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Heart Rate Variability of Polo Horses in Malta in Different Training Periods

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Table of Contents

List of Abbreviations

HR	Heart Rate
HRV	Heart Rate Variability
CVD	Cardiovascular Disease
ANS	Autonomic Nervous System
SNS	Sympathetic Nervous System
PNS	Parasympathetic Nervous System
ECG	Electrocardiogram
HRM	Heart Rate Monitor
SDNN	Standard Deviation of the Interbeat interval of Normal Sinus Beats
NN50	The Number of adjacent NN intervals that differ from each other by more than 50ms
pNN50	The Percentage of adjacent NN intervals that differ from each other by more than 50ms
RMSSD	Root Mean Square of Successive Differences between normal heartbeats
RR	Mean R to R interval in milliseconds (ms)
SD1	Standard Deviation of the distance of each point from the y=x axis of the Poincaré plot
SD2	Standard Deviation of the distance of each point from the y=x + mean RR interval of the Poincaré plot
IBI	Inter-Beat interval

Introduction

Background

The use of Heart Rate Variability (HRV) in Equine athletes is a rapidly developing field of research. HRV is a non-invasive way of measuring the autonomic regulation of the heart rate. HRV has been a helpful tool in establishing the basis of appropriate welfare and well-being of non-verbal domestic animals. It is believed that with further research changes in HRV can be better understood, with the aim of using HRV as a diagnostic tool for several medical and physiological conditions in the same way as it is currently being used in humans. The understanding of HRV parameters in the horse is still quite limited and further research is still required on how different physiological and environmental factors may have an effect on HRV in the equine species. Although some similarities between human and equine studies have been revealed, there are also several differences that need to be accounted for and thus a lot more information specific to the HRV of equine species is still to be discovered.

Project Motivations

The aim of the study was to assess how HR and HRV parameters respond to an increase in exercise and training over an entire training season. Several studies have investigated the short-term effect of exercise on HRV in horses, but very few assess the long-term effects exercise and fitness have on the horse. Whilst similar studies have been conducted on humans, to our knowledge none have investigated the effect that exercise, training and over all increased fitness may have on Equine Polo athletes.

Project Objectives

The study was conducted in Malta, a small island in the Mediterranean Sea. Thirteen Polo horses were analysed during the study. The study investigated the horses from prior to training for the new season, in August of 2018, to the peak of their season in April 2019. HRV recordings were collected from all the horses at three different time-points; August 2018, January 2019 and April 2019. HR, RR interval and HRV parameters were analysed and data from each time point was compared with the aim to gain a better understanding on how parameters may alter as an effect of increased exercise and fitness. Factors such as gender, age, breed and rider were also analysed and their effect on how HRV is affected by exercise was investigated.

Project Outline

The *literature review* is a collection of findings and conclusions made about the different aspects of HRV. It discusses the most relevant and modern uses of HRV in medicine and sport of both human and domestic animal species. The advantages and drawbacks of the different methods of measuring and analysing the HRV are discussed in detail, with special regard to the adoption of HRV analysis for the equine species. Several different HRV parameters are explored and their relevance to this study were outlined.

The next chapter describes the *methodology* of the study, the protocol used for measuring and analysing the raw data, as well as, a brief explanation of the different parameters chosen to be analysed. The statistical analysis carried out in the study is also explained.

The *results* offer the reader the chance to visualise the findings using tables, line graphs and bar graphs to gain a better understanding regarding the data collected and the results that were drawn from it. Special regards is given to statistical significant finings, however non-significant findings were also included for the sake of completeness, since these could also give valuable information on the effects of exercise on HRV.

The results are then discussed in further detail in the *Discussion* chapter. Here, some conclusions are drawn from the results of the study and these are compared with literature from previous research on the relevant subjects.

The final chapter draws the final *conclusion* of this study and suggests further topics of research on the subject.

Literature Review

2.1. REGULATION OF THE HEART RATE

The heart is intrinsically regulated by different cardiac tissues having pacemaker properties. In addition, the Autonomic Nervous system (ANS) has a major role in regulating the electrical and contractile activity of the myocardium (Sztajzel, 2004). The ANS consists of the Sympathetic and Parasympathetic systems. The Sympathetic Nervous System (SNS) can be associated with the 'fight or flight' concept and thus increases the heart rate. It acts on the heart via the sympathetic trunk and the neurotransmitter acetylcholine. On the other hand, the Parasympathetic Nervous System (PNS) is associated with 'rest and digest' and thus decreases the heart rate. It acts on the heart via the vagus nerve and the neurotransmitter noradrenaline. At rest, the PNS is the dominant acting system (Kuwahara et al, 1996). Both the PNS and SNS influence the ion channel activity involved in the depolarisation of the cardiac pacemaker cells. The lower the rate of depolarisation, the slower the heart rate. The PNS promotes homeostasis and optimal function of the internal viscera, whilst the SNS responds to extrinsic factors challenging the body.

An autonomic imbalance of increased sympathetic activity and reduced parasympathetic activity has been linked to the pathophysiology of the generation of arrhythmias and sudden cardiac death (Sztajzel, 2004).

2.2. HEART RATE VARIABILITY

Heart Rate Variability (HRV) is a simple, non-invasive technique to measure the autonomic nervous system (Sztajzel, 2004). HRV is the change in time intervals between consecutive heart beats, known as RR intervals or interbeat intervals (IBIs) (Shaffer and Ginsberg, 2017). RR intervals can also be defined as the intervals between QRS complexes seen on an ECG. Even if the heart rate (number of beats per minute) is constant, there is always some degree of difference in RR intervals between each beat (Kinnunen et al, 2006). HRV is an emergent property of interdependent regulatory systems that operates on different time scales in order to help us adapt to environmental and psychological challenges (McCraty and Shaffer, 2015). A number of different physiological systems play a role in regulating heart function. HRV can therefore be described as a measure of neurocardiac function, taking into account both the heart-brain interactions and autonomic nervous system dynamics (McCraty and Shaffer, 2015).

HRV can be influenced by gender, age, circadian rhythm, respiration, body position and health status. It has been found that following moderate physical activity, autonomic regulation is improved (Kinnunen, 2006).

2.3. HEART RATE VARIABILITY IN MEDICINE AND SPORT

HRV has been mainly used in human medicine as an indicator of health. There is a link between cardiovascular disease (CVD) and cardiac autonomic neuropathy. The use of HR and HRV can be used to give prognostic information in clinical CVD patients. HR determines cardiovascular mortality risk, whilst HRV may be used as a predictor of disease outcome (Grant et al., 2014). It has also been linked to diabetic neuropathy (Risk et al., 2001), myocardial infarction and congestive heart failure (Sztajzel, 2004). Furthermore, HRV is being used as a biomarker to determine the positive or negative effects of certain foods on the body, without having to wait years for the effects of diet to be visible on health. HRV seems to decrease with the development of diabetes, cardiovascular disease, inflammation, obesity and psychiatric disorders (Young and Benton, 2018). Young and Benton described how high intakes of saturated or trans-fat and high glycemic carbohydrates reduce the HRV. Inflammation causes the liver to produce C-reactive protein, which has an inverse relationship with HRV.

Nowadays, its popularity amongst athletes is increasing. Several studies have been carried out on human athletes to determine their fitness level, recovery status and to investigate ways to help with avoiding over-reaching and over-training. Athletes tend to have a lower resting heart rate, this could partly be due to a stronger vagal tone that comes from endurance training. Athletic training is based on gradually increasing the workload in order to improve the athletes' performance by continuously exceeding their previous workload. This is considered as a positive stressor. However, if the workload is too high or the training is too frequent, exhaustion can result, leading to over conditioning syndrome. This will in turn cause an imbalance of the ANS and thus cause changes in HRV (Aubert et al. 2003). HRV has also been used in human medicine as well as in farm and companion animal research to evaluate physical and psychological stress (Pohlin et al., 2017).

A few recent studies have been carried out on equine athletes in order to establish better suited training methods and avoid overtraining. Although it is a fairly new area in equine sport, it's popularity is increasing. It has been concluded that HRV of elite horses is significantly higher than the HRV of non-elite horses (Kinnunen et al., 2006). Kinnunen et al. also found that intense exercise decreased the HRV of trotters during recovery. It was observed that the HRV was highest when the horses were in better physical fitness, more regularly trained and were allowed more time to recover after a bout of exercise. HRV was lowest during the intense competition period, the most stressful part of the whole training period. Intense exercise increases the sympathetic tone and thus decreases the HRV of the trotters during the recovery period. A decrease in HRV despite an increase in HR may be an early sign of overreaching, which may lead to over-conditioning syndrome in human athletes (Kinunen et al, 2006). One can conclude that during the recovery phase following an intense training session, the HRV will be lowered, but following endurance training for a longer period of time, which thus increases the fitness of an athlete, HRV will be higher at rest.

However, in a study conducted by Lorello et al. (2017), competing eventing horses were found to have an overall higher resting HR and lower HRV values than non-competing controls. Lorello et al. hypothesised that these results could be due to increased stresses of being a competition horse, and thus increased the sympathetic tone of the animals. These results may also be due to the fact that eventing horses are more closely related to thoroughbreds than other warmblood sport horses, and it was previously found that HRV variables of racehorses may also decrease as training intensifies during the peak of their competition period (Kinnunen et al. 2006). The stressors involved in the training of sport horses increases the sympathetic tone, decreases the parasympathetic tone, or both, and thus reduces the HRV variables, however HRV variables increase again soon after the rider mounts and the horse starts moving and lowers it's heart rate; thus indicating that it is mostly the mounting of the rider that acts as a stressor for the young horses. Salivary cortisol levels were also monitored and a significant increase was also seen during the initial training of young sport horses (Schmidt et al., 2010).

Seasonality and outdoor temperature and humidity can also be a form of stress for horses, and in turn manipulate the HRV results. RMSSD (one of the HRV variables denoting parasympathetic activity) and HR were both lower in winter than in summer, however this could be a result of the limited resources available to the free-ranging Przewalski horses during the winter, rather than a direct effect of environmental temperature (Pohlin et al., 2017). It was determined that HRV is negatively affected by ambient temperature, and that horses are thought to be more sensitive to heat-stress than to cold-stress (Pohlin et al., 2017). Pohlin et al. found that the effect of ambient temperature on HRV was quadratic, and the minimum RMSSD was found at a temperature of around 18°C, any deviation from this increased the HRV. A rise in ambient temperature is believed to increase the deviation of IBIs (Shin, 2015). It is also evident that horses are calmer whilst being able to move than at rest, HRV at walk were found to be higher than at rest (Kinnunen et al., 2006; Pohlin et al., 2017).

Younes et al. conducted a study in 2016 whereby they compared several endurance horses aged 4 to 6 years old on four different training sessions, in order to establish means by which endurance horses could be selected for faster cardiac recovery by means of HRV analysis. Significant differences in RMSSD indicates that age and duration of exercise decrease the amplitude of HRV. Higher RMSSD led to a quicker recovery, and thus can be used to hand-pick the better endurance horses.

Test site was also found to be an influential factor of HRV analysis, therefore the tests for all horses should be performed on the same day and at the same place to obtain the most reliable results (Younes et al., 2016).

2.4. MEASURING HEART RATE VARIABILITY

As mentioned earlier, HRV is a measure of the changes of RR intervals or interbeat intervals. The most popular ways of measuring the HRV are by using an ECG or a heart rate monitor which continuously records the consecutive RR intervals. The ECG is still the gold-standard method for measuring HRV accurately and reliably, however, Polar heart monitors can be used in specific studies after artefact corrections.

The ECG, or electrocardiogram, works by recording the electrical activity of the heart and maps a graph of P waves, QRS complexes and T waves. This is then analysed using computer software to measure the RR intervals and determine the HRV variables based on the changes between each R wave. Heart rate monitors, such as the various models using the Polar system (Polar® Electro Oy, Finland) on the other hand do not record the entire ECG, but just the RR intervals for the whole duration of the recording. These recordings should then be analysed by Polar computer software and necessary corrections can be made by applying filters to clear any artefacts. Artefacts may occur as a result of ectopic or non-ectopic origin, such as poor conductance between the skin and the electrode, technical problems, noise from muscle movement, electrical interference, first and second-degree AV blocks, premature beats and cardiac arrhytmias. The corrected recording is then imported into an HRV analysis software such as Kubios (HRV software, Kubios Oy, Finland) and the HRV variables are extracted and calculated.

The advantage with ECG is that one can view the entire electrical signal of the heart, and thus notice any abnormal electrical conductivity such as premature beats, ectopic beats, AV-blocks and arrhythmias. This abnormal activity can then be manually edited out of the recording, so as to obtain more accurate HRV measurements. The disadvantage however is that ECGs can be rather expensive equipment and are not the most practical method to obtain recordings in an ambulatory setting. A recent study in pigs revealed that Polar recordings showed specific, measurement-related errors that were determined to be of non-ectopic origin based on the fact that none of these errors were present in the traditional ECG recordings (von Borrell et al., 2007). These errors were classified into 5 types. Type 1 errors were described as single-point discrepancies between ECG and Polar IBI recordings. Types 2 and 3 were identified as a long IBI followed by a short IBI and a short IBI followed by a compensatory long IBI, respectively. Type 4 errors were identified to be large peaks in the place of multiple IBIs, whilst Type 5 errors were characterised as two or more IBIs instead of one single IBI. These 5 types of errors were also found in IBI recordings of other animals, including horses. In addition to this, the equine species are known to have a more dominant resting parasympathetic effect which makes ectopic beats a common finding in horses (von Borrell et al., 2007). One unique feature of the Equine ECG is the pronounced T-wave, which may be misread as an additional R-peak by RR detectors such as the Polar HRMs (Parker et al., 2010). Fortunately, most of these errors can be successfully corrected using automatic algorithms available on HRV analysis software (von Borrell et al., 2007). Despite the HRMs not being able to detect any abnormalities in the heart's electrical activity, the use of Polar heart rate monitors in clinical studies is rising, particularly due to them being much less expensive and more portable than ECGs.

In a human study conducted to test the validity of a Polar V800 HRM, it was established that the Polar V800 HRM can in fact produce RR interval recordings that are comparable to that of an ECG (Giles et al., 2016). Another study evaluated the two-week test-retest reliability of the Polar RS800CX HRM which indicated a moderate reliability, parallel to previous studies that validated the use of HRMs to measure and analyse HRV. They further concluded that it can also be considered a reliable way of measuring HRV, especially when using the auto-regressive transformation method (Williams et al., 2016).

Ille et al. carried out a study comparing HR and HRV parameters obtained from HRMs and traditional ECG recording on non-exercising horses. The study revealed that corrected Polar IBI data and corrected ECG data not only correlated, but were also considered interchangeable when the recordings were taken while the horses were in their stalls (Ille et al., 2014). A similar study in 2010 concluded that the relation between data collected by HRM and that collected by ECG decreased as movement of the horses increased (Parker et al., 2010). In 2017, a study by Lenoir et al. compared the results obtained from an ECG to those obtained from an HRM from horses during exercise. It was concluded that although mean HR and RR intervals were in agreement, all other measures of HRV differed. The study displayed how their agreement decreases as the speed of the exercise increases. Thus, concluding that although previous studies conducted on horses at rest resulted in nearly identical HR, RR interval and other HRV measures between ECG and HRM recordings, this is not the case when the HRV is being evaluated during exercise. This could be due to the fact that as the horse starts to move faster, the number of artefacts on the recording increases drastically (Lenoir et al., 2017).

The interchangeability of ECG with HRMs is therefore dependent on the nature of the study, the subjects to be used, the data to be analysed and the environment that the data will be recorded in. In studies involving horses at rest or stationary, HRMs and ECG can be used interchangeably, since the number of artefacts while a horse is standing at rest are very low and will not interfere with the results. On the other hand, during exercise or competition, an ECG is better adapted and preferred over HRMs. However, if the study only requires raw HR and RR interval measurements, then both ECG and HRMs can be used, since these were found to be significantly comparable both at rest and during exercise. In any case, HRM data can only be used successfully after having carefully selected and applied the most appropriate artefact filter specific for the data that has been collected in each individual study and having examined all the data and very carefully selecting the best possible segment to be analysed.

2.5. ANALYSIS OF HEART RATE VARIABILITY

After collection of data using either ECG or HRM, the data should be analysed by a dedicated software to extract the HRV variables. The software used in this study was Kubios® HRV standard, which takes the raw data and computes all the RR intervals in the selection made. There are two main methods of analysing HRV data. The linear metrics including time-domain analysis and frequency-domain analysis, and non-linear metrics such as the Poincare plot (Shaffer and Ginsberg, 2017). Each has their own advantages and drawbacks. Time-domain indices give the amount of HRV observed during monitoring periods, whereas frequency-domain variables give us the absolute or relative amounts of signal energy within the set component bands. Non-linear metrics analyse the unpredictability and complexity of a recording of RR intervals (Shaffer and Ginsberg, 2017). Parameters obtained from non-linear analysis were found to be indicative of changes in sympatho-vagal balance. HRV can be influenced by several different factors simultaneously. These feedback and feed-forward systems, together with further effects can elicit non-linear chaotic behaviour, a key feature of elaborate dynamic systems (von Borrell et al., 2017).

HRV recordings can be 24 hours long, short-term; meaning around 5 minutes of recording, or ultra-short term; which is less than 5 minutes of recorded data. Longer measurements give a much better representation of slower fluctuating processes, such as the cardiovascular system's response to external variables, hence these cannot be replaced by short or ultra-short recordings. The advantage of short-term recordings is that they are fairly simple to record, and thus, when 24-hour long recordings are not feasible they can help evaluate physiological changes. Short-term recordings are generated by two over-lapping processes, the complex relationship between sympathetic and parasympathetic branches and regulatory mechanisms such as the bare receptor reflex that control the heart rate (Shaffer and Ginsberg, 2017).

Time-domain indices include mean NN interval, SDNN, RMSSD, NN50, pNN50, and HRV triangular index. These are more easy to interpret than frequency-domain indices, however they result in less specific values for the parasympathetic and sympathetic activities of the nervous system. It is important that values such as SDNN are all obtained from measurements of the same length, as the total variance of HRV increases with longer duration of recording, both 5 minute recordings and 24-hour long term recordings seem to be appropriate options to measure SDNN (Electrophysiology, 1996). While SDNN gives an estimate of overall HRV, RMSSD provides an estimate of the short-term components of HRV. It describes vagal effect similarly to HF in the frequency-domain analysis, but with less respiratory component. NN50 and pNN50 are both used to assess beat-to-beat variation. NN50 represents the number of adjacent NN intervals that differ from each other by more than 50ms, whilst pNN50 indicates the percentage of adjacent NN intervals that differ from each other by more than 50ms. Both parameters are highly correlated to RMSSD and can therefore also be representative of the vagal activity (von Borrell et al., 2007). RMSSD is preferred over NN50 and pNN50 because it has better statistical properties (Electrophysiology, 1996).

The interpretation of frequency-domain indices is more complex, however they offer a more specific view of the overall sympathetic-parasympathetic balance. Ideally short-term recordings without any ectopic beats, arrhythmias, missing data or noise effects should be used for frequency-domain interpretation (Electrophysiology, 1996). Frequency-domain indices are much more sensitive to artefacts, and this results in the total power and HF power components to be over-estimated, whilst LF power components tend to be underestimated (Stucke et al., 2015). Frequency-domain indices include ULF (ultra-low-frequency) Band, VLF (very-low-frequency) Band, LF (low-frequency) Band, HF (high frequency) Band, LF/HF ratio. LF fluctuations mainly reflect the baroreceptor activity in resting conditions. LF power is said to be produced by both SNS and PNS, and baroreflex regulation of the blood pressure, or even by the bar reflex activity alone. The SNS is not believed to produce rhythms higher than 0.1 Hz, whilst the PNS is thought to produce rhythms as low as 0.05 Hz. Furthermore, at rest, LF band reflects baroreflex activity rather than cardiac sympathetic innervation. The HF fluctuations are brought about by parasympathetic nervous system activity, and corresponds with the heart rate variations related to the respiratory cycle. (Shaffer and Ginsberg, 2017). ULF and VLF fluctuations are a result of posture and the hormonal control, respectively (Marr and Bowen, 2010).

It is important to correct any excessive variation between beats caused by mis-detected prematurity or movement artefact, because "HRV is used to assess fluctuations as a result of autonomic control, not due to abnormal conduction within the myocardium (dysrhythmia). Since physiological changes in heart rate are not sudden, acute changes are likely to represent artefact, pre-mature complexes, prolonged pauses and second-degree atrioventricular block. However, filtering all episodes of second-degree AV-blocks from an equine ECG recording will remove important information about autonomic control of the equine heart since second-degree AV block is brought about by similar mechanisms that induce respiratory sinus arrhythmia in other species; vagal outflow results in blocked conduction through the AV node, rather than slowing discharge from the sinoatrial node. Although removing second degree AV-blocks from HRV calculations will remove large amounts of information, including them would result in a greater amount of heterogeneity of HRV in normal animals. The author recommends that time-based filters are set to remove intervals that are less than 65% or more than 175% of preceding intervals" in order to remove dysrhythmias including physiological second degree AV-blocks (Marr and Bowen, 2010).

In the case of short-term recordings, frequency-domain measures are preferred over time-domain methods, and 5-minute recordings are an ideal length as a standard for studies (Electrophysiology, 2016). However, SDNN and RMSSD values, amongst other time-domain methods are also possible for investigating short-term recordings, but frequency-domain method would offer more interpretable results on physiological regulations (Electrophysiology, 1996).

Horses have lower resting HR than other domestic species and thus, a longer recording time might increase the accuracy of HR and HRV analysis in the horse. In fact, correlation between data obtained using HRM and data collected by ECG was improved as length of recording increased, but a minimum recording length of 2 minutes is always required (Ille et al., 2014). 5 minute recordings are recommended for standardisation reasons, since research showed that analysing 5 minute segments gave similar, or better results than analysing 24 hours long recordings (von Borrell et al., 2007).

Non-linear measurements may give valuable information about the physiological interpretation of HRV and for the risk assessment of sudden death (Electrophysiology, 1996). These are represented as the Poincaré plot, made up of S, SD1, SD2 and SD1/SD2 ratio. This is essentially a scatter plot of each RR interval plotted against the prior interval. It allows for the visual search for pattern hidden within a time series.

When working with HRV, it is important to record the context of the readings, such as the gender, age, physical training of the individuals, as well as the duration of the recordings and whether the readings were taken during exercise or at rest, indoors or outdoors, etc. (Shaffer and Ginsberg, 2017), since all the above mentioned factors can influence the HRV values obtained. Physiological rhythm irregularities, which are induced by increased vagal activity in order to control atrial pressure, are present in a vast majority of healthy horses at rest and may compromise the HRV data. These irregularities may include sinus arrhythmias, sinus bradycardia, first and second degree atrio-ventricular (AV) block and Sino-atrial block.

The necessary data corrections can be done by applying automatic artefact filters available on HRV analysis softwares such as Kubios® being used to analyse the data. The filters vary between very low (0.45sec), low (0.35sec), medium (0.25sec), strong (0.15sec) and very strong (0.05sec). One can also apply a custom filter and set it at the required setting according to the data at hand. A study on professional footballers resulted in no significant differences between HRV indices without filter, and indices with filter, except when the very strong filter was applied, however this could be because the study assessed top athletes having high variability which may not be as sensitive to the interpolations made for the filter (Aranda et al., 2016).

Time-domain indices are highly resilient to artefacts and mis-detected complexes, and therefore this makes them very useful in the horse. On the other hand, frequency-domain analysis is very susceptible to the effects of artefacts, and therefore is usually only used on 2-5 minute long intervals in human, however in horses it would be better to use 1024 N-N intervals than a selection of 5 minutes from a recording (Marr and Bowen, 2010). A selection of a recording can be made in two ways, either by time, for instance a 2 minute section or a 5 minute section, or by determining the number of N-N intervals to be analysed. By choosing a section with 1024 N-N intervals, there should be enough data to quantify the effects of both the high frequency and low frequency fluctuations in heart rate (Marr and Bowen, 2010).

2.6. EXERCISE DEMANDS OF NON-ELITE POLO HORSES

The equestrian sport of polo is split between periods of high intensity and periods of low intensity, the horses are required to gallop, stop, change direction in an instant and get back to galloping from an almost stand still. This requires both muscular and articular work. The game is divided into four or six 7-minute periods of playing time, called a chukka, with an interval between each chukka. The horses can play a half or full chukka, but must be altered during each interval. Horses with greater stamina tend to play a full chukka, whilst other horses who may not be as fit, or may require a break either physically or mentally, can be changed during a chukka, making them half-chukka horses. Studies have shown that there is no significant loss of body weight after a polo match and that blood electrolytes Na^+, CL^- and K^+ do not reduce significantly, meaning the animals do not experience any significant dehydration caused by excessive sweating. Low handicap polo is hence not considered prolonged or intense, and is classified as a short-duration exercise with varying intensity, since it does not evoke a significant loss of ions through sweating (Araújo et al., 2014). Low-goal or non-elite polo causes moderate to high stress on the cardiovascular system (Marlin and Allen, 1999). Full chukker horses recover faster than half chukka horses playing in consecutive chukkas (Fiander, 2014), this could likely be because full chukka horses are trained to have better developed stamina and are therefore fitter than the other horses who need to be changed mid-chukka. Training of polo horses should aim at developing the horses stamina well enough for them to be able to play an entire chukka, thus this should require a level of endurance training that will enable the horses to reach the necessary level of fitness.

Materials and Methods

The aim of this study was to use heart rate variability to determine if there was a change in fitness level of non-elite polo horses throughout their training season. This study took place in Malta, during the training season of August 2018 to April 2019. In Malta, the competitive polo season runs from October to June in order to avoid the hottest months of summer. The riders start to prepare their horses for the upcoming season at the end of August, they start with long hacks twice or three times a week whilst gradually increasing the duration of trot and canter during each ride. By September the horses are usually ridden four times per week, for a total of 40 minutes. Training intensity increases gradually until October, when they will be trained 4 times a week for 1 hour. From then on, their training will consist of chukka practice twice a week and a further two moderate exercise sessions during the week. Once competition season starts, a weekly match is held.

Using data collected at three different time points during the training season, the researcher wanted to try gain a better understanding of how the heart rate variability might change in response to higher exercise intensity and improved fitness level from the first measurements taken in August, before the beginning of their pre-competition training, to the last measurement taken in April during the peak of their competition period. The study was aimed to answer the following questions;

- Is there any HRV data that correlates at any time point with the horse parameters: gender, breed, age, rider?
- How do HRV parameters change with increasing exercise intensity and fitness level?

3.1. HORSES

The study included a total of seventeen horses, however four of these were either retired or sold during the investigation period and therefore had to be excluded, resulting in a total of thirteen (n=13) horses being a part of the entire investigation. The horses' ages ranged from nine to twenty years old and they were all thoroughbreds and Argentinian breeds. The horses belonged to three different riders all having a similar level of skill and all the horses play full chukkas. The horses are kept stabled during the night and left out onto their paddocks during the day, they are fed a concentrate-based diet consisting of pelleted feed and sugar beet twice a day, given plenty of hay throughout the day and have fresh water available at all times. A data sheet (Annex 1) was completed for each of the horses with individual information such as gender, age, feed, any known medical conditions, etc. Any comments, changes and information about the horses were written on their respective sheet, and this was kept updated all throughout the investigation.

Horses used in the study	
Total number of horses (n)	13
Mares	10
Geldings	3
10 years and under	7
11 years and over	6
Thoroughbred	4
Argentinian	9

3.2. EQUIPMENT

A Polar ® RS800CX heart rate monitor was used to record the heart rate and RR intervals for a period of 30 minutes. The points on the horse where the electrodes were placed were moistened with water and the electrodes were carefully placed onto the appropriate locations on the horse. The electrodes were placed on the left upper and lower areas of the thorax. An elastic surcingle was then used to keep the electrodes in the correct place and provide better stability to reduce their movement for the duration of the measurement. The recording device was fixed around the surcingle close to the electrodes so as to avoid any signal interference and for it to remain close to the electrodes, since the horses were allowed to walk around their stall during the period of recording.

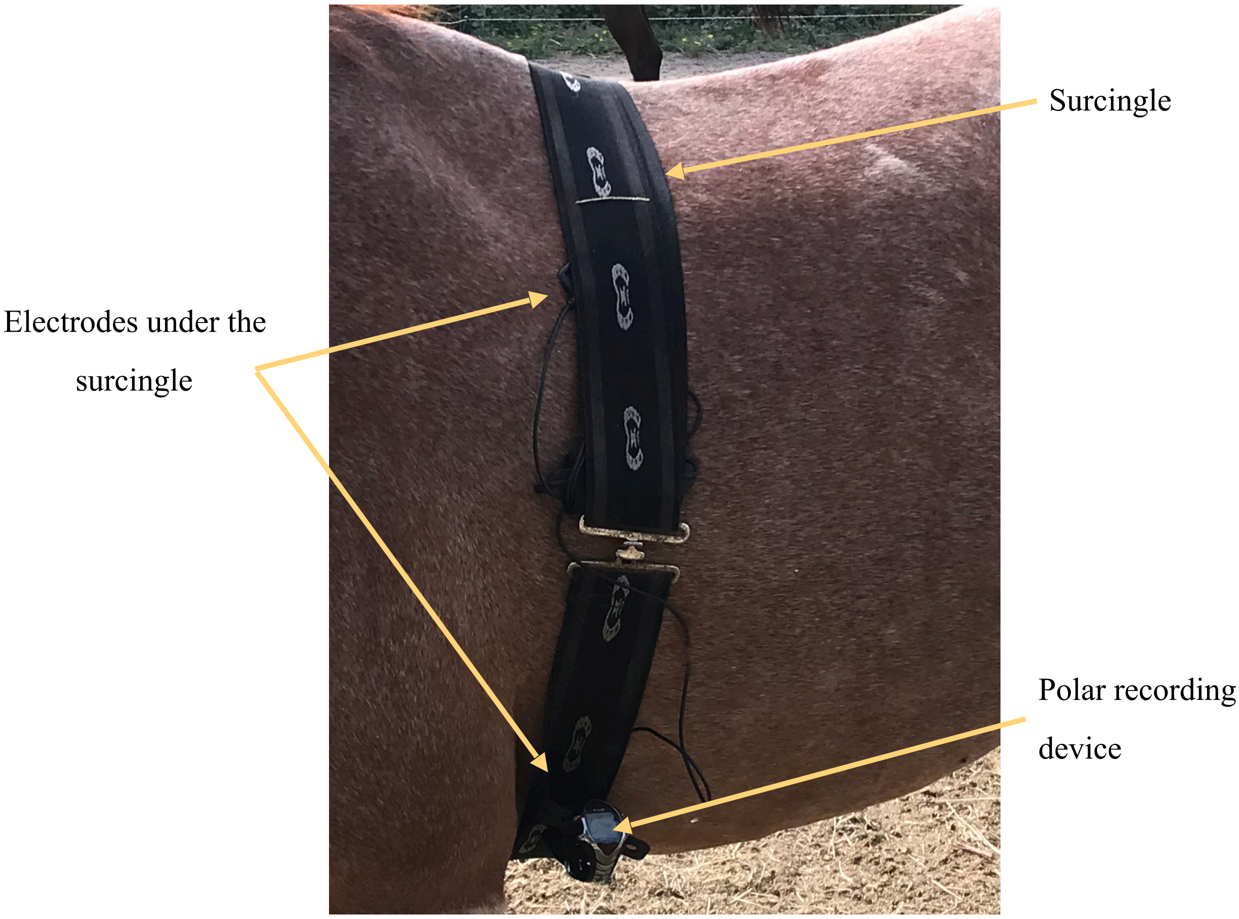


Figure 1: Placement of electrodes and equipment

3.3. EXPERIMENTAL PROTOCOL

HRV measurements were recorded at three different time points during the training period. The first recording took place in **August of 2018**, the second recording occurred in **January 2019** and the third and final recording took place in **April 2019**. These three time points were chosen to represent the pre-training phase, the early competition phase and the peak competition phase, respectively. The idea was to collect measurements from three distinctive phases during the whole training period, so as to obtain an accurate representation of the heart rate variability at rest of the same horses at different training intensities and thus different levels of fitness.

All measurements were recorded at rest whilst the horses were either in their stall or individual paddocks. The horses were not tethered during the recordings and were allowed to move freely in their restricted space. This was allowed so as to have the horses in a relaxed state, without being confined in any way. It is important to note that the recordings took place at different locations, and not all the recordings were obtained on the same day, since not all the horses belonged to the same rider. Measurements were also taken at different times during the day, since only one heart rate monitor was available. The last recordings in April were done outdoors, although the horses were accustomed to the area and noise level around them, this may have had an influence on the HRV parameters when compared to the other measurements which were taken indoors.

3.4. POLAR RECORDINGS

Recordings of thirty minutes were taken for each horse at each time point, thus resulting in three recordings for each horse. A thirty minute recording was required so as to increase the chances of having a continuous five minute segment without too many artefacts that could be used for analysis.

Following each measurement, the recorded data was transferred to a computer directly from the Polar recording device via infrared technology. The data was then opened using the Polar ® ProTrainer5 Equine Edition computer software. A text file of the entire recording was exported and opened on Kubios® HRV standard version 3.1.0.1. The Kubios software was used for the HRV analysis of the data. The manufacturer suggests to set the RR deviation recognition to 30%. Firstly, the average RR interval value for all the recordings was determined, and 30% of the average value was calculated to be 513ms. The artefact correction threshold was therefore set to 0.55 seconds (30% of the average RR interval). Removal trend component settings were set to smoothn priors and lambda 500. HRV frequency bands were set to 0.001-0.005Hz for Very Low Frequency (VLF), 0.005-0.07Hz for Low frequency (LF) and 0.07-0.5Hz for High Frequency (HF), as described by Marr and Bowen, 2010. The best five minute section of each recording was selected based on the section having the least amount of artefacts, which are typically represented by sharp spikes in the graph. It is important to try to eliminate artefacts and excessive deviations from the norm, because these will result in less accurate data and results. After carefully selecting the best five minute section, the results were exported and saved in PDF format (Annex 2). The values were then inputted into an Excel sheet and a dataset was prepared.

3.5. HRV PARAMETERS

Kubios® HRV standard gives results for over twenty-five HRV parameters. Out of these, the parameters which according to research papers have the highest relevance in horses and are appropriate for this particular study were selected for statistical analysis. Aside from the mean HR and mean RR intervals, the SDNN, RMSSD, NN50, pNN50, SD1, SD2, SD1/SD2 ratio, LF, HF and LF/HF ratio were selected as the HRV parameters to be analysed.

Frequency-domain variables should be preferred in the case of short-term recordings of five minutes, these are more complex to interpret than time-domain variables and given that SDNN and RMSSD are also significant for five minute short-term recordings, it was decided to include both time-domain variables and frequency-domain variables. SDNN was selected due to its high correlation with the Low Frequency (LF) power in the frequency domain analysis which is highly influenced by the SNS, and thus SDNN is thought to represent the SNS. RMSSD on the other hand has a high correlation with the High Frequency (HF) power, which is more influenced by the PNS, and thus is said to be representative of it. NN50 and pNN50 were also used, but these carry less statistical properties, therefore are not as important as SDNN and RMSSD.

The powers in LF and HF bands were also selected for analysis, together with the LF/HF ratio. The power in the High Frequency (HF) band is representative of vagal activity, and that in the Low Frequency (LF) band is representative of sympathetic activity. The LF/HF ratio is therefore considered to be a measure of sympatho-vagal balance. The power in the VLF band should only be interpreted in long-term recordings, therefore it was not included in this study.

SD1, SD2 and the ratio between them (SD1/SD2) were also selected for statistical analysis. SD1 correlates with HF band power and is identical to RMSSD. SD2 correlated with LF band power. Their ratio is a measure of the unpredictability of the RR time series and represents the autonomic balance when the recording is long enough and sympathetic activation is also present. This also correlates with the LF/HF ratio.

3.6. STATISTICAL ANALYSIS

Once all the data from the three time points had been collected, analysed and inputted into a dataset, IBM® SPSS® Statistics version 23 was used to carry out the necessary statistical analysis. After consulting with a professor of statistics from the University of Malta, it was decided to opt for Paired Sample t-tests and one-way analysis of variance (ANOVA) to compare each parameter between each time point, and also to compare the parameters based on the different factors such as gender, age, rider and breed.

The paired sample t-test was used to compare mean parameters measured at two different time points, for example August 2018 and January 2019. The null hypothesis specifies that the mean parameters vary marginally between the two time points and is accepted if the p-value exceeds the 0.05 level of significance. The alternative hypothesis specifies that the mean parameters vary significantly between the two time points and is accepted if the p-value is less than the 0.05 level of significance.

The one-way ANOVA test was used to compare mean parameters between different groups clusters by the gender, breed, age or rider of the horse. This comparison was carried out for each time point separately. The null hypothesis specifies that the mean parameters vary marginally between the groups and is accepted if p-value exceeds the 0.05 level of significance. The alternative hypothesis specifies that the mean parameters vary significantly between the groups and is accepted if the p-value is less than the 0.05 criterion.

A paired sample t-test for each HRV parameter (mean HR, mean RR, SDNN, etc.) was first run for all horses in the dataset. The three time points were compared by running 3 paired sample t-tests simultaneously. August 2018 was compared with January 2019, January 2019 with April 2019 and then August 2018 with April 2019. The test was then repeated in the same way for each factor, for example by gender (geldings only, then mares only), by breed (Thoroughbreds only, then Argentinian breed only) and by age group (10 years or less and 11 years or more). Since the riders were of similar skill this factor was omitted from the paired sample t-test analysis.

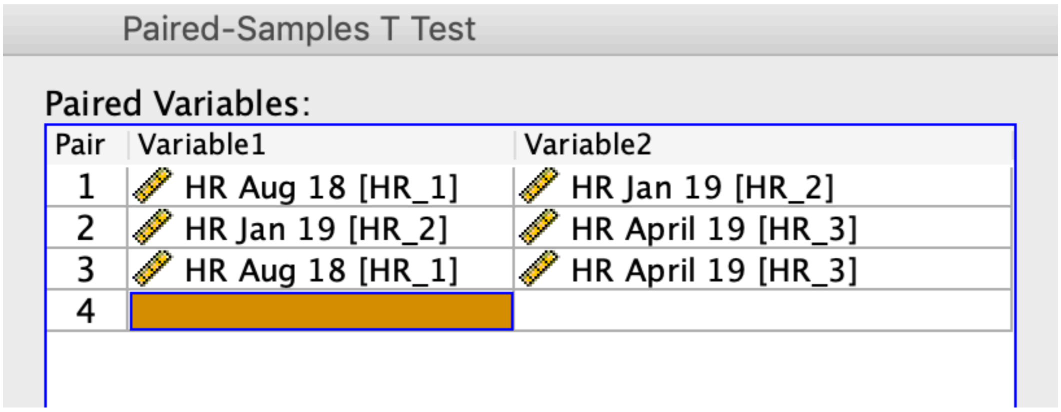


Figure 2: Example for paired sample t-test

The results from the paired sample t-test give information about how each variable (mean HR) might change from one time point to another, both in general and with regard to each factor (gender, age group, breed) individually. In this way one can determine if there is a difference between how geldings react to increased exercise intensity when compared to mares.

On the other hand, the one-way ANOVA test gives information on the difference between geldings and mares at each time point separately. For instance the mean HR of males vs. the mean HR of mares for the recordings taken in August of 2018, or the mean HR of Thoroughbreds vs. the mean HR of Argentinian for the recordings taken in January 2019.

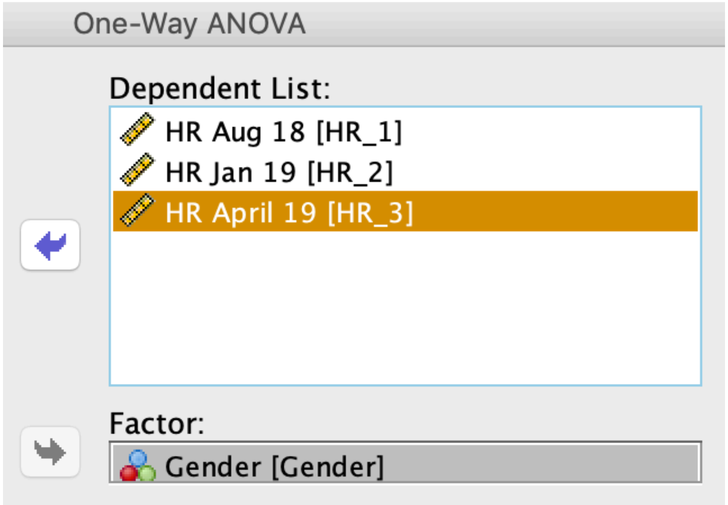


Figure 2: Example for one-way ANOVA

This would indicate if there was a more significant difference between different genders, age groups or breeds at one time point more than another. For instance there might be a greater difference between the mean HR of geldings and the mean HR of mares in August 2018 than in January 2019.

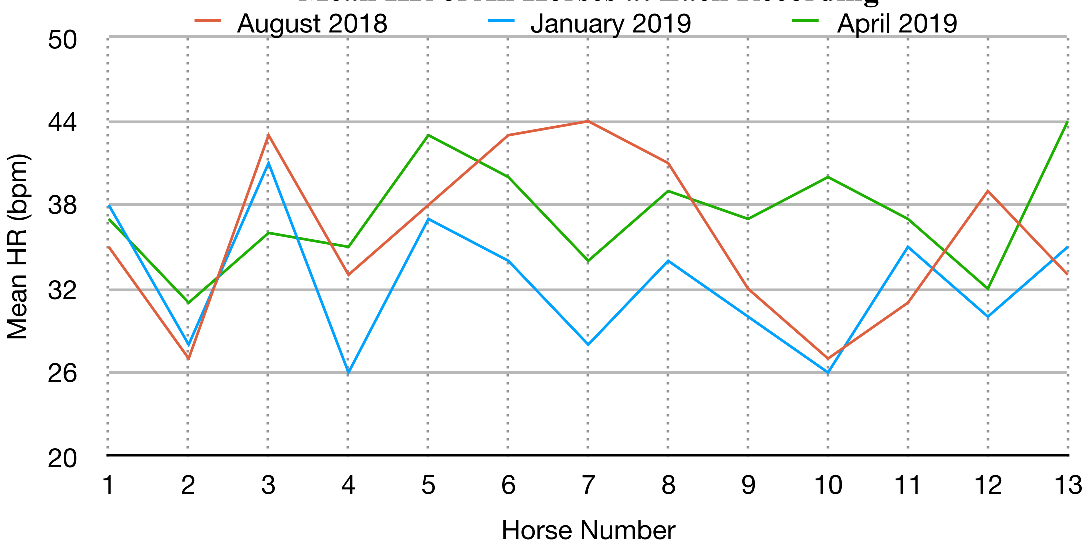
The level of significance for both tests was set at 5%, therefore when p-values were less than 0.05, the change in value was said to be statistically significant.

Results

4.1. MEAN HR

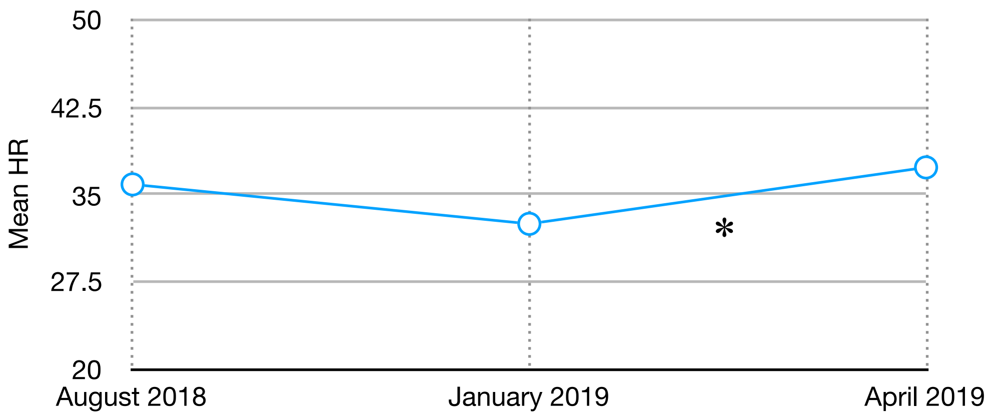
The first graph demonstrates the resting mean HR of each horse during each of the recordings.

Mean HR of All Horses at Each Recording



The second graph shows the average mean HR at each time point, where we can see a decrease between August and January (p-value 0.058), and a significant increase of mean HR from January to April (p-value 0.003).

Average Mean HR at Each Time Point

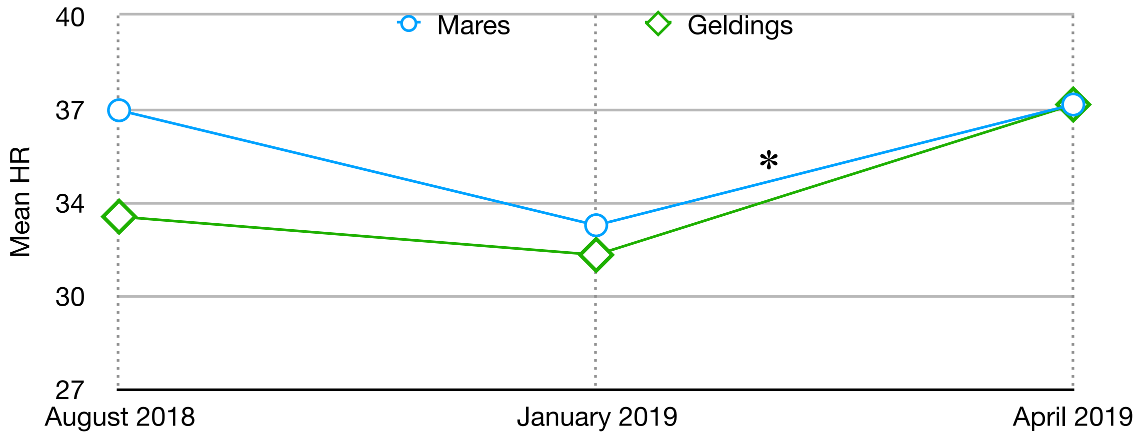


**Significance <0.05*

The changes in mean HR were more evident in female horses than in males. Mean HR for mares was 36.70 bpm in August 2018, 32.70 bpm in January 2019 and

36.90 bpm in April 2019. Male horses had a mean HR of 33 bpm in August 2019, 31.67 bpm in January 2019 and 38.67 bpm in April 2019.

Average Mean HR for Mares and Geldings



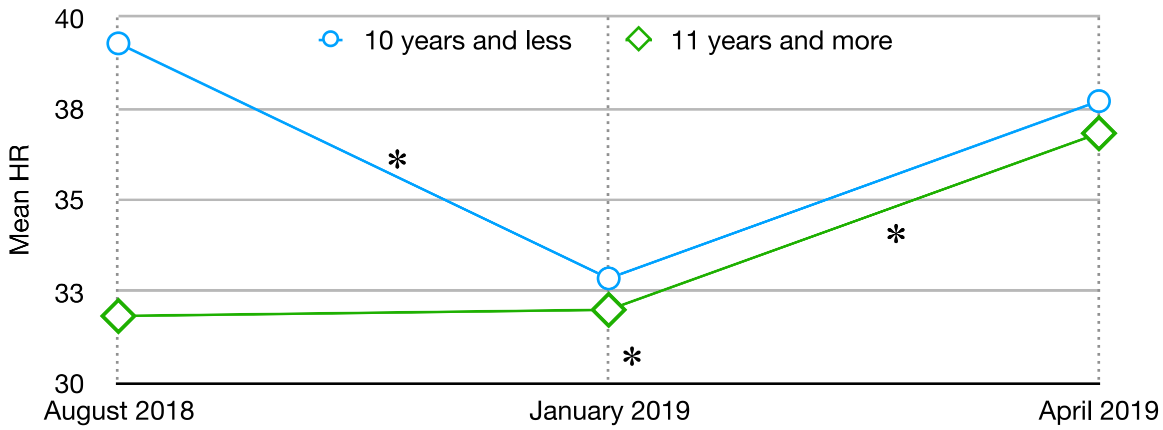
**Significance <0.05*

P-values for the changes in mean HR by Gender

	Mares	Geldings
August 2018 - January 2019	*0.66*	0.716
January 2019 - April 2019	**0.16**	0.192
August 2018 - April 2019	0.927	0.321

Interestingly, horses aged 10 years or younger showed a more significant *decrease* of mean HR from August 2018 to January 2019 (p-value 0.022). Whilst horses aged 11 years or older showed a more significant *increase* of mean HR from January 2019 to April 2019 (p-value 0.035), with an overall increase in HR occurring from August 2018 to April 2019 (p-value 0.015). These changes can be visualised in the graph below;

Average Mean HR for Each Age Group



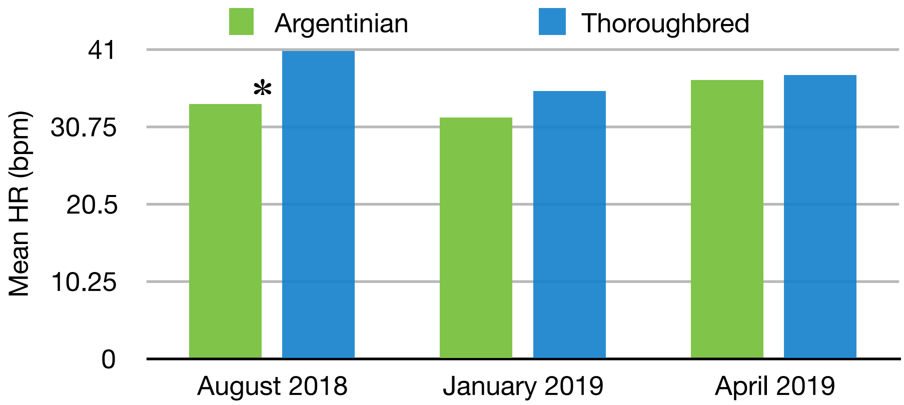
**Significance <0.05*

P-values for the changes in mean HR by Age Group

	10 years and less	11 years and more
August 2018 - January 2019	**0.022**	0.924
January 2019 - April 2019	*0.064*	**0.035**
August 2018 - April 2019	0.624	**0.015**

One-way ANOVA analysis of the mean HR showed no significant differences as a result of gender or rider at any of the three time points. In the first recording prior to the training period in August 2018, Argentinian horses showed a significantly lower mean HR than thoroughbred horses (p-value 0.040) and horses 10 years or younger appeared to have a higher mean HR than horses 11 years or older (39.29 bpm and 31.83 bpm, respectively, with a p-value of 0.016).

Comparison of the Mean HR of Argentinian and Thoroughbred Horses at Each Time Point



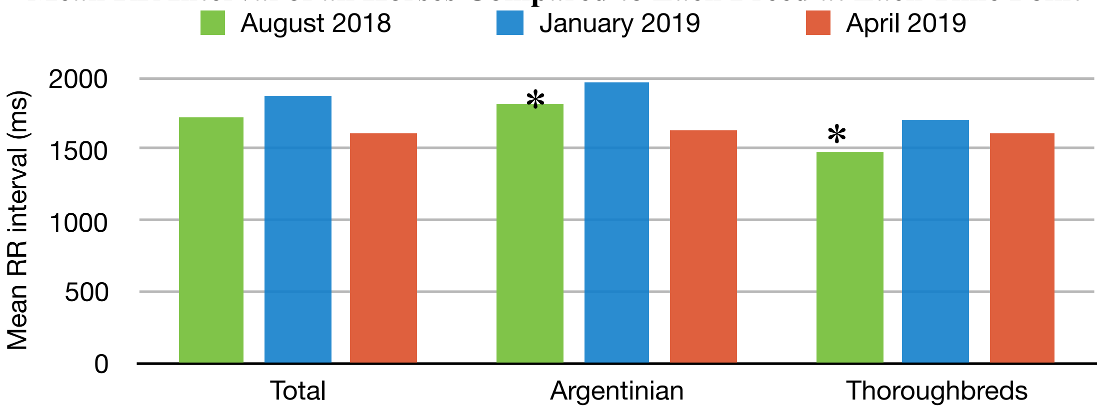
**Significance <0.05*

4.2. MEAN RR INTERVALS

When all data was analysed collectively, paired sample t-test revealed a significant decrease in mean RR intervals, measured in milliseconds (ms), from the recordings taken in January 2019 to the recordings taken in April 2019 (p-value 0.004). This change was more evident in female horses than in males (p-vale 0.014 and 0.235, respectively), also reflected in the mean HR results displayed above. Argentinian horses showed a more significant decrease in mean RR intervals than thoroughbreds for the same period mentioned (p-value 0.005 and p-value 0.456, respectively).

One-way ANOVA showed that Argentinian horses had higher values of mean RR interval before the start of the training season (August 2018), than Thoroughbred horses (p-value 0.054*).

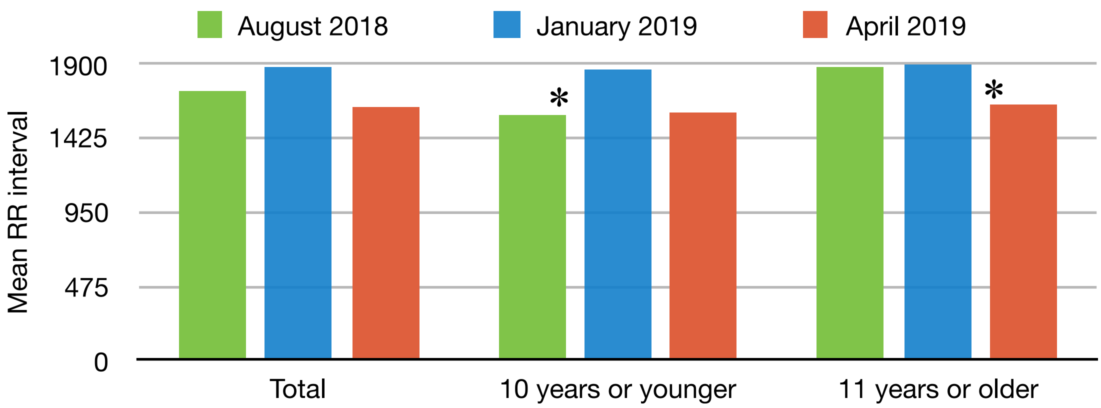
Mean RR Interval of all Horses Compared to Each Breed at Each Time Point



**Significance = 0.054*

When factoring in the age, younger horses (up to 10 years) had marginally significant lower mean RR interval values in August 2018 when compared to horses 11 years old or more (p-value 0.054). Horses 10 years old or younger showed a significant *increase* of mean RR interval from August 2018 to January 2019 (p-value 0.31). On the other hand, Horses 11 years old or more showed a significant *decrease* of mean RR interval from January 2019 and April 2019 (p-value 0.039) and more so from the start of the study to the end (p-value 0.007), this overall decrease of mean RR was not seen in younger horses. These changes can be visualised in the graph below.

Mean RR Interval of all Horses Compared to the Different Age Groups at Each Time Point



**Significance <0.05*

4.3. HRV PARAMETERS

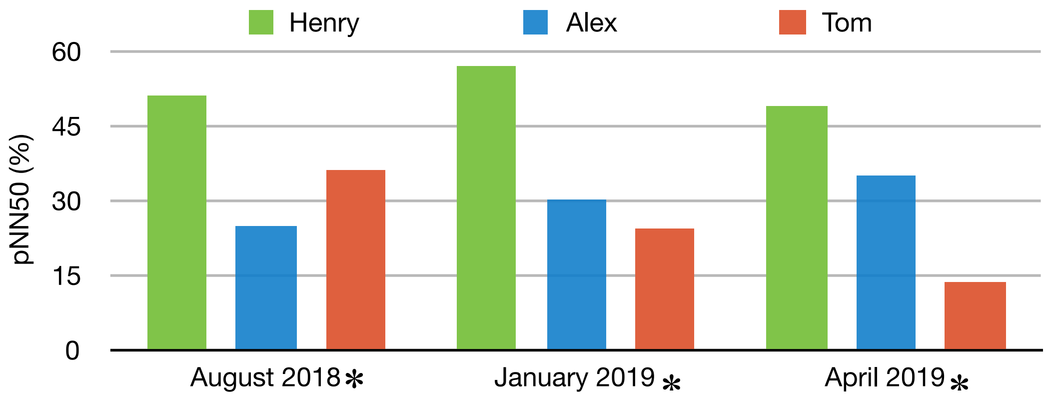
Time-domain analysis revealed no significant changes between any of the three time points, when the mean parameters were analysed by paired sample t-test. SDNN, RMSSD, NN50 and pNN50 were analysed collectively as well as by each factor individually. Gender, age, breed and rider were taken into consideration. Mean values and the corresponding standard deviations (SD) are presented in the table below. All p-values were more than 0.05 and thus not considered significant.

Average Time-domain Parameter Values for Each Time Point

Parameter	August 2018	SD	January 2019	SD	April 2019	SD
SDNN	73.82	37.65	74.57	51.07	77.06	29.98
RMSSD	81.90	39.34	85.90	59.84	87.49	33.54
NN50	70.80	27.92	63.46	32.25	69.57	32.93
pNN50	39.42	16.19	41.14	22.78	36.54	18.84

One-way ANOVA, which compares mean parameters between different groups clustered by gender, breed, age group and rider at each time point separately was also carried out. No significant differences in any of the parameters were found between different genders, age groups or breeds at any of the three time-points. However, Henry's horses were found to have higher SDNN, RMSSD, NN50 and pNN50 values than horses belonging to and ridden by Tom and Alex. SDNN and RMSSD showed a significant difference in January 2019, with p-values 0.029 for SDNN and 0.043 for RMSSD. NN50 was significantly different in January 2019 (p-value 0.047) and April 2019 (p-value 0.039). The differences in pNN50 between horses belonging to different riders was found to be significant at all three time points (p-values all <0.05).

pNN50 Values for All Horses According to Different Rider



*Significance <0.05

In frequency-domain analysis, HF and LF showed no significant differences at any time point, nor when comparing different factors (p-values all > 0.05).

Average LF and HF Values for Each Time Point

Parameter	August 2018	January 2019	April 2019
LF (ms^2)	1818.08	2981.85	1706.77
HF (ms^2)	3594.38	3044.23	4379.46

The ratio of LF/HF, however, showed significant changes that reflect the changes in mean HR and mean RR intervals. Improvement is seen from August 2018 to January 2019, which then declines once again from January 2019 to April 2019.

Change in LF/HF ratio Between Each Time Point

LF/HF	Mean	Sample Size	Std. Deviation	P-value
August 2018	1.13	13	0.235	**0.029**
January 2019	1.13	13	0.756	
January 2019	1.13	13	0.756	**0.001**
April 2019	0.55	13	0.437	
August 2018	0.53	13	0.235	0.903
April 2019	0.55	13	0.437	

The increase of LF/HF ratio from August 2018 to January 2019 is of similar significance for both males and females, but the decline from January 2019 to April 2019 is more significant in female horses.

Change in LF/HF ratio Between Each Time Point for Geldings

LF/HF	Mean	Sample Size	Std. Deviation	P-value
August 2018	0.34	3	0.238	0.111
January 2019	1.36	3	0.883	
January 2019	1.36	3	0.883	0.182
April 2019	0.50	3	0.363	
August 2018	0.34	3	0.238	0.429
April 2019	0.50	3	0.363	

Change in LF/HF ratio Between Each Time Point for Mares

LF/HF	Mean	Sample Size	Std. Deviation	P-value
August 2018	0.59	10	0.211	0.138
January 2019	1.06	10	0.752	
January 2019	1.06	10	0.752	**0.005**
April 2019	0.57	10	0.474	
August 2018	0.59	10	0.211	0.906
April 2019	0.57	10	0.474	

Argentinian horses showed a more significant increase from August 2018 to January 2019 and decrease from January 2019 to April 2019 than thoroughbreds.

Change in LF/HF ratio Between Each Time Point for Argentinian

LF/HF	Mean	Sample Size	Std. Deviation	P-value
August 2018	0.50	9	0.212	0.025
January 2019	1.28	9	0.809	
January 2019	1.28	9	0.809	0.004
April 2019	0.63	9	0.491	
August 2018	0.50	9	0.212	0.505
April 2019	0.63	9	0.491	

Change in LF/HF ratio Between Each Time Point for Thoroughbreds

LF/HF	Mean	Sample Size	Std. Deviation	P-value
August 2018	0.61	4	0.297	0.709
January 2019	0.79	4	0.564	
January 2019	0.79	4	0.564	0.252
April 2019	0.38	4	0.255	
August 2018	0.61	4	0.297	0.420
April 2019	0.38	4	0.255	

Age group had no particular effect on LF/HF ratio. The most significant difference for both age groups was the decrease from January 2019 to April 2019.

Change in LF/HF ratio Between Each Time Point for Horse 10 Years Old and Less

LF/HF	Mean	Sample Size	Std. Deviation	P-value
August 2018	0.60	7	0.243	0.196
January 2019	1.07	7	0.686	
January 2019	1.07	7	0.686	0.038
April 2019	0.45	7	0.271	
August 2018	0.60	7	0.243	0.428
April 2019	0.45	7	0.271	

Change in LF/HF ratio Between Each Time Point for Horses 11 Years Old and More

LF/HF	Mean	Sample Size	Std. Deviation	P-value
August 2018	0.46	6	0.221	0.108
January 2019	1.19	6	0.894	
January 2019	1.19	6	0.894	0.017
April 2019	0.68	6	0.579	
August 2018	0.46	6	0.221	0.412
April 2019	0.68	6	0.579	

Non-linear analysis of the data, specifically SD1, SD2 and SD1/SD2 ratio representing the Poincaré plot, showed no significant changes between any of the time points. Different gender, breed, age and rider revealed no significant changes between any of the time points.

Change in SD1 Between Each Time Point

SD1	Mean	Sample Size	Std. Deviation	P-value
August 2018	57.72	13	27.922	0.787
January 2019	60.97	13	42.484	
January 2019	60.97	13	42.484	0.923
April 2019	62.05	13	23.790	
August 2018	57.72	13	27.922	0.630
April 2019	62.05	13	23.790	

Change in SD2 Between Each Time Point

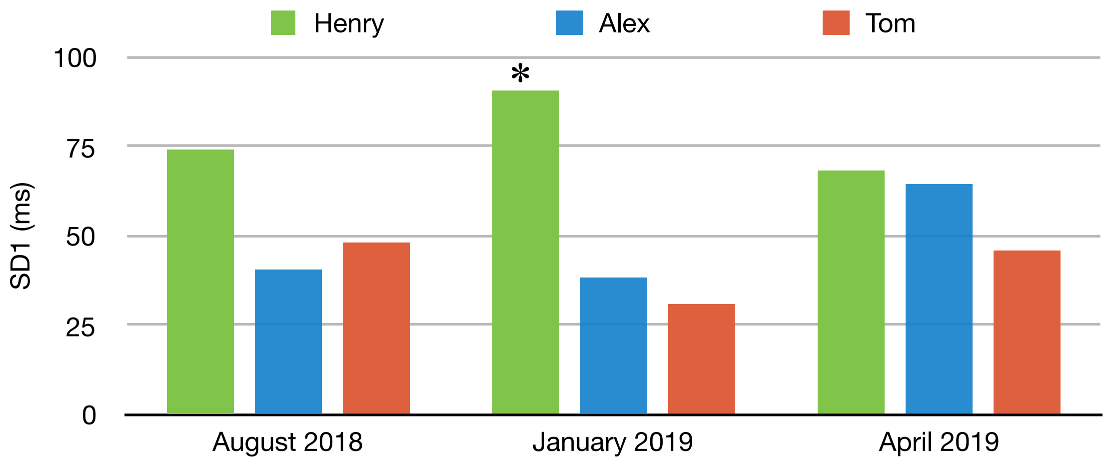
LF/HF	Mean	Sample Size	Std. Deviation	P-value
August 2018	85.96	13	47.292	0.973
January 2019	85.35	13	59.286	
January 2019	85.35	13	59.286	0.847
April 2019	88.99	13	37.066	
August 2018	85.96	13	47.292	0.815
April 2019	88.99	13	23.790	

Change in SD1/SD2 Between Each Time Point

LF/HF	Mean	Sample Size	Std. Deviation	P-value
August 2018	1.44	13	0.438	0.982
January 2019	1.44	13	0.385	
January 2019	1.44	13	0.385	0.940
April 2019	1.43	13	0.356	
August 2018	1.44	13	0.438	0.968
April 2019	1.43	13	0.356	

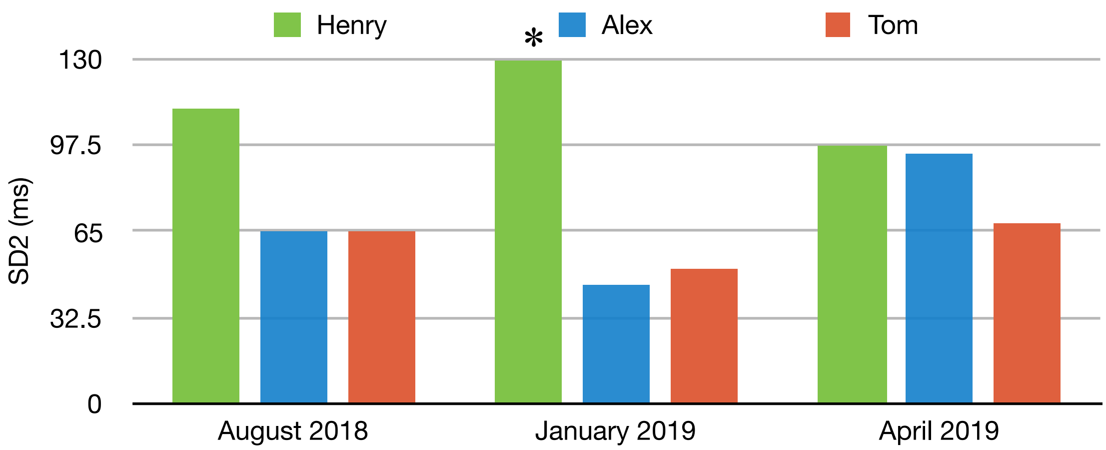
It is worth mentioning that one-way ANOVA test revealed no significant differences between genders, age groups or breeds at any of the time points. However, Henry's horses seemed to have higher values of SD1 (p-value 0.043) and SD2 (p-value 0.025) than Tom and Alex's horses, more so in January 2019.

SD1 Values for All Horses According to Different Rider



**Significance <0.05*

SD2 Values for All Horses According to Different Rider



**Significance <0.05*

Discussion

HR and RR interval parameters demonstrated an improvement from the beginning of the study towards the middle, and then a decline from the middle to the end of the study. This change was also reflected in the LF/HF parameter of the frequency analysis indices. The LF/HF ratio indicates sympathetic dominance, which occurs when we engage in fight-or-flight behaviours or in the case of parasympathetic withdrawal. In theory, with decreasing resting HR one would expect an increase in HF and a decrease in LF/HF ratio, however the opposite is evident in the results obtained from this study. Time-domain and non-linear parameters showed no significant changes throughout the study.

A decrease in resting HR from the pre-training phase in August 2018 to the mid season phase in January 2019 was likely to be a result of training. The horses have started the training period and are therefore becoming fitter, resulting in a decreased resting HR (Marr and Bowen, 2010; Hodgson, McKeever and McGowan, 2013). The improvement was more evident in female horses, when compared to males. Younger horses also showed greater improvement from August 2018 to January 2019 in comparison with horses in the older age group, suggesting that parasympathetic, or vagal, control may decrease with age. There are a few studies describing the effect of age on HRV in horses. In this study, mares originally had higher HR than geldings, however the change during the study was more significant in mares, and this resulted in mares and geldings having a similar HR at the end of the study. This could either be as a result of mares being on average younger than geldings or else mares may have been less trained than geldings and therefore had a greater capacity for improvement. Average ages were 11.7 and 12.7 years for mares and geldings, respectively, and training for all horses started at around the same time, however this does not exclude the possibility that geldings may have started the season fitter than mares. Younes et al. (2016) concluded that HRV amplitude decreased as age increased, however they did not find any significant difference between HR at rest as a result of age. In contrast, this present study indicated a significantly higher resting HR in horses 10 years or younger than horses 11 years or older. This discrepancy could be due to the different age range available in each study. The previous study by Younes et al. investigated endurance horses aged 4 to 6 years old, whereas the present study included horses from 8 to 20 years old. A study which investigated age as an effect of HR and HRV parameters in Thoroughbred horses concluded that resting HR decreases with age, from birth to geriatric horses (Ohmura and Jones, 2017). Ohmura and Jones also suggested that older horses (over 25 years old) may have different autonomic balance than younger horses. Further studies are needed to determine the exact effect age and gender may have on HRV parameters in horses.

During the second half of the study, January 2019 to April 2019, the overall performance seemed to decrease. This decrease in HR and RR values was more pronounced in older horses and Argentinian horses. This drop could be a sign of exhaustion, since the HRV parameters are known to decrease as a result of increased competition intensity when compared to parameters in the pre-competition period, furthermore a decrease in HRV despite an increase in HR may be sign of over-reaching (Kinnunen et al, 2006). In previous studies competing horses were found to have higher resting HR and lower HRV than non-competing horses, and the stresses that come with being a competition horse were believed to be the reason behind this finding (Lorello et al., 2017). During the second half of this study, frequency of matches was increased and training sessions were more frequent. In addition to this, the results may also be an effect of seasonality, given that outdoor temperatures in Malta start to increase rather quickly from March onwards, with temperatures in April ranging from 20-25°C. Horses are more sensitive to warmer temperatures than colder temperatures. In Przewalski horses, HRV parameters were lower in winter and decreased further in April (Pohlin et al., 2017). An accumulative effect of the positive effect of training on HRV parameters together with the negative effect of seasonality may explain why no significant change in HRV was detected during the study.

The improved parameters in January 2019, could also be due to the horses being allowed more recovery time between training sessions. In the winter season during the study Malta experienced a higher rainfall than normal, making the ground conditions of the sand-filled polo field suboptimal and resulting in several matches in November and December being cancelled. Since there is only one polo field available, frequency of polo training was also affected. This resulted in the horses having more time to rest between the more intensive training sessions and also longer periods between matches. This also meant that some matches were postponed to the spring period. The decreased recovery time between training and matches in the second half of the study could also explain the increase in HR and decrease in RR interval values presented from January to April 2019.

Thoroughbred horses showed a more pronounced improvement from August 2018 to January 2019, whilst Argentinian horses showed a more evident decline in the second half of the study. It is believed that hot-blooded horse breeds may have reduced HRV parameters compared to other breeds (von Borrell, 2007). However, the effect of breed on HR and HRV parameters should be researched further since recent studies were based on the use of a homogenous group of horses (Stucke et al, 2015). However it is important to mention that in this present study, thoroughbred horses all fell within the younger age group, so the changes between the different time-points could be due to the effect of age rather than breed. All the horses used in this study had previous experience with polo, and none were new to the sport, therefore experience was not considered as an influential factor when discussing the results. A more elaborate study with equal amounts of thoroughbreds and Argentinian horses of similarly varying ages would be needed to really determine the effect breed may have on HR and HRV parameters.

Time-domain indices and non-linear HRV parameters did not vary significantly throughout the study. SDNN, RMSSD, NN50 and pNN50 were investigated as part of the time-domain analysis. SD1, SD2 and SD1/SD2 represent the Poincaré plot of non-linear analysis. SD1 represents the short-term variability, measured in ms, and correlates with baroreflex sensitivity (BRS), which is the change in IBI duration per unit change in blood pressure and HF power. SD2 is representative of long-term variability, and therefore its analysis in this study may not be suitable since the evaluation period may not be long enough. SDNN is believed to represent the sympathetic system and has more significant information in long-term recordings because “in short-term recordings the primary source of variation is parasympathetic-mediated RSA” (Shaffer and Ginsberg, 2017). SDNN is more accurate when calculated over a 24-hour period rather than short-term recordings.

During long-term (24 hours) recordings, in addition to the cardiorespiratory regulation, the effect of workload, circadian rhythm and influences of classical conditioning on the CNS can all be factored in and evaluated, and thus will give a much more accurate understanding of the SNS involvement in HRV (Shaffer and Ginsberg, 2017). The RMSSD is thought to be more representative of the parasympathetic system and is identical to the non-linear SD1, and therefore both reflect short-term HRV. During this study, no significant change was seen in these parameters.

Despite all parameters not being significantly affected by the increased training during the season, they were all found to be significantly higher in Henry's horses, especially in the data collected in January 2019. Henry's six horses are all Argentinian, with ages ranging from 8 to 16 years. These horses appeared to have higher RMSSD, NN50, pNN50 and SD1 when compared to the horses belonging to the other two riders. Alex's four horses were all 9 year old Thoroughbreds, whilst Tom had three Argentinian horses with ages ranging from 12 to 20 years old. Henry's horses also had significantly higher pNN50 values at all three time points. Given that the three riders use similar training systems, it might be the style of riding or the horses level of experience that may have had an influence, however it is unclear what the exact cause of these results could be.

In frequency-domain analysis, LF, HF and LF/HF ratio were analysed. The power in the high frequency (HF) band is representative of vagal activity, and that in the low frequency (LF) band is representative of sympathetic activity. However, the PNS strongly influences both LF and HF, whereas the SNS only affects the LF power in horses (Stucke et al., 2015). Therefore, LF power is influenced by both SNS and PNS, as well as some smaller influences by unspecified factors. The LF/HF ratio is therefore considered to be a measure of sympatho-vagal balance, however one must keep in mind that this measure may also be affected by other functions such as thermoregulation (von Borrell et al., 2007). A low LF/HF ratio reflects parasympathetic dominance. Therefore, an increased LF/HF ratio may be due to increased sympathetic activation, a depressed parasympathetic effect or a simultaneous effect of both (Shaffer and Ginsberg, 2017). It is always important to keep in mind that the relationship between the SNS and the PNS is complex, non-linear and most often non-reciprocal, and that the SNS and PNS contribution to the LF/HF ratio may be made more complex through respiration mechanics.

The power in LF and HF bands measured in ms^2 showed no significant changes throughout the duration of the study. The power in LF band increased from August to January and decreased further from January to April, however the change was not considered significant (p-value > 0.05). On the other hand, the power in HF band decreased in the first half of the study and then increased in the second half, however these changes were also not considered to be of any statistical significance (p-value > 0.05). The changes in LF/HF ratio, however, had a p-values lower than 0.05 and thus were considered significant. The LF/HF ratio increased from August 2018 to January 2019 (p-value 0.029) and decreased from January to April 2019 (p-value 0.001). This indicated a higher sympathetic activation in January 2019, since HF decreased and LF increased. Training is believed to increase LF power and LF/HF ratio in the long term, meaning increased sympathetic activation in resting conditions (Stucke et al., 2015).

However, whilst the HF/LF ratio increased during the first half of the study, the resting HR decreased. These are contradictory of each other, since decreased HR would mean a predominant PNS effect rather than an SNS effect. These results could be due to the artefact filter applied to the data. Since resting HR was decreased in response to increased training, this shift to a more dominant PNS could have led to a greater number of vagal arrhythmias which the filter may have detected as abnormal and excluded from analysis. However, this may introduce a level of bias by excessively reducing the HRV since the ‘abnormal’ RR intervals would reflect the correction of lower blood pressure after vagal influence and thus may result in HRV which is lower than the original. Additionally, the filter may affect more beats than the two RR intervals on either side of the abnormal beat (Shaffer and Ginsberg, 2017).

It is important to note that the recordings for this study were taken on different days, and thus some horses may have exercised the day before whilst others may have had a day of rest. This may have had an effect on inter-individual differences of HR and HRV. Circadian rhythm is another influential factor of HR and HRV. HR decreases at night and is lowest in the early morning. Researchers describe a stronger parasympathetic control during the night and early morning than in the afternoon. This means that the time of day may be an important factor in equine HRV analysis (Stucke et al., 2015). In the present study, most measurements were taken in the late morning to early afternoon, however a few were taken later during the day. Another factor that may influence the HRV analysis is the horse's environment at the time of recording. The August and January recordings were taken while the horses were indoors in their stalls, while the recordings in April were taken while the horses were outdoors in their individual paddocks. The size of the paddocks is just slightly larger than an indoor stall, but all horses remained calm outdoors. The environmental noise levels outdoors might be higher than inside. The horses were allowed to remain outside during the time of the recording because they were all very calm and taking them into their stables in the middle of the day might have upset their daily routine. However, this variation may have had an influence on the resting HR.

It would have been more ideal if recording conditions such as time of day, the previous day's exercise and location of recordings were kept constant, however, some of these were inevitable. Since only one recording device was available, recordings had to be taken one at a time. If the same time of day was kept, the different recordings would have had to be taken over a period of two weeks and this would have also influenced the HR and HRV. For this reason, a period of time during the day was chosen, in which most recordings were taken. Despite this a minor number of recordings were still taken outside of this time period, mainly because the owners might have not been able to be at the stables at that particular time of day.

Conclusion

The aim of the study was to investigate how the HR and HRV parameters might vary throughout the training season. The resting heart rate was significantly lower in January 2019 when compared to August 2018, prior to training. This can be explained by the long-term effect training and exercise has on the ANS. It is believed that as a horse becomes fitter, the resting HR decreases. This is evident in the results obtained in this study. However, during the same period LF/HF ratio was significantly increased. An increased LF/HF ratio is believed to represent the net activity of the ANS, thus increase in sympathetic activation and/or a decrease of parasympathetic activity.

From January 2019 to April 2019, a decrease in performance was evident through an increased resting HR and an increased RR interval. These changes could be explained by the increase in frequency of training and more matches during the second half of the study, which may have led to slight exhaustion. At the same time, LF/HF ratio decreased during this period of the study. This suggests a decreased sympathetic activation together with an increased parasympathetic effect.

The improved changes from August 2018 to January 2019 were more pronounced in younger, female and thoroughbred horses. Whereas the decline during the second half of the study was more pronounced in older horses and Argentinian horses. Younger horses may react quicker and more efficiently to training than older horses. Older horses on the other hand may have a higher risk of exhaustion.

A drawback of this study was the small sample size that was available to work with. A larger sample may have provided a more accurate and reliable representation. Studies on the long-term effects on HRV as a result of training and sport in the equine species are still rather limited and not a lot of information is available on the subject. Previous studies seem to focus more on the acute effects of training versus rest on the HRV and physiology of the body by comparing the HRV measured the day after different training intensities or rest days. Further study is required to elaborate further on how exercise may have a long term effect on HRV parameters. A study with a larger sample size with equal variations or studies with uniform sample of breed, age and gender may give further insight as to how exercise, training and over all increased fitness might alter the HRV of equine athletes. It would have been interesting to also analyse the HRV of the horses after a week of rest from the very last match of the season, this would have allowed the horses to recoverer from any stress whilst still being at competition-level of fitness. Conducting a similar study in racing horses, who undergo more intensive training and competition would also be an interesting area of research.

Summary

HRV is a non-invasive way of measuring the autonomic regulation of the heart rate. It is believed that with further research changes in HRV can be better understood, with the aim of using HRV as a diagnostic tool for several medical and physiological conditions in the same way as it is currently being used in humans. Further research is still required on how different physiological and environmental factors may have an effect on HRV in the equine species. The aim of the study was to assess how HR and HRV parameters respond to an increase in exercise and training over an entire training season by investigating the effect that exercise, training and over all increased fitness may have on Equine Polo athletes.

Thirteen Polo horses were analysed during the study. The study investigated the horses from before they started training for the new season, in August of 2018, after a few months of training, in January 2019 and then at the peak of their season in April 2019. A Polar ® RS800CX heart rate monitor was used to record the heart rate and RR intervals for a period of 30 minutes. All measurements were recorded at rest whilst the horses were either in their stall or individual paddocks. The data was then opened using the Polar ® ProTrainer5 Equine Edition computer software and exported for analysis to Kubios® HRV standard version 3.1.0.1. The artefact correction threshold was therefore set to 0.55 seconds. Aside from the mean HR and mean RR intervals, the SDNN, RMSSD, NN50, pNN50, SD1, SD2, SD1/SD2 ratio, LF, HF and LF/HF ratio were selected as the HRV parameters to be analysed. Paired sample t-tests and one-way ANOVA were run to determine the statistical significance of the changed parameters.

HR and RR interval parameters demonstrated an improvement from the beginning of the study towards the middle, and then a decline from the middle to the end of the study. Time-domain indices and non-linear HRV parameters did not vary significantly throughout the study. The power in LF and HF bands measured in ms^2 showed no significant changes throughout the duration of the study. The changes in LF/HF ratio, however, had a p-values lower than 0.05 and thus were considered significant.

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Annex

10.1. ANNEX 1

Horse Data **Name:**____________________ **No.**_____

Sex: **Mare** **Gelding** **Stallion**

Age: _________

Breed: ___________________________

Half or **Full** Chukker horse

Nutrition:

____

Any known diseases or conditions:

____

Rider:____________________________

Date of First Readings: _______________

Training: **1 - 2 - 3 - 4 - 5 - 6 - 7** days per week

Level of exercise: **Low** **Medium** **High**

Duration of Exercise: _____________________

Notes:

____

Date of Second Readings: _______________

Training: **1 - 2 - 3 - 4 - 5 - 6 - 7** days per week

Level of exercise: **Low** **Medium** **High**

Duration of Exercise: _____________________

Notes:

____

Date of Third Readings: _______________

Training: **1 - 2 - 3 - 4 - 5 - 6 - 7** days per week

Level of exercise: **Low** **Medium** **High**

Duration of Exercise: _____________________

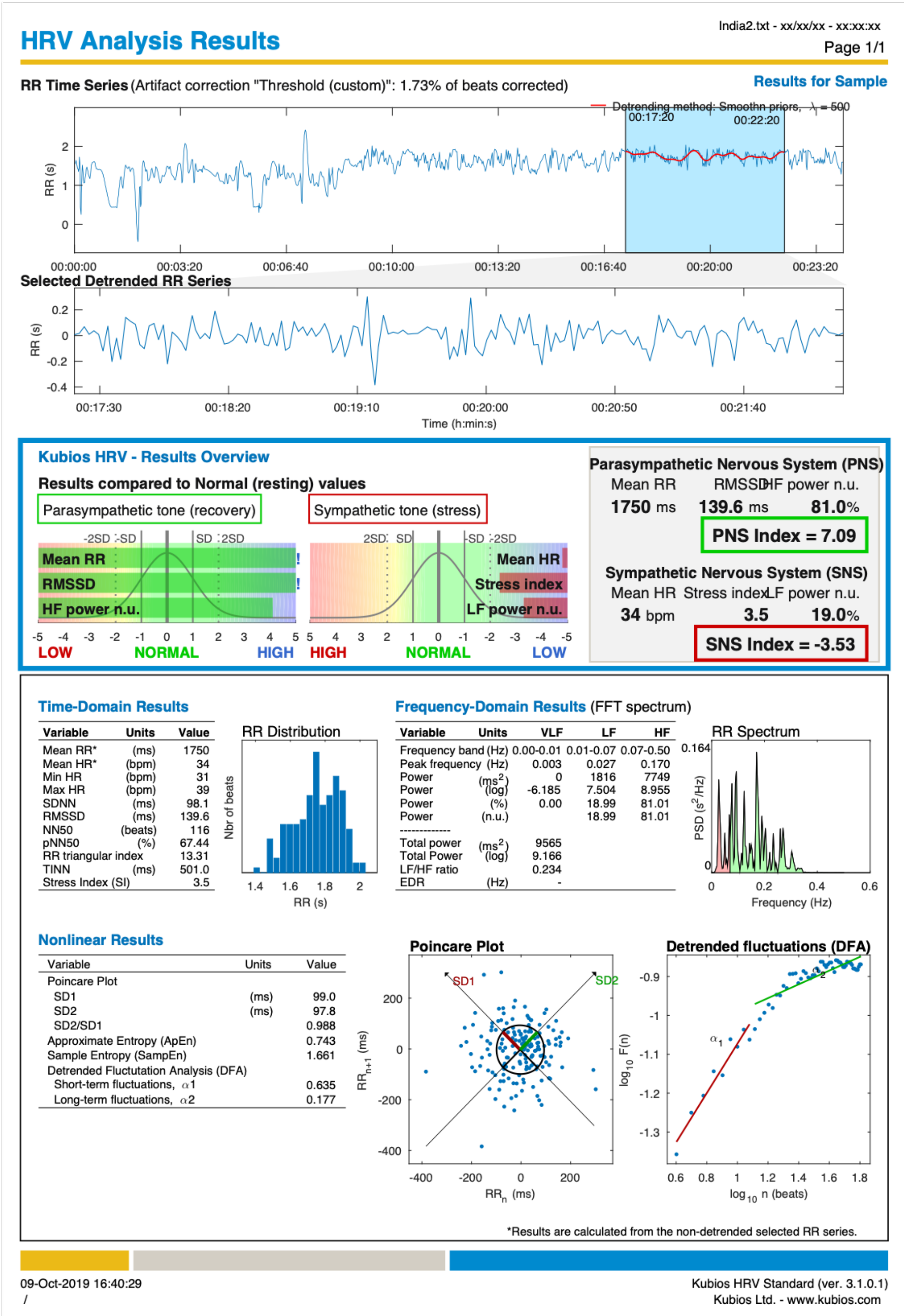
Notes:

____

Data Sheet for Each Individual Horse

10.2. ANNEX 2

Kubios results for horse no.7 (India2) for April 2019 before artefact correction



HRV Analysis Results

India2.txt - xx/xx/xx - xx:xx:xx
Page 1/1

RR Time Series (Artifact correction "Threshold (custom)": 1.73% of beats corrected)

Results for Sample

Kubios HRV - Results Overview

Results compared to Normal (resting) values

Parasympathetic tone (recovery) — Mean RR, RMSSD, HF power n.u.

Sympathetic tone (stress) — Mean HR, Stress index, LF power n.u.

LOW NORMAL HIGH | HIGH NORMAL LOW

Parasympathetic Nervous System (PNS)

Mean RR	RMSSD	HF power n.u.
1750 ms	139.6 ms	81.0%

PNS Index = 7.09

Sympathetic Nervous System (SNS)

Mean HR	Stress index	LF power n.u.
34 bpm	3.5	19.0%

SNS Index = -3.53

Time-Domain Results

Variable	Units	Value
Mean RR*	(ms)	1750
Mean HR*	(bpm)	34
Min HR	(bpm)	31
Max HR	(bpm)	39
SDNN	(ms)	98.1
RMSSD	(ms)	139.6
NN50	(beats)	116
pNN50	(%)	67.44
RR triangular index		13.31
TINN	(ms)	501.0
Stress Index (SI)		3.5

RR Distribution

Frequency-Domain Results (FFT spectrum)

Variable	Units	VLF	LF	HF
Frequency band	(Hz)	0.00-0.01	0.01-0.07	0.07-0.50
Peak frequency	(Hz)	0.003	0.027	0.170
Power	(ms^2)	0	1816	7749
Power	(log)	-6.185	7.504	8.955
Power	(%)	0.00	18.99	81.01
Power	(n.u.)		18.99	81.01
Total power	(ms^2)	9565		
Total Power	(log)	9.166		
LF/HF ratio		0.234		
EDR	(Hz)	-		

RR Spectrum

Nonlinear Results

Variable	Units	Value
Poincare Plot		
SD1	(ms)	99.0
SD2	(ms)	97.8
SD2/SD1		0.988
Approximate Entropy (ApEn)		0.743
Sample Entropy (SampEn)		1.661
Detrended Fluctutation Analysis (DFA)		
Short-term fluctuations, α1		0.635
Long-term fluctuations, α2		0.177

Poincare Plot

Detrended fluctuations (DFA)

*Results are calculated from the non-detrended selected RR series.

09-Oct-2019 16:40:29

Kubios HRV Standard (ver. 3.1.0.1)
Kubios Ltd. - www.kubios.com

Kubios results for horse no.7 (India2) for April 2019 after artefact correction

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