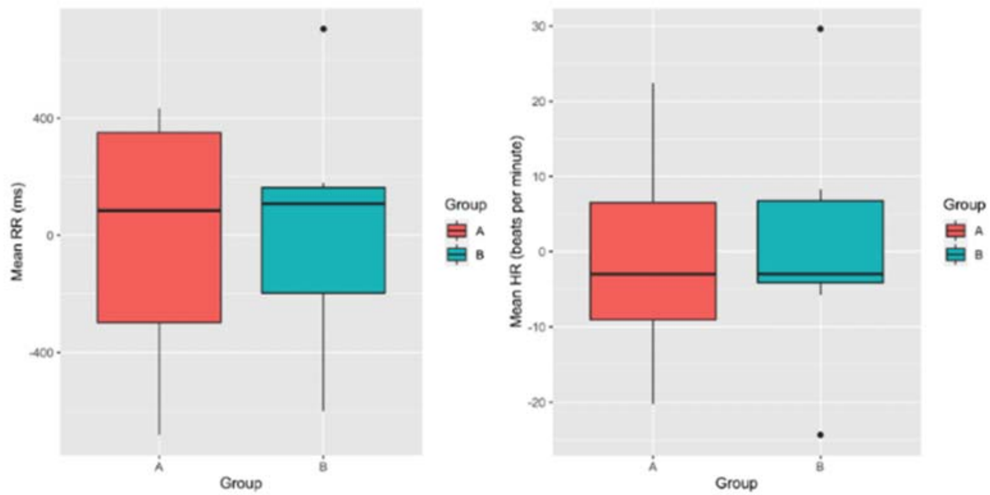

Figure 12: Boxplots showing heart rate measures determined by comparing the delta (difference of pre- and post-treatment measurements) of Group A of the thoroughbred racehorses subjected to three acupuncture sessions and control Group B

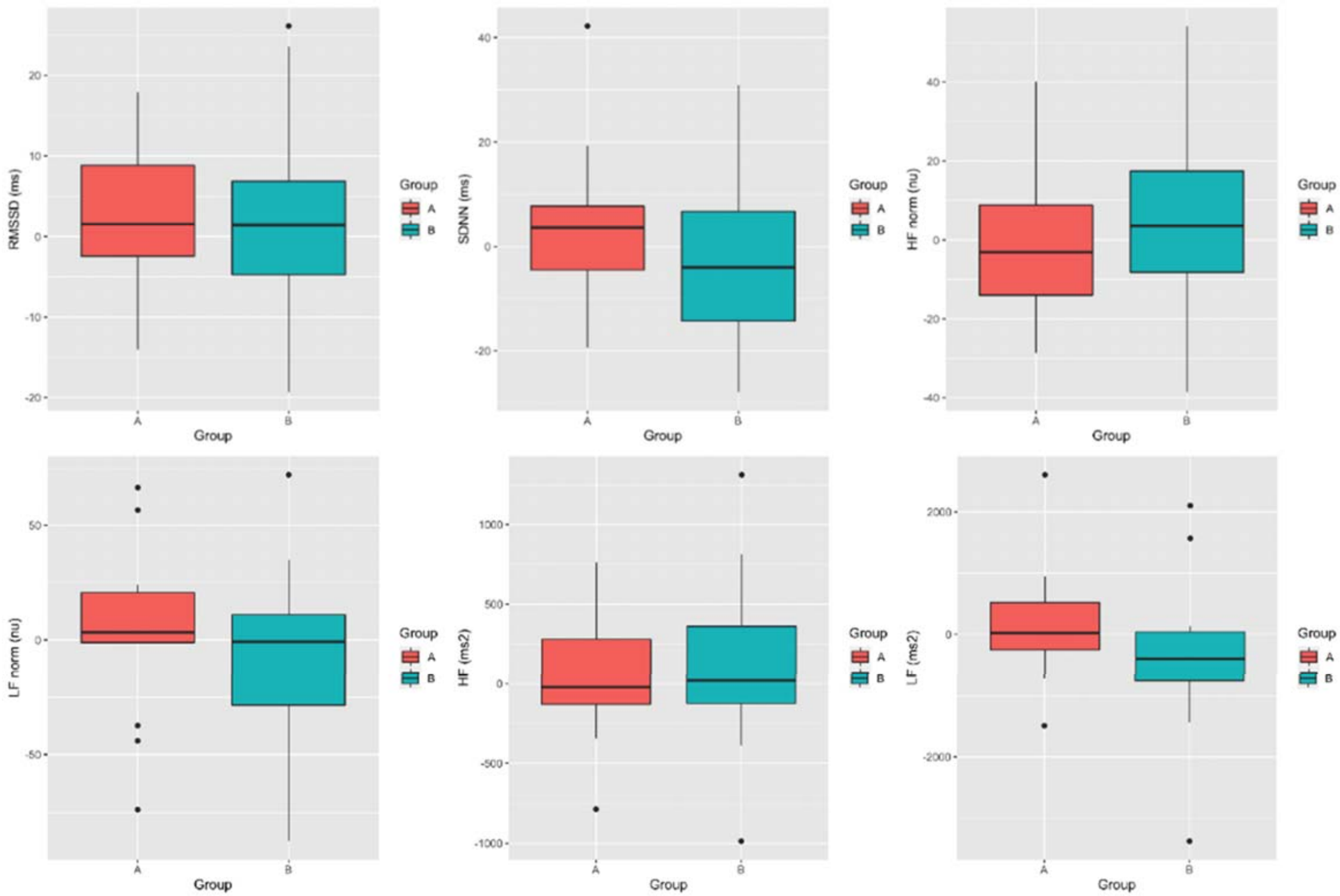

Figure 13: Boxplots showing RMSSD, SDNN, HF/LF, LF norm, HF, LF determined by comparing the delta (difference of pre- and post-treatment measurements) of Group A of the thoroughbred racehorses subjected to three acupuncture sessions and control Group B

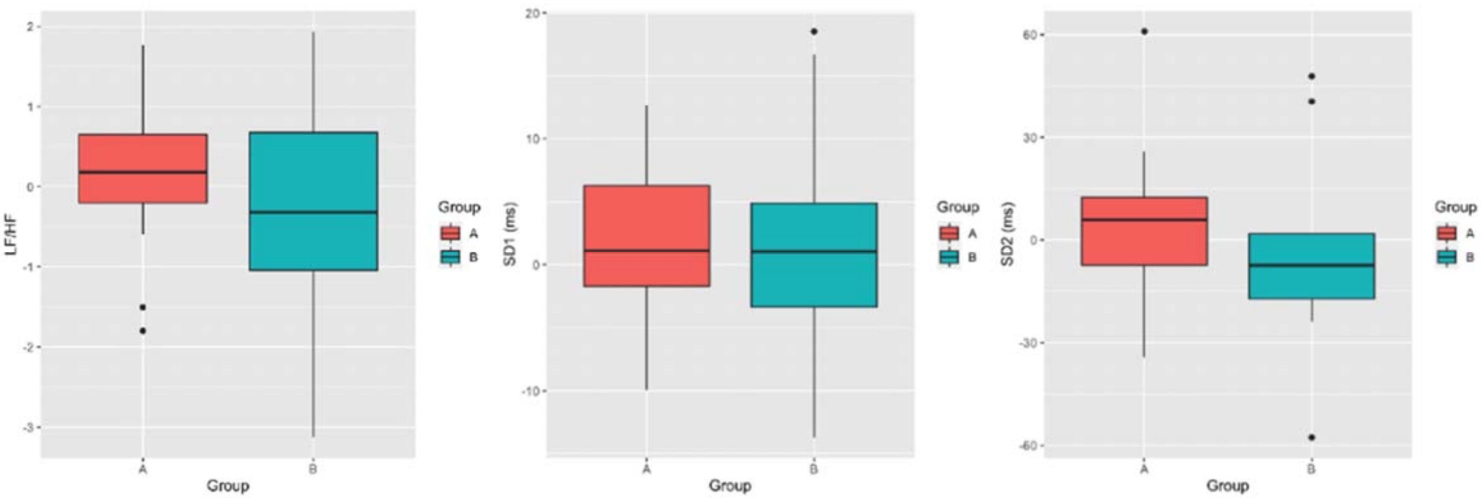

Figure 14: Boxplots showing LF/HF, SD1, SD2 determined by comparing the delta (difference of pre- and post-treatment measurements) of Group A of thoroughbred racehorses subjected to three acupuncture sessions and control Group B

During the randomised allocation of the treatment and control groups all the fillies / mares ended up in the same treatment group (A). This created an unequal distribution of gender in the groups influencing the results. To calculate results with a more accurate distribution the statistics were recalculated without the fillies' / mare's results. Table 10 shows the difference (delta) between the treatment group and control group comparing to pre- and post-acupuncture treatment results of the HRV and heart rate measures. The boxplot for each difference (delta) for every indicator without the fillies' / mare's results is given below (refer to Figure 15-17).

Table 10: Descriptive statistics of heart rate measures and heart rate variability indicators determined by comparing the delta (difference of pre- and post-treatment measurements) of the treatment Group A without the fillies / mares subjected to three acupuncture sessions and the control Group B

	Combined data (n = 20)	**Group A (n = 10)**	**Group B (n = 10)**	
	Median (iqr)	**Median (iqr)**	**Median (iqr)**	**Independent Mann Whitney U test P-value**
Heart Rate Measures				
Mean HR (beats per minute)	0.37 (-4.89, 8.42)	3.96 (-7.70, 10.65)	-3.00 (-4.13, 6.77)	0.85
Mean RR (ms)	-11.35 (-336.10, 168.91)	-169.72 (-490.11, 287.21)	107.54 (-197.88, 162.73)	0.48
HRV Indicators				
SDNN (ms)	0.28 (-12.20, 9.85)	4.39 (-3.94, 10.34)	-4.00 (-14.28, 6.72)	0.35
RMSSD (ms)	-1.25 (-5.37, 8.21)	-2.19 (-4.61, 9.50)	1.47 (-4.73, 6.88)	0.80
LF (ms^2)	-178.45 (-542.98, 581.67)	179.48 (-178.70, 594.25)	-397.26 (-754.78, 41.82)	0.17
Hf (ms^2)	-12.82 (-175.10, 447.45)	-37.19 (-188.31, 475.61)	19.42 (-123.72, 360.50)	0.80
LFf Norm (nu)	7.31 (-24.36, 20.99)	14.23 (0.36, 23.08)	-0.80 (-28.46, 10.96)	0.44
Hf Norm (nu)	-0.47 (-12.09, 16.34)	-4.78 (-22.58, 9.40)	3.54 (-8.19, 17.46)	0.53
LF/HF	0.14 (-0.75, 0.87)	0.26 (-0.08, 0.92)	-0.32 (-1.04, 0.68)	0.48
SD1 (ms)	-0.90 (-3.84, 5.85)	-1.56 (-3.30, 6.76)	1.04 (-3.37, 4.90)	0.80
SD2 (ms)	-0.33 (-14.91, 16.14)	8.55 (-7.14, 16.30)	-7.48 (-17.26, 1.74)	0.28

Independent Mann Whitney U test = Significance set at $p < 0.05$. Treated group A = dry needling for 20 minutes at points GB21, ST36, LI10, Bai Hui, Shen Shu, Shen Peng, Shen Jiao, Yan Chi, BL40, GB27, LU1, BL54, SI9; Group B = haltered in stable for 20 minutes. ; iqr = interquartile range; HRV = heart rate variability; RR = beat-to-beat interval; HR = heart rate; SDNN = standard deviation of RR-interval; RMSSD = root mean square of successive differences in RR intervals; HF = high frequency components; LF = low frequency components; LF/HF = autonomic balance; LF norm = low frequency power normalised units; HF norm = high frequency power normalised units; SD1 = standard deviation of short-term variability; SD2 = standard deviation of long-term variability

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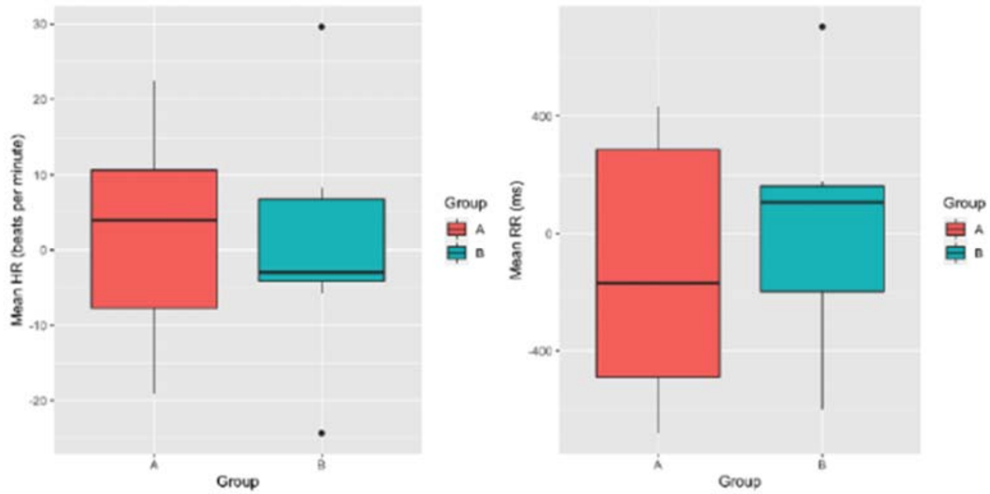

Figure 15: Boxplots of heart rate measures determined by comparing the delta (difference of pre- and post-treatment measurements) of Group A without the fillies / mares subjected to three acupuncture sessions and the control Group B

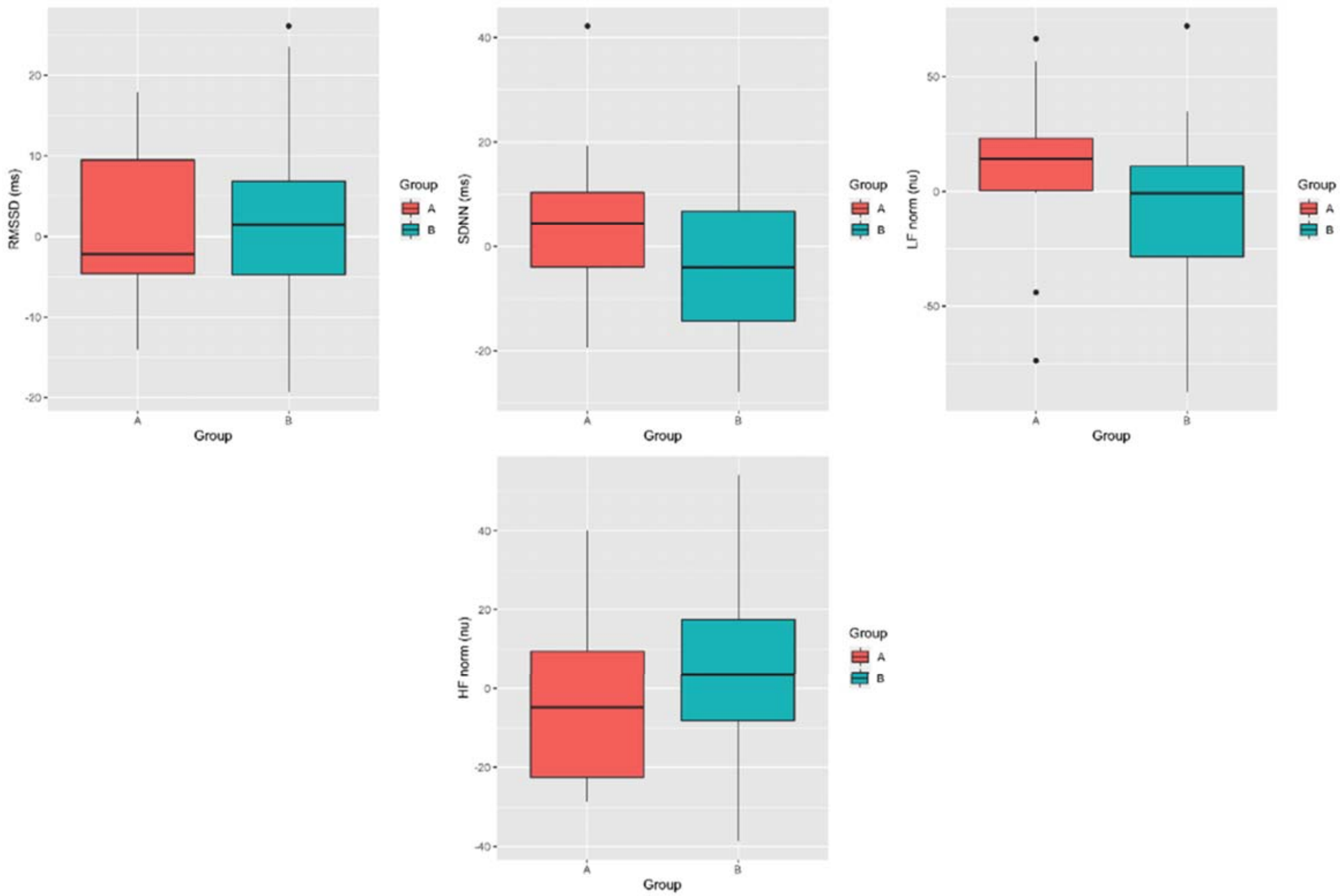

Figure 16: Boxplots showing RMSSD, SDNN, LF norm, HF norm determined by comparing the delta (difference of pre- and post-treatment measurements) of Group A without the fillies subjected to three acupuncture sessions and the control Group B

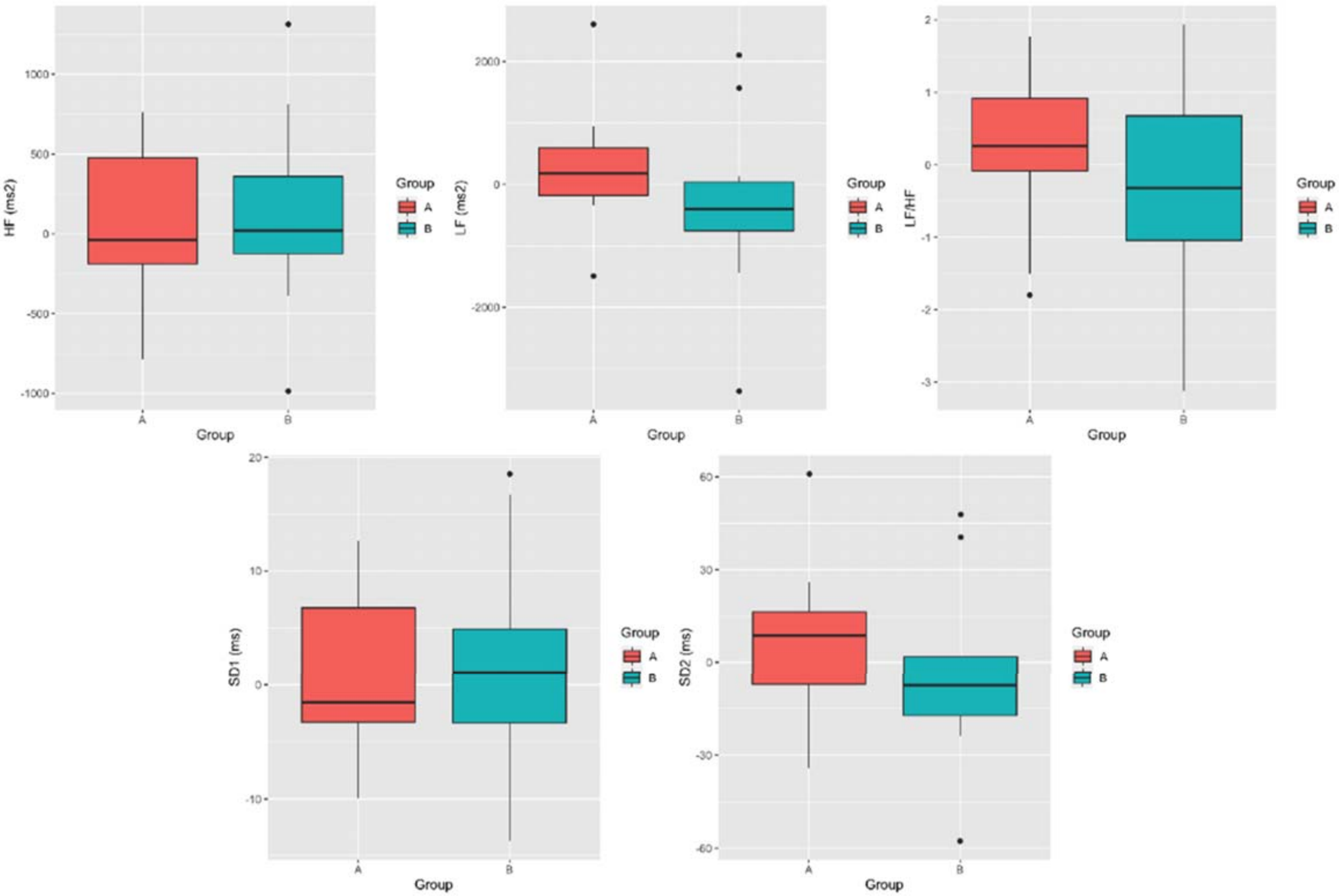

Figure 17: Boxplots showing HF, LF, LF/HF, SD1, SD2 determined by comparing the delta (difference of pre- and post-treatment measurements) of Group A without the fillies / mares subjected to three acupuncture sessions and the control Group B

4.3 Stride parameters results

Statistical indicators (median and interquartile range) for the SP for Groups A and B are given in the Tables below. Table 11 gives results pre-acupuncture treatments; Table 12 shows the results post-acupuncture treatments and Table 13 shows the difference (delta) in the change indicators between the treatment group (A) and control group (B). The independent Mann Whitney U test was used to determine the p-value for statistically significant changes. Boxplots giving a visual representation of the SP for Groups A and B are shown below the Tables (refer to Figure 18-23).

Table 11: Descriptive statistics of the stride parameters in both Group A and B pre-acupuncture sessions

	Combined data (n = 25)	**Group A (n = 13)**	**Group B (n = 12)**
Stride Parameters	**Median (iqr)**	**Median (iqr)**	**Median (iqr)**
SL Max (m)	7.01 (6.60, 7.21)	7.03 (6.60, 7.23)	6.88 (6.66, 7.13)
SL Avg (m)	6.21 (5.99, 6.40)	6.06 (5.99, 6.49)	6.24 (6.12, 6.39)
SF Max (stride/sec)	2.56 (2.44, 2.63)	2.50 (2.38, 2.63)	2.59 (2.50, 2.63)
SC Avg	32.46 (31.60, 33.95)	33.43 (31.28, 33.95)	32.44 (31.80, 33.14)
SC Max	37.71 (36.17, 41.29)	37.49 (36.05, 41.41)	37.84 (36.42, 38.24)
Max Speed (km/h)	62.10 (61.30, 63.50)	61.80 (61.20, 62.70)	62.85 (61.83, 65.08)
SC Max Speed	29.98 (29.41, 31.47)	29.93 (29.41, 31.83)	30.43 (29.35, 31.44)
SL Max Speed (m)	6.78 (6.40, 6.98)	6.81 (6.35, 7.01)	6.67 (6.41, 6.85)
Avg Speed (km/h)	52.00 (49.00, 53.40)	51.60 (46.50, 52.30)	52.45 (49.38, 53.40)

Treated Group A = dry needling for 20 minutes at points GB21, ST36, LI10, Bai Hui, Shen Shu, Shen Peng, Shen Jiao, Yan Chi, BL40, GB27, LU1, BL54, SI9; Group B = haltered in stable for 20 minutes; iqr = interquartile range; SL Max (m)= maximum stride length achieved during gallop, SL Avg (m) = average stride length in the gallop , SF Max (strides/s) = maximum stride frequency in the gallop, SC Avg = average stride count in the gallop, SC Max = maximum stride count during the gallop, Max Speed (km/h) = maximum speed achieved in the gallop, SC Max Speed = stride count achieved at maximum speed, SL Max Speed (m) = stride length at maximum speed, Avg Speed (km/h) = average speed during the gallop

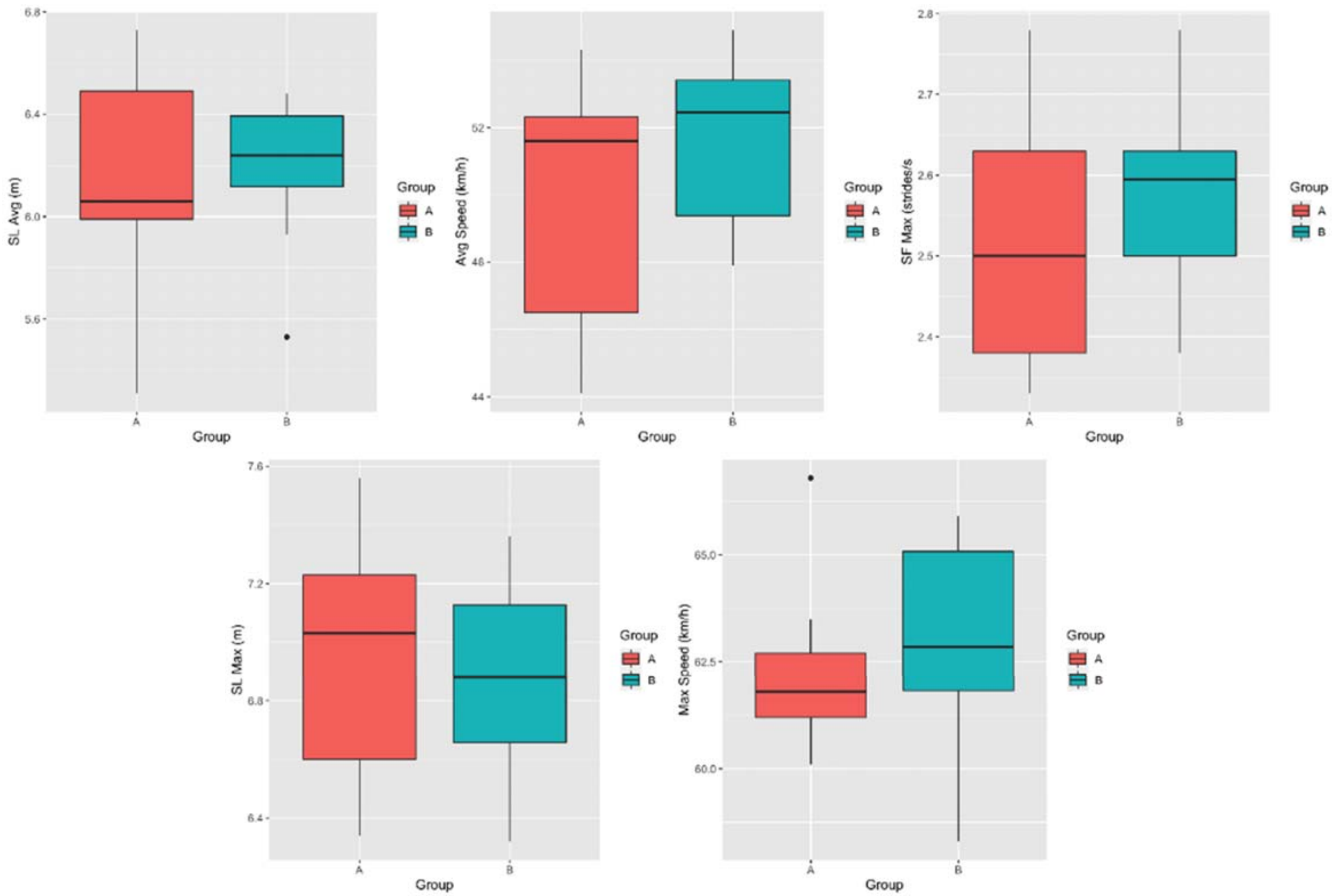

Figure 18: Boxplots showing SL Avg, Avg Speed, SF Max, SL Max, Max Speed for both the treatment Group A and control Group B pre- acupuncture treatments

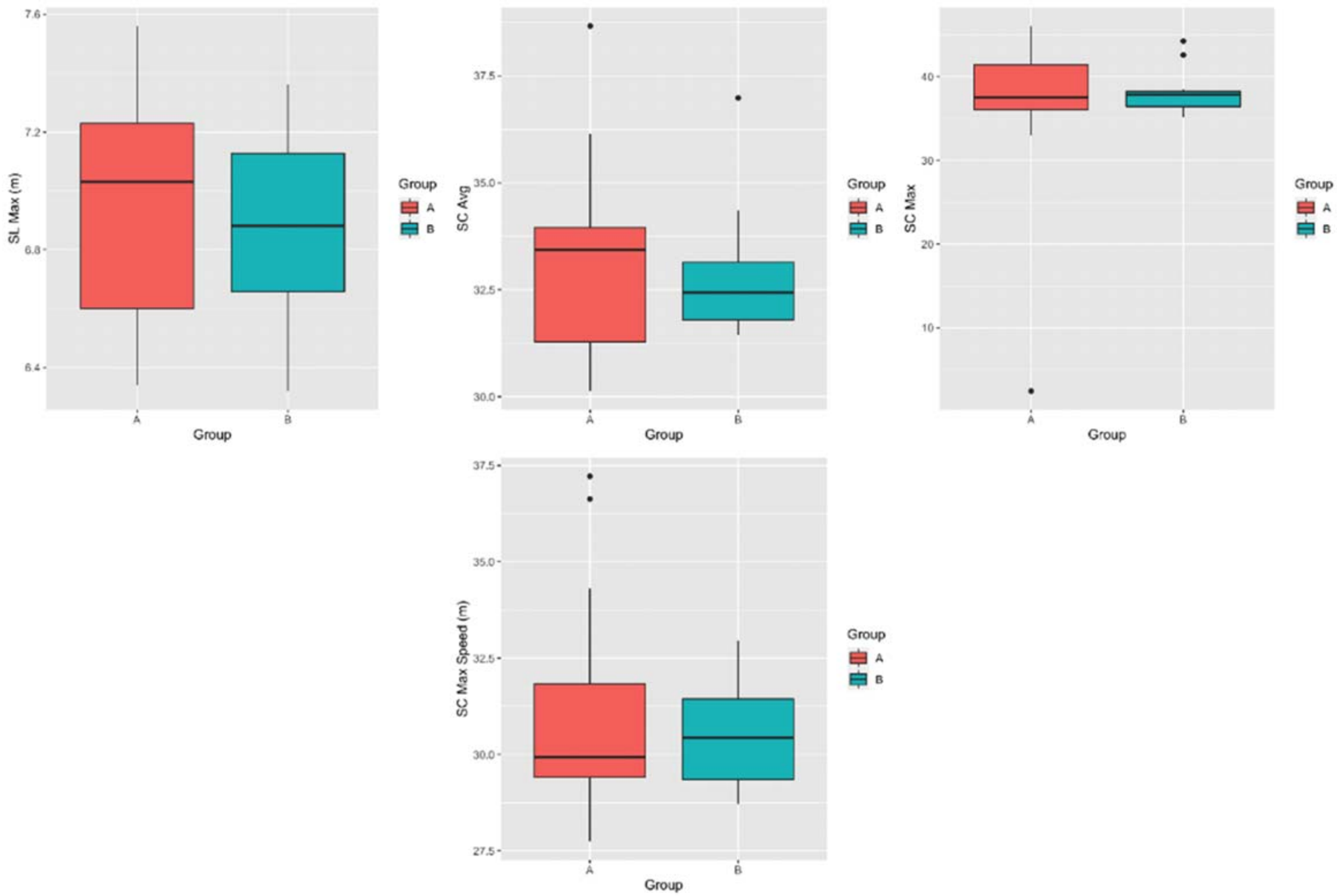

Figure 19: Boxplots showing SL Max, SC Avg, SC Max, SC Max Speed for both the treatment Group A and control Group B pre- acupuncture treatments

Table 12: Descriptive statistics of the stride parameters measured in both Group A and B after three acupuncture sessions

	Combined data (n = 25)	Group A (n = 13)	Group B (n = 12)
Stride Parameters	**Median (iqr)**	**Median (iqr)**	**Median (iqr)**
SL Max (m)	6.98 (6.82, 7.27)	6.98 (6.74, 7.27)	6.96 (6.90, 7.25)
SL AVG (m)	6.17 (5.98, 6.38)	6.11 (5.96, 6.43)	6.25 (6.06, 6.31)
SF Max (strides/sec)	2.56 (2.44, 2.56)	2.50 (2.44, 2.56)	2.56 (2.50, 2.63)
SC Avg	32.89 (31.73, 33.88)	33.3 (31.61, 34.14)	32.47 (32.24, 33.59)
SC Max	37.07 (33.40, 39.10)	33.40 (29.49, 39.04)	37.22 (36.76, 39.44)
Max Speed (km/h)	62.20 (60.80, 63.60)	61.70 (60.80, 62.50)	63.45 (60.67, 64.32)
SC Max Speed	30.54 (29.49, 31.86)	31.45 (29.49, 31.91)	30.26 (29.73, 30.70)
SL Max Speed (m)	6.65 (6.44, 6.93)	6.64 (6.40, 6.93)	6.65 (6.55, 6.90)
Avg Speed (km/h)	50.60 (49.40, 51.50)	49.50 (49.20, 51.40)	51.25 (50.10, 51.55)

Treated Group A = dry needling for 20 minutes at points GB21, ST36, LI10, Bai Hui, Shen Shu, Shen Peng, Shen Jiao, Yan Chi, BL40, GB27, LU1, BL54, SI9; Group B = haltered in stable for 20 minutes; iqr = interquartile range; SL Max (m)= maximum stride length achieved during gallop, SL Avg (m) = average stride length in the gallop , SF Max (strides/sec) = maximum stride frequency in the gallop, SC Avg = average stride count in the gallop, SC Max = maximum stride count during the gallop, Max Speed (km/h) = maximum speed achieved in the gallop, SC Max Speed = stride count achieved at maximum speed, SL Max Speed (m) = stride length at maximum speed, Avg Speed (km/h) = average speed during the gallop

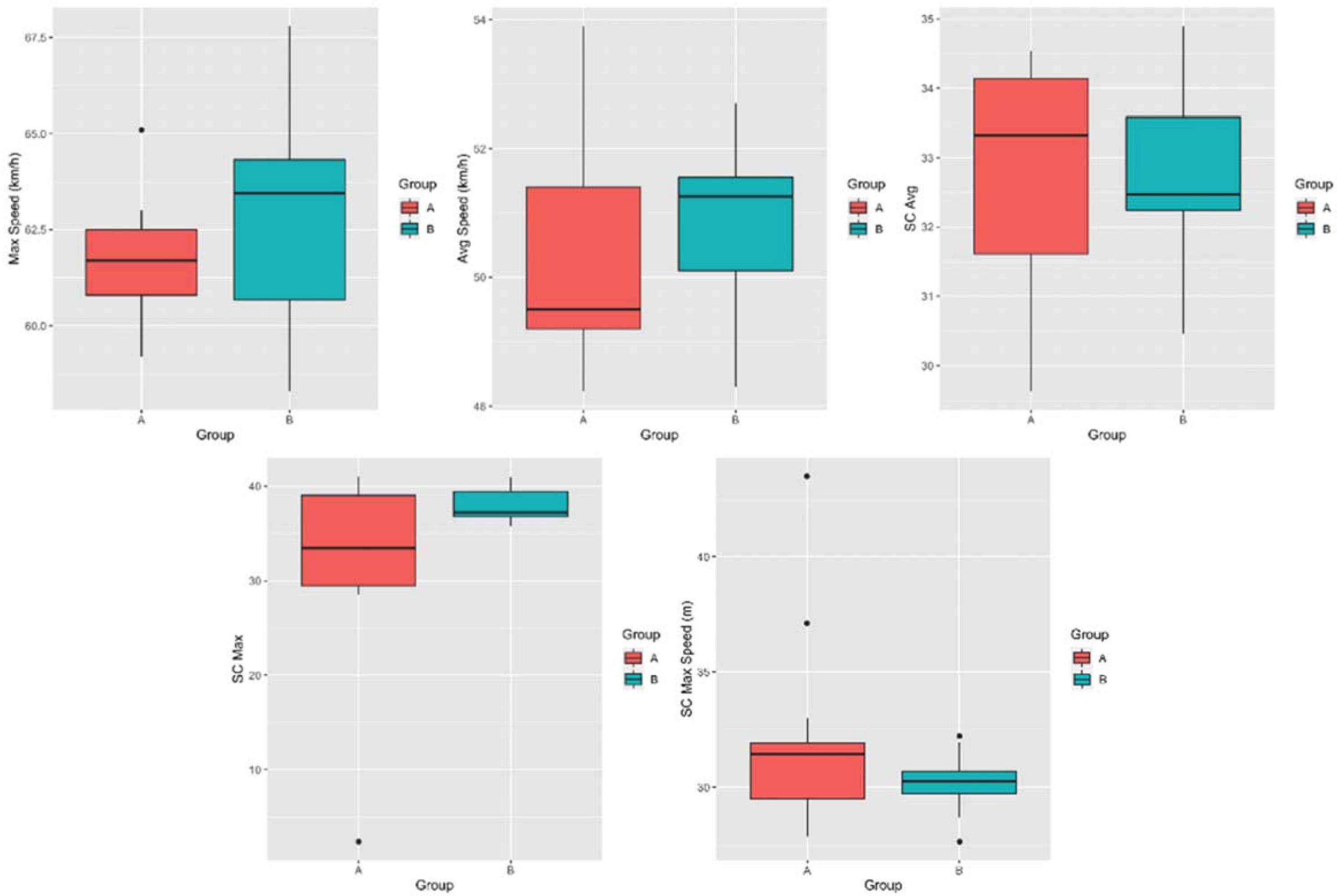

Figure 20: Boxplots showing Max Speed, Avg Speed, SC Avg, SC Max, SC Max Speed for both treatment Group A and control Group B post-acupuncture treatment.

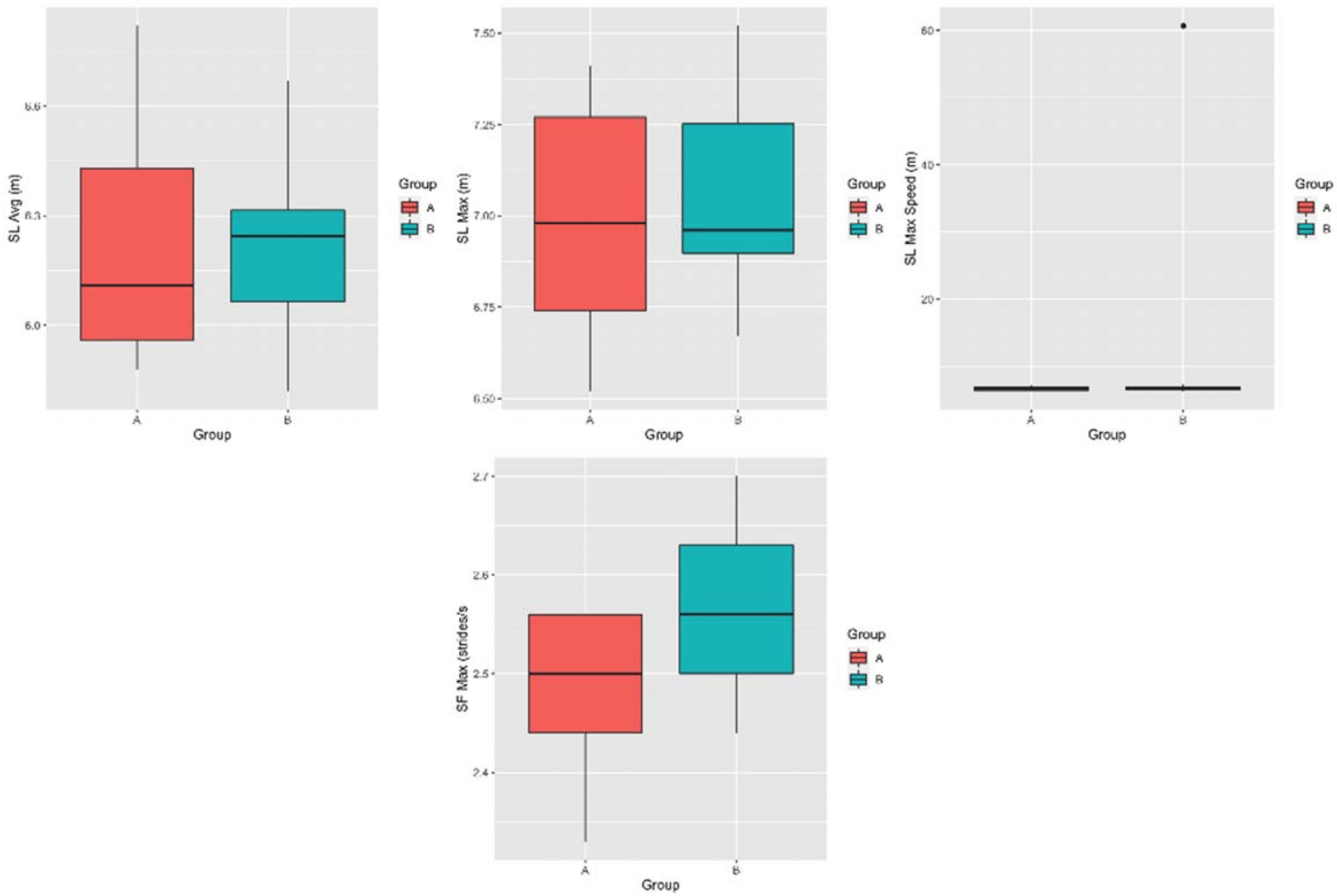

Figure 21: Boxplots showing SL Avg, SL Max, SL Max Speed, SF Max for both treatment Group A and control Group B post - acupuncture treatments

Table 13: Descriptive statistics for stride parameters determined by comparing the delta (difference of pre- and post-treatment measurements) of a treatment group of thoroughbred racehorses subjected to three acupuncture session and a control group

	Combined data (n = 25)	**Group A (n = 13)**	**Group B (n = 12)**	
Stride Parameters	**Median (iqr)**	**Median (iqr)**	**Median (iqr)**	**Independent Mann Whitney U test p-value**
SL Max (m)	0.14 (-0.19, 0.36)	0.10 (-0.42, 0.32)	0.25 (-0.03, 0.36)	0.26
SL Avg (m)	-0.02 (-0.13, 0.28)	0.07 (-0.13, 0.31)	-0.06 (-0.14, 0.13)	0.53
SF Max (strides/sec)	0.00 (-0.11, 0.06)	0.00 (-0.13, 0.06)	-0.03 (-0.07, 0.02)	0.78
SC Avg	-0.07 (-1.84, 0.77)	-0.29 (-2.00, 0.37)	0.31 (-0.50, 0.78)	0.38
SC Max	-1.33 (-3.64, 1.13)	-2.44 (-7.76, -1.33)	0.84 (-1.37, 1.57)	0.07
Max Speed (km/h)	0.10 (-1.80, 1.70)	-1.40 (-1.80, 0.77)	1.30 (-1.88, 1.75)	0.38
SC Max	-0.09 (-0.87, 1.16)	-0.09 (-0.59, 2.17)	-0.23 (-0.93, 0.78)	0.50
SL Max Speed (m)	0.07 (-0.26, 0.24)	-0.03 (-0.41, 0.12)	0.13 (-0.10, 0.25)	0.25
Avg Speed (km/h)	-0.40 (-2.20, 1.40)	-0.10 (-1.50, 3.00)	-0.90 (-2.35, 0.50)	0.46

Independent Mann Whitney U test = Significance set at $p < 0.05$

Treated Group A = dry needling for 20 minutes at points GB21, ST36, LI10, Bai Hui, Shen Shu, Shen Peng, Shen Jiao, Yan Chi, BL40, GB27, LU1, BL54, SI9; Group B = haltered in stable for 20 minutes; iqr = interquartile range; SL Max (m)= maximum stride length achieved during gallop, SL Avg (m) = average stride length in the gallop , SF Max (strides/sec) = maximum stride frequency in the gallop, SC Avg = average stride count in the gallop, SC Max = maximum stride count during the gallop, Max Speed (km/h) = maximum speed achieved in the gallop, SC Max Speed = stride count achieved at maximum speed, SL Max Speed (m) = stride length at maximum speed, Avg Speed (km/h) = average speed during the gallop.

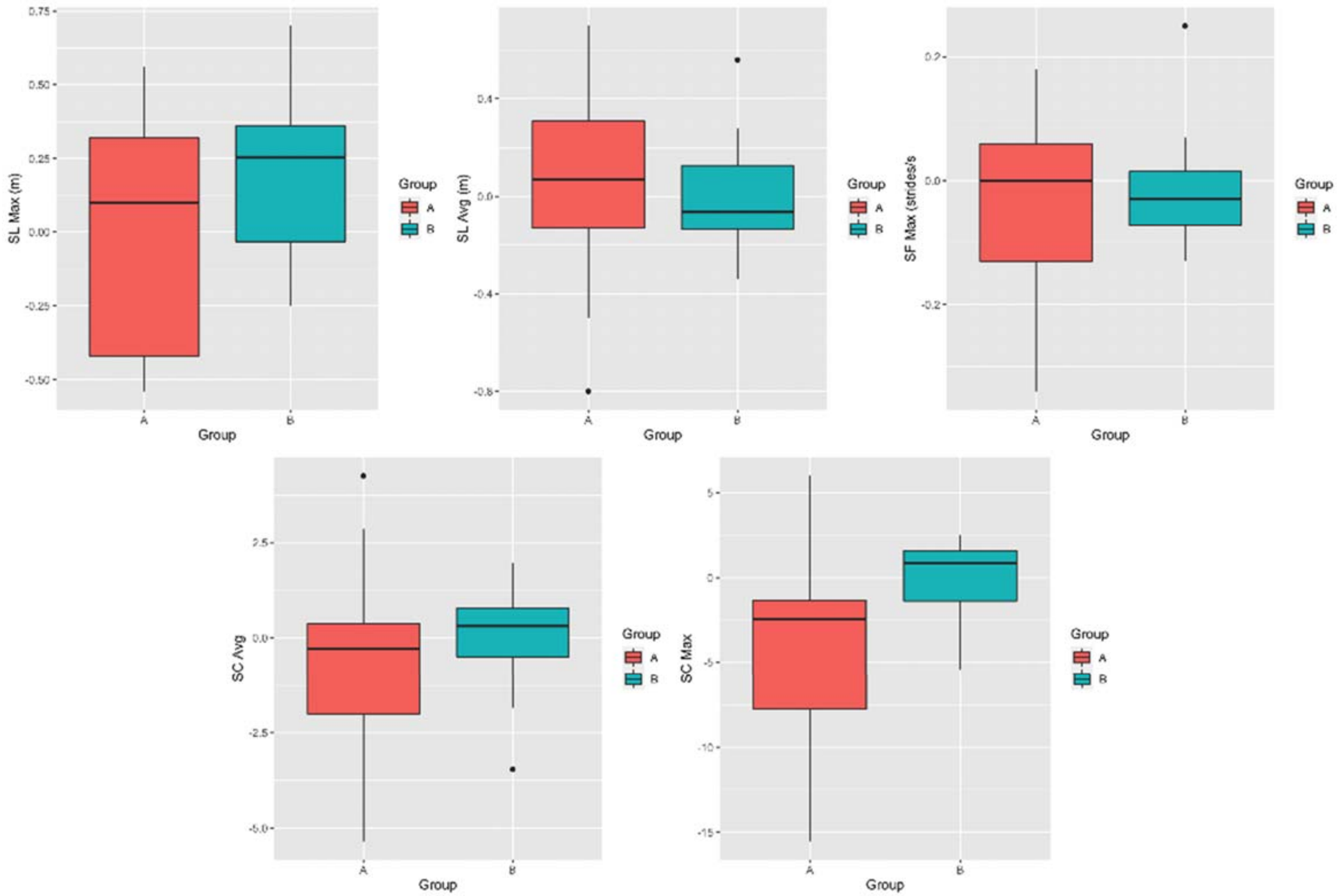

Figure 22: Boxplot results showing SL Max, SL Avg, SF Max, SC Avg, SC Max parameters determined by comparing the delta (difference of pre- and post-treatment measurements) of the treatment Group A of thoroughbred racehorses subjected to three acupuncture sessions and control Group B

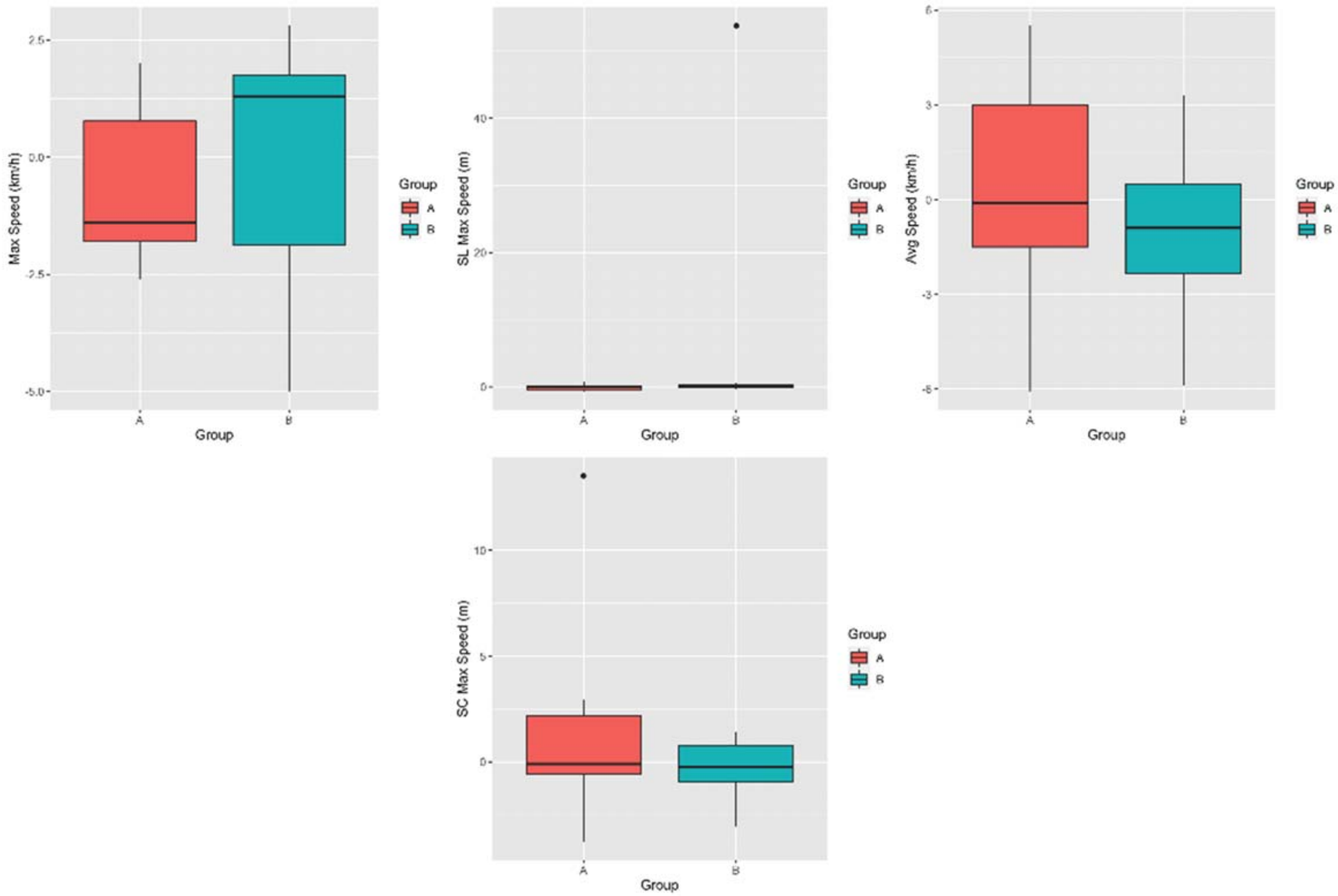

Figure 23: Boxplot results showing stride parameters determined by comparing the delta (difference of pre- and post-treatment measurements) of the treatment Group A of thoroughbred racehorses subjected to three acupuncture sessions and control Group B

4.4 Results excluding the filly data.

As already mentioned, during the randomised allocation of the treatment and control groups all the fillies / mares ended up in the same treatment group (A). This created an unequal distribution of gender in the groups influencing the results. The statistical indicators (median and interquartile range) for the SP were recalculated without the results from the fillies / mares in Group A to allow for a more uniform study group. The treatment group thus included seven geldings and three colts while the non-treatment group included five geldings and five colts.

The results describing the difference (delta) between the groups' comparison pre-and post-treatments is given in Table 14. The boxplots illustrating the changes for each SP is given below the Table (refer to Figure 24-25).

Table 14: Descriptive statistics of the stride parameters measured in a gallop training session determined by comparing the delta (difference of pre- and post-treatment measurements) of treatment Group A without fillies / mares subjected to three acupuncture sessions and control Group B

	Combined data (N = 20)	**Group A (N=8)**	**Group B (N=12)**	
Stride Parameters	**Median (iqr)**	**Median (iqr)**	**Median (iqr)**	**Independent Mann Whitney U test p-value**
SL Max (m)	0.19 (-0.13, 0.36)	0.12 (-0.19, 0.34)	0.25 (-0.03, 0.36)	0.54
SL Avg (m)	-0.02 (-013, 0.23)	0.05 (-0.08, 0.33)	-0.06 (-0.14, 0.13)	0.37
SF Max (strides/sec)	-0.03 (-011, 0.06)	-0.05 (-0.14, 0.08)	-0.03 (-0.07, 0.02)	0.67
SC Avg	0.03 (-1.20, 0.58)	-0.26 (-1.96, 0.04)	0.31 (-0.50, 0.78)	0.24
SC Max	-1.64 (-4.15, 0.97)	-5.70 (-10.24, -2.38)	0.84 (-1.37, 1.57)	0.004*
Max Speed (km/h)	0.25 (-1.85, 1.70)	-0.80 (-1.85, 0.60)	1.30 (-1.88, 1.75)	0.59
SC MaxSpeed	-0.20 (-0.93, 0.88)	-0.20 (-1.24, 2.22)	-0.23 (-0.93, 0.78)	0.68
SL Max Speed (m)	0.08 (-0.19, 0.25)	-0.01 (-0.31, 0.16)	0.13 (-0.10, 0.25)	0.40
Avg Speed (km/h)	-0.25 (-1.52, 2.25)	1.45 (-0.62, 4.95)	-0.90 (-2.35, 0.50)	0.18

Independent Mann Whitney U test = Significance set at $p < 0.05$. *Significant = < 0.05

Treated Group A = dry needling for 20 minutes at points GB21, ST36, LI10, Bai Hui, Shen Shu, Shen Peng, Shen Jiao, Yan Chi, BL40, GB27, LU1, BL54, SI9; Group B = haltered in stable for 20 minutes; iqr = interquartile range; SL Max (m)= maximum stride length achieved during gallop, SL Avg (m) = average stride length in the gallop , SF Max (strides/sec) = maximum stride frequency in the gallop, SC Avg = average stride count in the gallop, SC Max = maximum stride count during the gallop, Max Speed (km/h) = maximum speed achieved in the gallop, SC Max Speed = stride count achieved at maximum speed, SL Max Speed (m) = stride length at maximum speed, Avg Speed (km/h) = average speed during the gallop

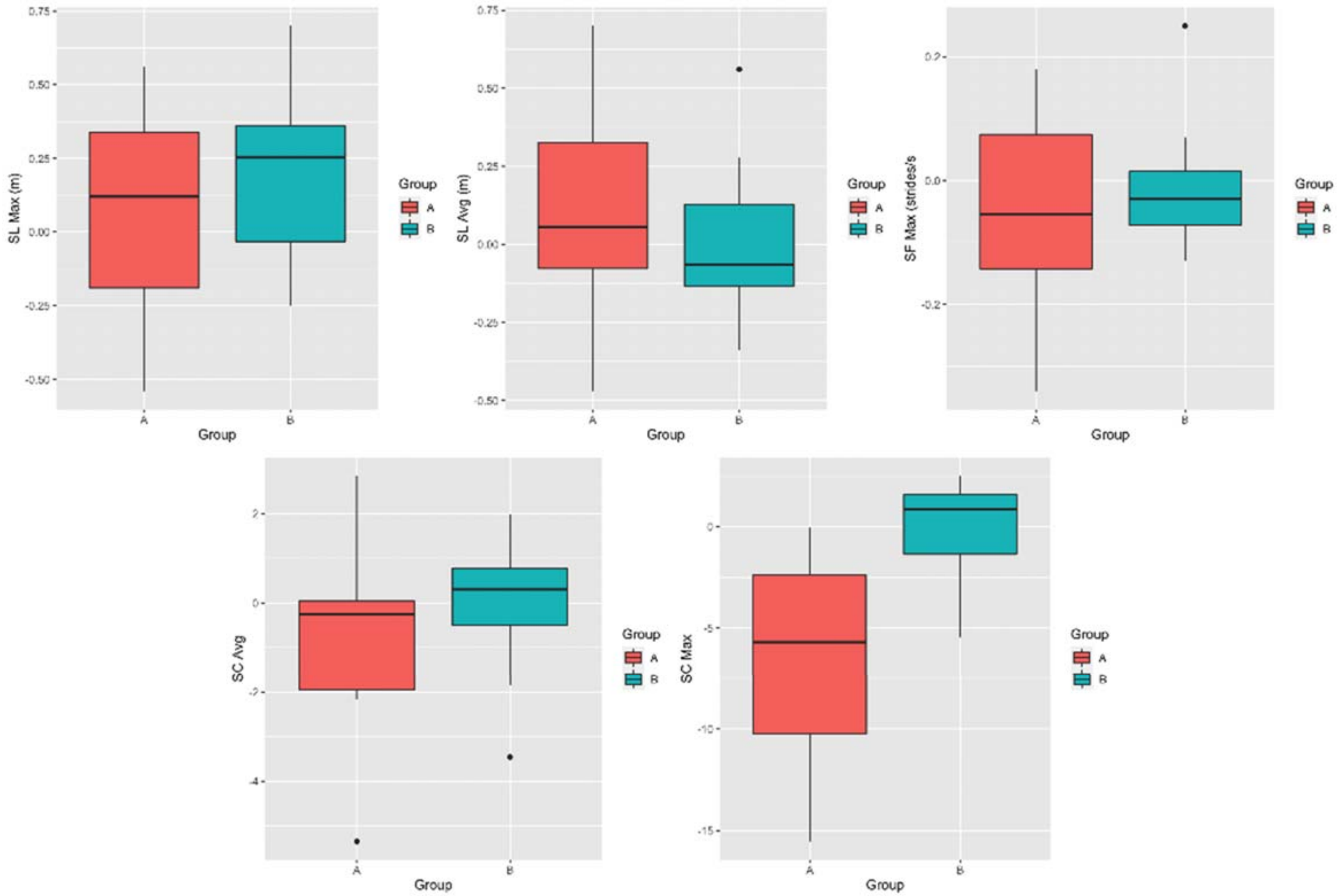

Figure 24: Boxplot results showing the SL Max, SL Avg, SF Max, SC Avg, SC Max parameters determined by comparing the delta (difference of pre- and post-treatment measurements) of treatment Group A without the fillies / mares subjected to three acupuncture sessions and control Group B

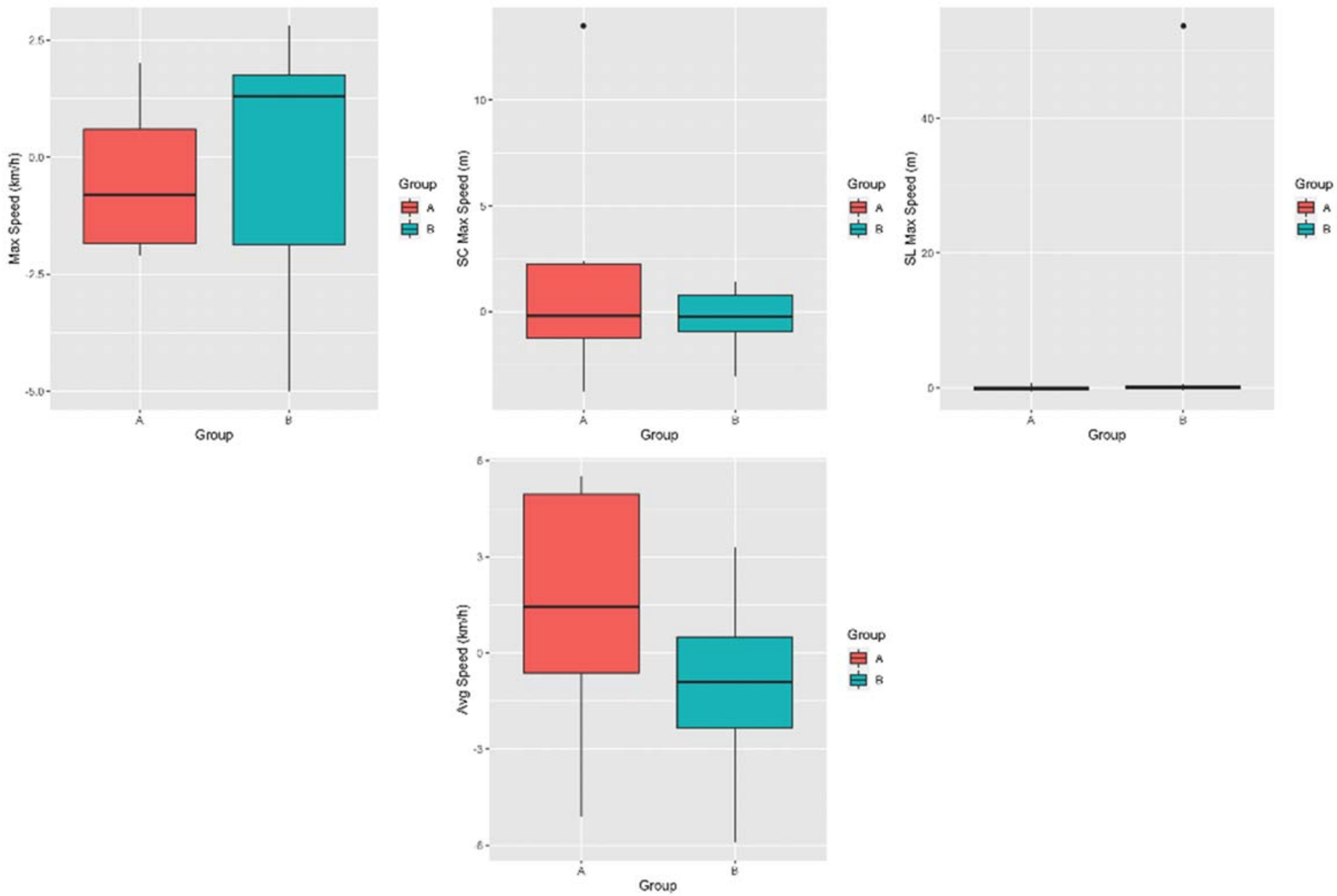

Figure 25: Boxplot results showing the Max Speed, SC Max Speed, SL Max Speed, Avg Speed parameters determined by comparing the delta (difference of pre- and post-treatment measurements) of treatment Group A without the fillies / mares subjected to three acupuncture sessions and control Group B

Table 15: Summary of directional changes (increase or decrease of median), of HRV indicators, heart rate measures and stride parameters following three weeks of acupuncture treatment in Group A, and control Group B (non-treatment) with the fillies' / mare's data included and excluded.

HRV indicators Stride parameter	**Increase (↑) or decrease (↓) of median**			**Comment on changes**
	Treatment group with fillies / mares	**Treatment group without fillies / mares**	**Control (non-treatment)**	
Heart rate measures associated with parasympathetic and sympathetic influence on the heart				
Mean HR	↓	↑	↓	Opposite directions when fillies / mares excluded.
Mean RR	↑	↓	↑	Opposite directions when fillies / mares excluded.
HRV indicators associated with vagal (parasympathetic) cardiac control				
RMSSD	↑	↓	↑	Opposite directions when fillies / mares excluded = decrease in PNS cardiac control in the treatment group.
HF	↓	↓	↑	**Opposite directions*.** Decrease in the PNS cardiac control in the treatment group (with or without fillies / mares).
SD1	↑	↓	↑	Opposite directions when fillies / mares excluded = decrease in PNS cardiac control in the treatment group.
HRV indicators associated with parasympathetic and sympathetic influence on the heart				
SDNN	↑	↑	↓	**Opposite directions*.**
LF	↑	↑	↓	**Opposite directions*.**
SD2	↑	↑	↓	**Opposite directions*.**
Cardiac autonomic balance				
LF/HF	↑	↑	↓	**Opposite directions*.**
HF (norm)	↓	↓	↑	**Opposite directions*.**
LF (norm)	↑	↑	↓	**Opposite directions*.**

HRV indicators Stride parameter	Increase ↑ or decrease ↓ of median			Comment on changes
	Treatment group with fillies / mares	**Treatment group without fillies / mares**	**Control (non-treatment)**	
SL Max	↑	↑	↑	Same directions with or without fillies / mares.
SL Avg	↑	↑	↓	**Opposite directions*.** Increase in the average SL in a gallop in the treatment group.
SF Max	0 (no change)	↓	↓	Same directions (or slight differences) with or without fillies / mares.
SC Avg	↓	↓	↑	**Opposite directions*.** Decrease in the average SC in the treatment group.
SC Max	↓	↓	↑	**Opposite directions*.** Decreased in the Maximum SC in the treatment group.
Max Speed	↓	↓	↑	**Opposite directions*.** Decrease in the Maximum Speed reached during the gallop in the treatment group.
SC Max Speed	↓	↓	↓	Decrease in both groups of SC during maximum speed.
SL Max Speed	↓	↓	↑	**Opposite directions*.** Decrease in the SL during maximum speed in the treatment group.
Avg Speed	↓	↑	↓	Opposite directions when fillies / mares excluded. Increase in the average speed during the gallop (excluding fillies / mares) in the treatment group.

Opposite directions* = median values moving in opposite direction when comparing the non-treatment group with both treatment sub-groups (fillies / mares included or fillies / mares excluded)

Treated group A = dry needling for 20 minutes at points GB21, ST36, LI10, Bai Hui, Shen Shu, Shen Peng, Shen Jiao, Yan Chi, BL40, GB27, LU1, BL54, SI9; Group B = haltered in stable for 20 minutes. ↑↓ = delta median direction (post-treatment median minus pre-treatment median); ↑ = post-treatment median higher than pre-treatment median (increase); ↓ = pre-treatment median higher than post-treatment median (decrease); HRV = heart rate variability; RR = beat-to-beat interval; HR = heart rate; SDNN = standard deviation of RR-interval; RMSSD = root mean square of successive differences in RR intervals; HF = high frequency components; LF = low frequency components; LF/HF = autonomic balance; LF norm = low frequency power normalised units; HF norm = high frequency power normalised units; SD1 = standard deviation of short-term variability; SD2 = standard deviation of long-term variability; ; SL Max (m)= maximum stride length achieved during gallop, SL Avg (m) = average stride length in the gallop , SF Max (strides/sec) = maximum stride frequency in the gallop, SC Avg = average stride count in the gallop, SC Max = maximum stride count during the gallop, Max Speed (km/h) = maximum speed achieved in the gallop, SC Max Speed = stride count achieved at maximum speed, SL Max Speed (m) = stride length at maximum speed, Avg Speed (km/h) = average speed during the gallop

Chapter 5: Discussion

In this study, the effect of acupuncture treatments on HRV indicators and SP were assessed in thoroughbred racehorses subjected to three DN acupuncture treatments over a three-week period. HRV indicators and SP were compared between the treatment and control group (non-treatment group) regarding the delta values (difference in the changes) of pre- and post-treatment. There were no significant changes in any of the HRV indicators or SP when compared with the non-treatment group.

5.1. Heart rate variability

There were no significant changes in any of the HRV indicators when comparing the delta (post-treatment minus pre-treatment) of the two groups. In addition, the indicators associated with the PNS (RMSSD, SD2 and HF) did not show a clear pattern, i.e., the delta median for the non-treatment group showed a shift towards the PNS while the treatment group showed mixed results.

In view of the fact that the randomised allocation of the treatment and control groups resulted in fillies / mares in the treatment group (A), it was decided to recalculate all stats in the treatment group excluding the fillies / mares from the entire study. As gender can influence HRV results (von Borell et al., 2007) and fillies or mares may be more prone to stress during training (Kędzierski et al., 2012; Schmidt et al., 2010) the statistical analysis was also performed by excluding the fillies / mares. This also resulted in more uniform groups i.e., ten horses in each group.

The new statistics completed indicated that, although still not significant ($p < 0.05$), all the pure vagal (parasympathetic) indicators in the treatment group showed a shift towards inhibition of the vagal system after the intervention. Thus, there was less PNS cardiac influence present when measured post-treatment in the acupuncture group compared with increased PNS cardiac influence after three weeks measured in the non-treatment group.

The delta median of the HRV indicators, associated with the combined effect of the PNS and SNS, decreased after three weeks in the non-treatment group and in the treatment group a shift occurred in the opposite direction, namely an increase after the acupuncture treatments (fillies / mares included). Because the pure parasympathetic indicators decreased, these results imply that the sympathetic cardiac component increased with the treatment group.

The HRV indicators associated with cardiac autonomic balance also showed a shift in the opposite direction when comparing the delta median for the non-treatment group and the treatment group.

Finally, the heart measures also showed opposite patterns when comparing the delta median of the non-treatment group and the treatment group especially, when the fillies / mares were excluded from the comparison. It is important to note that although there were no significant differences, the results of the two groups (treatment and non-treatment) moved in opposite directions after a training period of three weeks.

To summarise, the delta median results showed a reciprocal effect after the intervention (compared to no intervention) on the autonomic control of the heart. Contrary to the hypothesis, the treatment group showed inhibition of the PNS post-treatment and activation of the SNS (HRV indicator's association with SNS with a PNS component). This apparent activation of the SNS may in part be due to the inhibition of the PNS. Contrary to the hypothesis that the non-treatment group would show a shift towards SNS (increased stress due to training level), they showed a shift towards PNS. Also, it was expected that SP in the non-treatment group would improve as the training programme progresses unless subclinical injuries existed. The results supported this hypothesis.

This concurs with a study conducted by le Jeune et al. (2014) on the effect of acupuncture on HRV, where a similar decrease in heart rate, decrease in HF and increase in LF/HF were observed over time (comparing three acupuncture treatments). The authors postulated that these changes may have been due to stress, as the horses were haltered. In the present study both groups were handled identically by the same people during the ECG-measurements and the routines during the treatments (except for inserting needles in the non-treatment group) were the same. All the horses were used to being haltered. Thus, the handling procedures could not explain the differences in results. The authors (le Jeune et al., 2014) also hypothesise that acupuncture point selection may have influenced the results and the manipulation of the needles. In the present study, the needles were manipulated (turned), but the point selection may be of relevance. As acupuncture needles are placed in anatomical points with a larger concentration of nerve endings (Shmalberg and Xie, 2011, Slabber, 2021) it is possible that some of these points may have an effect on the sympathetic ANS. Acupuncture studies in humans have shown that the stimulation of different acupuncture points activates different brain activity patterns (Choi et al., 2012).

To limit test variables in the study and standardise the treatment, a set of pre- determined acupuncture points were selected namely GB21, LU1, LI10, SI9, BL23, GB29, Bai Hui, Shen Shu, Shen Jiao, Shen Peng, Yan Chi, BL40, ST36 and BL54 (Table 5). These points were chosen to treat the big muscle groups most often affected by injury in racehorses as well as some points that are thought to have performance enhancement effects (Xie and Preast, 2013). Ideally each horse should be examined, and the trauma or painful area diagnosed, followed by points selection based on the diagnosis. Furthermore, a total of three treatments over three weeks were given, as this is the standard number of treatments given to animal patients in practice. The three treatments may have been inadequate to have a significant effect on HRV and / or the high intra-horse and / or inter-horse variability may have masked the results.

It is important to consider the effect that insertion of needles may have on the ANS response of the horse. None of the horses treated in the study showed any immediate behavioural changes such as aggression towards the acupuncturist, that could be attributed to an aversion or sensitivity to needles. As the sessions were restricted to 20 minutes, the horses would have experienced acute stress, which would be unlikely to cause an effect on the ANS three days later as seen in studies measuring HRV and cortisol as stress indicators, where cortisol levels decrease quickly after an acute stressful event in horses (van Vollenhoven et al., 2017; van Vollenhoven et al. 2018).

5.2. Stride parameters as performance indicators in the thoroughbred racehorse.

There were no significant changes in any of the SP when comparing the delta (post-treatment minus pre-treatment) of the two groups. However, it is important to note that the delta median of the following parameters moved in different directions:

average stride length (SL Avg), average stride count (SC Avg), maximum stride count (SC Max), maximum speed (Max Speed), stride length at maximum speed (SL Max Speed) and average speed (Avg Speed) when comparing the treatment and non-treatment groups. As the SP may differ in fillies compared to colts or geldings (Seder and Vickery, 2003), the fillies / mares were removed from the data set and the stride parameters were statistically analysed again. By excluding the fillies / mares, SF Max and Avg Speed were influenced. Thus, the median delta of the following parameters consistently moved in different directions for the two groups, with or without the removal of the fillies / mares. Of importance is that SC Max (maximum stride count) was marginally significant with the fillies / mares included ($p = 0.07$) and significant ($p = 0.004$) with the fillies / mares excluded. Thus, the non-treatment group significantly increased their stride count during a 600 m gallop after three weeks compared to the acupuncture treatment group with the fillies / mares excluded.

The gallop test was done five days after the last acupuncture treatment and no other changes or variables were reported in the groups. These results may indicate that acupuncture has a potential negative residual effect on the running performance five days post-DN causing a decrease in the number of strides given in a 600 m gallop.

In addition, as the racing performance of the thoroughbred is ultimately determined by the amount of winnings accumulated throughout its career, it is an ongoing challenge for owners and trainers to determine if a racehorse will have a successful career. There are very few parameters that can be objectively measured that will give an accurate indication of the horse's running potential and performance. With the advancement in GPS and accelerometer technology, several devices that measure real time stride parameters, heart rates and respiration have become available and are being used in the training for races (Morrice-West et al., 2020). Still, there seems to be little understanding on how to use the measurements in evaluating the performance of the racehorse as individual horses seem to have different strategies to increase velocity during a race (Morrice-West et al., 2020). This may explain the different results or interpretation of the parameters.

For example, SL is a function of body size and limb flexibility (Heglund and Taylor, 1988) and is negatively correlated with an increase in speed while an increase in

SF resulted in an increase in velocity (Barrey et al.,2001). In contrast, Ratzlaf et al., (1991) argue that in the gallop horse both the SL and SF increase. These inconsistencies may be because SF, SL, respiration and the extension range of the distal phalangeal joints also depend on the velocity (Seder and Vickery, 2003). There is significant inter-horse variation in the SP that it is not correlated with speed (Morrice-West et al., 2020) such as sex, age and conformation that had a bigger influence on the stride parameters. Training alone has very little effect on the SP, with the jockey, track surface and distance raced having a more significant effect on the SP (Fonseca et al., 2010). In addition, SC increases with an increase in muscle fibre reactivity and results in acceleration (Heglund and Taylor, 1988).

Although the interpretation of these parameters is far from clear, the non-treatment group had a decreased average stride length, an increased stride count, increased maximum stride count (significant or marginally significant), an increased maximum speed and an increased stride length at maximum speed. This may indicate that the non-treatment group outperformed the group treated with acupuncture with or without the exclusion of the fillies / mares.

Although the groups were small, the results showed that the treatment group had an increase in the average stride length and the average speed (fillies / mares excluded) in the 600 m gallop. This could be an indicator that acupuncture influences the flexibility and comfort of movement in the gallop, increasing both the average stride length and the average speed. The control group had an even more significant increase in the maximum stride count ($p = 0.004$) in the gallop. Thus, DN treatment decreased maximum stride count, an important finding that warrants further investigation.

During the study period, three horses were eliminated because of injuries in the non-treatment group and one in the treatment group. The horse eliminated from the treatment group received only one acupuncture treatment before injury. Thus, acupuncture could possibly prevent injuries associated with a training programme. This hypothesis should be investigated in a bigger sample population, over a longer period of training focusing on fatigue or overtraining and the potential benefits of acupuncture in preventing these syndromes.

5.3. Overall discussion

The null hypothesis cannot be rejected, i.e., Ho = Acupuncture treatments over a three-week period have no effect on HRV and SP measured before and after treatment in thoroughbred racehorses. There were no significant changes in any of the HRV indicators or SP when comparing the delta (difference in the changes) of pre-and post-treatment of the acupuncture treatment group with the non-treatment group. There was, however, a significant difference regarding the maximum stride count when the fillies / mares were removed from the analysis. The non-treatment group seemed to perform better (SP) and were calmer (PNS activated and possible decrease in SNS activity).

These findings suggest that DN may have a negative effect on the performance of a horse and thus, a withdrawal period may be appropriate. Although, acupuncture did not enhance performance it may play a role in keeping animals healthier during training by improving flexibility, preventing fatigue, or enhancing healing of subclinical injuries. Further research is necessary before a conclusion can be reach on the healing properties of acupuncture during a training program.

Acupuncture may thus have a positive and a detrimental effect on an animal, the same as any veterinary medication (Ernst and White, 2001). Thus, careful consideration regarding the withdrawal period and the methodology used are of utmost importance.

5.4. Limitations

Although the selection of the group allocations was random, all the fillies / mares used in the study were in the treatment group. This caused an uneven representation of sexes between the groups. To rectify this, the statistical analysis was again performed without the fillies' / mare's data set to determine if they unduly influenced the data analysis.

The sample size may not have been adequate to highlight significant changes, as the inter-horse variability (HRV) was large.

The acupuncture points may have influenced the results. Further investigation into the performance stimulating points (ST36, Yan Chi, LI10) is needed to determine if these points have a direct influence on the SNS.

The number of treatments and the three-week monitoring period may have been too short to show significant changes in some of the HRV indicators and SP.

The horses used in this study are experienced racehorses in full training and at the peak of their careers. The SD for SP in fit working horses in training is small and thus does not change significantly unless the horse is sick or injured, causing a major decline in running performance. A short course of acupuncture treatments has not been proven to enhance an already peak SL and SF performance. Other indicators of running performance should be investigated and used in future studies to measure physical well-being and performance.

Chapter 6: Conclusions

This study suggested that although not significant in most cases, three dry-needle acupuncture sessions over three weeks may have an influence on SP and HRV if compared to a non-treatment group. Acupuncture protocols and withdrawal periods should be carefully considered in thoroughbred horses during training and / or when competing in races.

Recommendations

TCVM philosophy relies on disease patterns to individualise treatment protocols. As standardisation is important in research, a “cookbook” approach was followed, i.e., one set of points used for all patients. TCVM practitioners believe that this approach seldom yields optimal results. Clinical studies should also aim to at least record the disease patterns seen and recommend points that could have added benefit.

HRV was used to assess the physiological wellbeing of the horses at rest. Acupuncture did not have any significant effect on these HRV indicators. Three treatments over three weeks may not have been enough to show a long-term effect on HRV. More research needs to be conducted on HRV as an indicator of fatigue and OTS in the equine athlete, as well as the number of acupuncture treatments needed to have a significant influence on HRV in the prevention of fatigue and overtraining. The different outcomes between DN and EA also need to be investigated as it is believed that EA will have a much quicker and better result when used during treatments (Salazar et al, 2017).

As mentioned above, the horses used in this study were experienced racehorses in full training. The SD for SP in fit working horses in training is small and thus does not change significantly unless the horse is sick or injured, causing a major decline in running performance. SL and SF may thus not be appropriate parameters to measure, but the maximum SC did show a marginal increase and should be included in future studies in combination with other indicators of running performance.

The non-treatment group showed marginally improved SP and HRV indicators. Thus, indicating an effect of acupuncture on the treatment group, even though it resulted in poorer outcomes. As post-treatment measurements were taken within three days for HRV (and within five days for the SP) of the last acupuncture treatment, it is possible that acupuncture has a withdrawal period of at least three days and horses should not be raced within this period as it may cause poor racing performance.

More injuries were reported within the non-treatment group during the study, i.e., three horses were eliminated from the non-treatment group vs. one in the treatment group. This is an incidental finding suggesting that acupuncture may have an injury protective effect when done regularly. Studies over a longer period with more participants will be needed to investigate this potential benefit of acupuncture treatments.

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Annexures

1. Allocation of Horses

Table A1 and Table A2 list the horses, their age and comments pertaining to the study and the pilot trial.

Table A1: The list of horse names and age of participants in the study

Horse **Group A**	**Age in years**	**Sex**	**Comments**
Apache Trail	3	Gelding	
Castellano	4	Filly	
Circle of Latitude	6	Mare	
Contrail	4	Colt	
Creation	4	Gelding	
Dynastic Light	4	Filly	
Electra Flying	4	Gelding	
Green Haze	5	Gelding	
Irrevocable Dream	4	Gelding	
Leading Lad	4	Colt	
Marygold	4	Filly	
Ocean Forest	4	Filly	
Pack Leader	6	Gelding	
Skiminac	6	Gelding	
Sumurai Jack	4	Colt	
Duke of Gold	4	Gelding	Eliminated due to injury

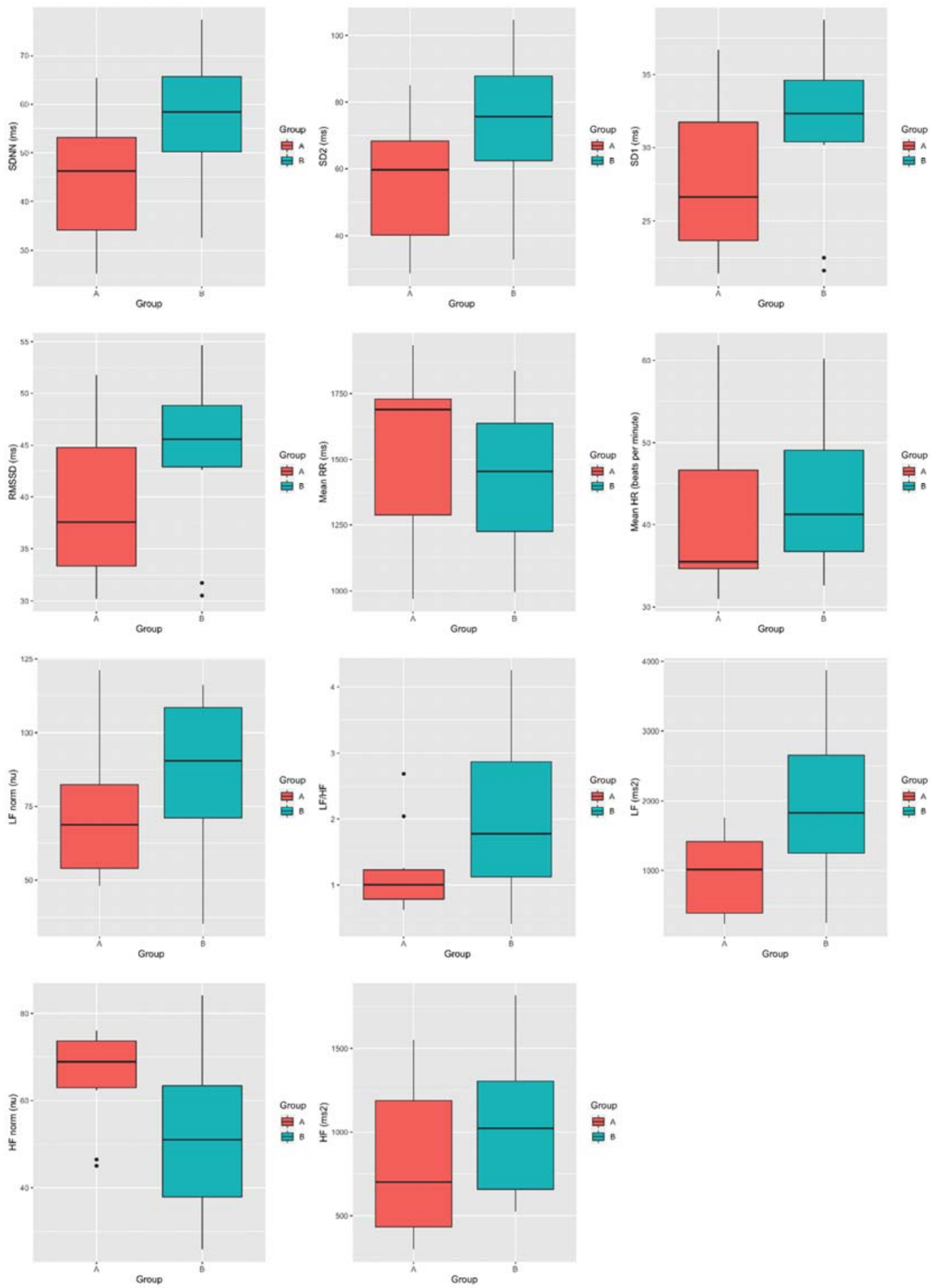
Horse Group B	Age in years	Sex	Comments
Free Wylie	3	Gelding	
Imperial Duke	3	Gelding	
Irish Rain	3	Colt	
Johnny Ripon	3	Colt	
Lear Jet	3	Colt	
Paperless Post	3	Gelding	
Royal Wulf	3	Colt	
Sea Viriscent	3	Colt	
Send of Gold	3	Gelding	
Time to Conquer	3	Gelding	
Only Him	3	Gelding	Eliminated due to injury
Campa	3	Colt	Eliminated due to injury
Countfonic Legacy	3	Colt	Eliminated due to injury
Crackon	3	Gelding	Eliminated due to poor data
Shangani	3	Colt	Eliminated due to poor data

Table A2: Names and age of horses participating in the pilot trial.

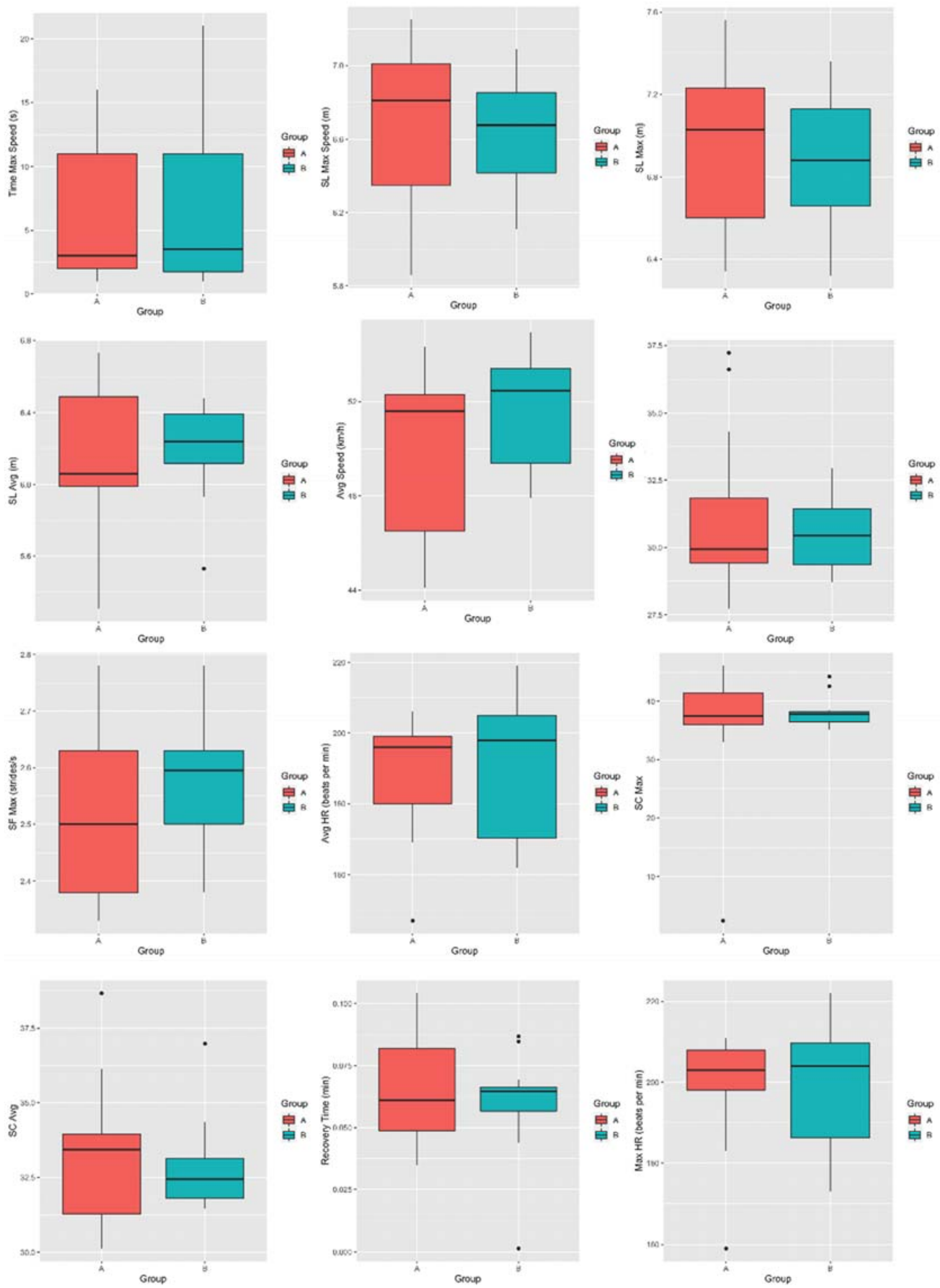
Horse Name	Age in years	Sex
GI Joe	4	Colt
Sumarai Jack	4	Colt
On Broadway	4	Filly
Miss Carusso	4	Filly

2. Boxplots illustrating the data sets pre- and post-treatments without the filly data

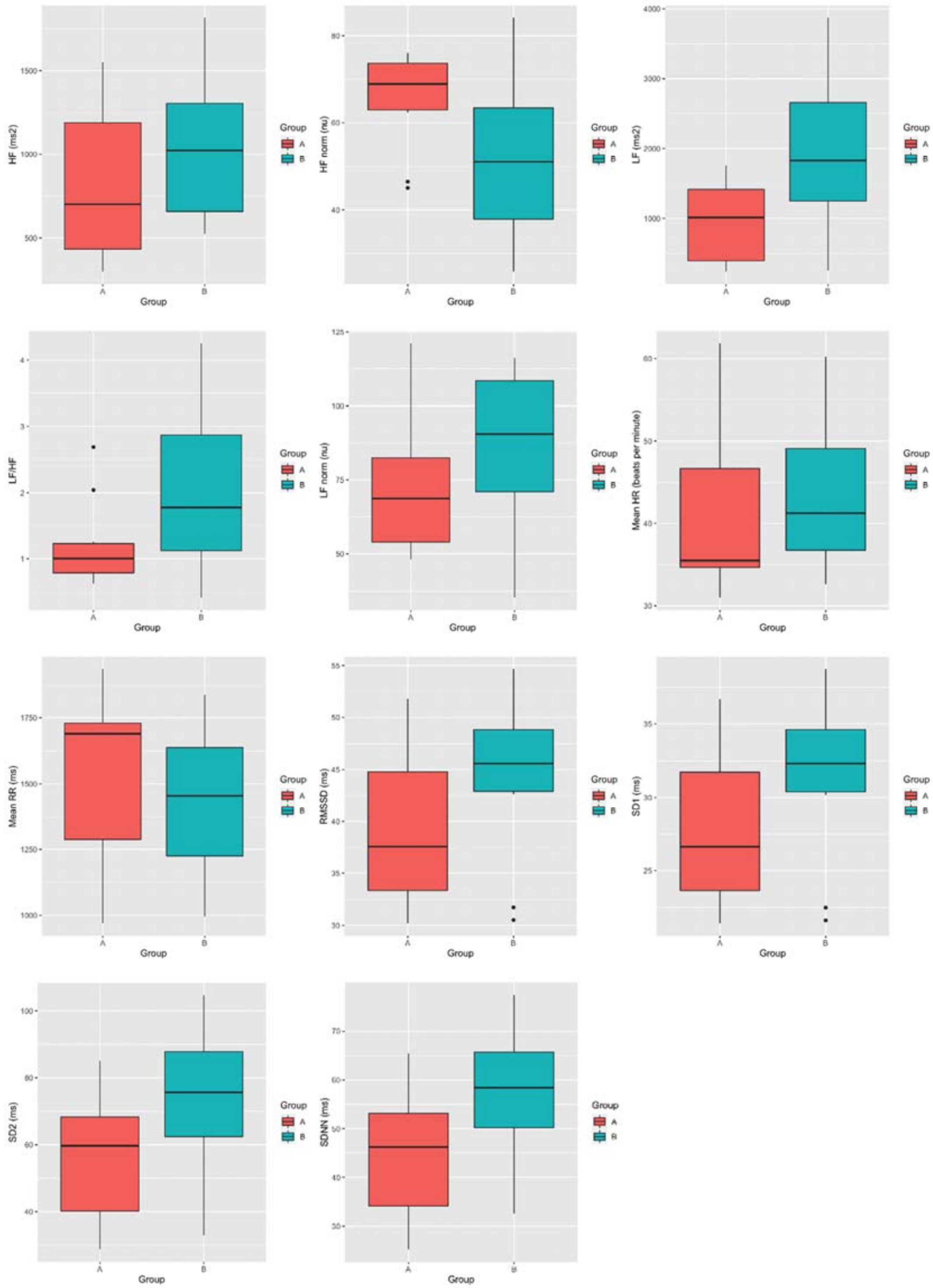
Boxplots indicating the distribution of heart rate measures and HRV indicators for Group A and Group B pre-treatments with data from the fillies / mares removed.



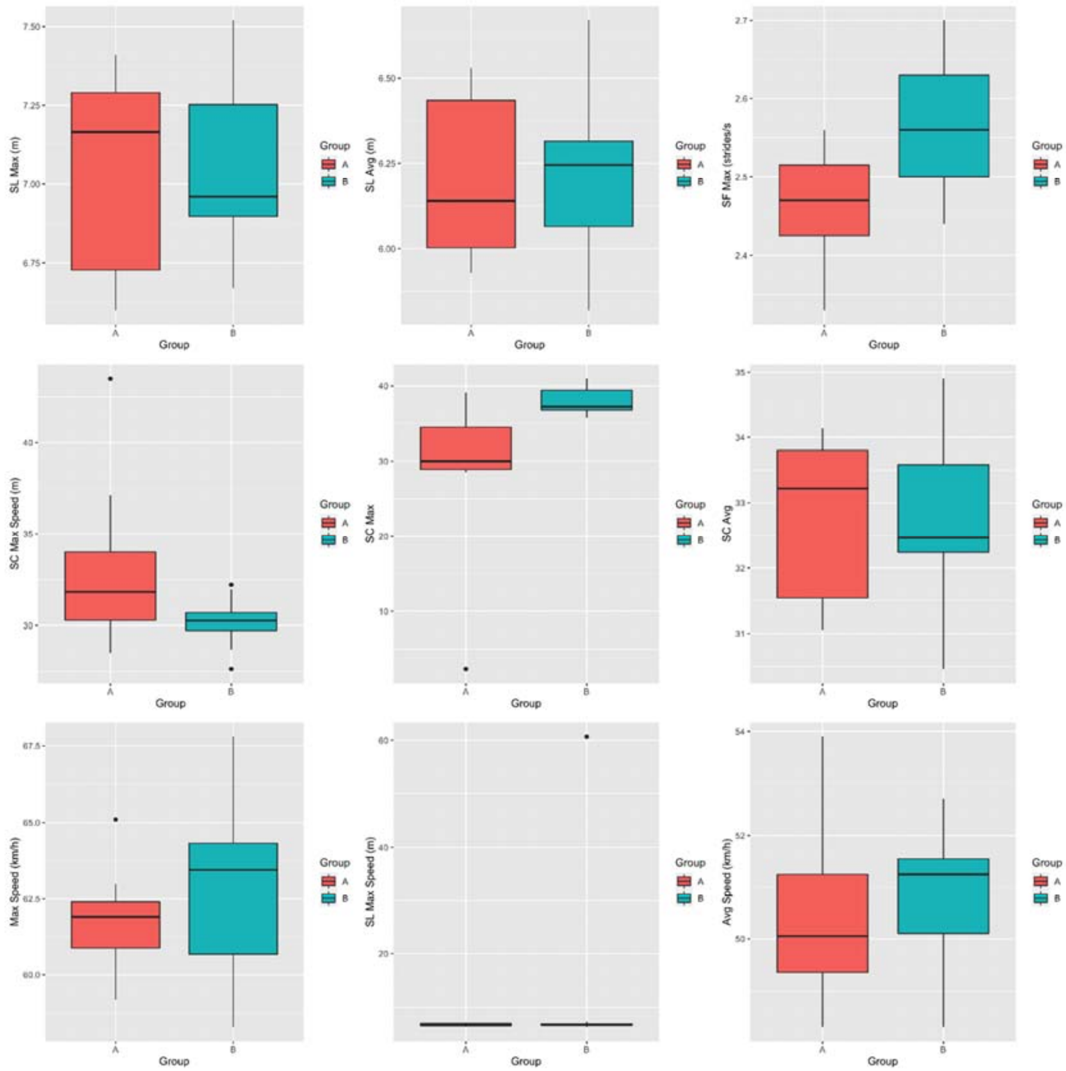
Boxplots indicating the distribution of the stride parameters measured for Group A and Group B pre-treatments with the data from the fillies / mares removed.



Boxplots indicating the distribution of heart rate measures and HRV indicators for Group A and Group B post-treatments with data from fillies / mares removed.



Boxplot indicating the distribution of the stride parameters for Group A and Group B post-treatments with the data from the fillies / mares removed.



3. Ethics committee approval letter

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science

Project Title	The effect of acupuncture treatments in the Thoroughbred racehorse on heart rate variability at rest and stride frequency and stride length during gallop.
Project Number	REC185-19
Researcher / Principal Investigator	Dr R Hartwigsen
DIissertation / Thesis submitted for	Masters

Supervisor	Dr E van Vollenhoven
APPROVED	Date: 2019-09-12
CHAIRMAN: UP Research Ethics Committee	Signature:

Office of the Chairman: Research Ethics Committee
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UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

15
October 2020 Approval Certificate

Annual Renewal (EXT1)

AEC Reference No.: REC185-19
Title: The effect of acupuncture treatments in the Thoroughbred racehorse on heart rate variability at rest and stride frequency and stride length during gallop.
Researcher: Dr R Hartwigsen
Student's Supervisor: Dr E van Vollenhoven

Dear Dr R Hartwigsen,

The **Annual Renewal** as supported by documents received between 2020-09-15 and 2020-10-02 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2020-10-02.

Please note the following about your ethics approval:

1. The use of species is approved:

Species and Samples	Number Available
Horses	34
Electronic measurements	

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2021-10-15.
3. Please remember to use your protocol number (REC185-19) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. As part of your approval, the committee requires that you record a **short video footage** of major animal procedures approved in your study. The committee may request them for monitoring purposes at any later point.

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Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.
Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee