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# Heart rate variability in Mangalarga Marchador horses after physical exercise

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**ABSTRACT**: The aim of this study was to evaluate the influence of physical exercise (marcha gait) on heart rate variability indices in Mangalarga Marchador horses. Twenty-five animals, fourteen females and eleven males, aged 6.4 ± 2.7 years and with a mean weight of 428.3 ± 24.7 kg, were used. Electrocardiograms were recorded at two different time points: rest and immediately after exercise (40 min of aerobic exercise, marcha gait). Time domain analyses of heart rate variability were made using vasovagal tonus index (VVTI) and standard deviation of normal intervals (SDNN). Variables were analysed for normality using the Shapiro-Wilk test and comparisons were made between T0 and T1 using the paired *t*-test (VVTI) or Mann-Whitney (SDNN), considering $P < 0.05$. The Pearson coefficient was calculated to evaluate the correlation between heart rate and VVTI and the Spearman coefficient was calculated for heart rate and SDNN. There was an increase in heart rate after exercise ($P < 0.0001$), from 45.7 ± 12.7 to 77.3 ± 13.5 beats/min, and a reduced VVTI ($P < 0.0001$), from 8.66 ± 1.45 to 4.65 ± 1.25 and a reduced SDNN ($P < 0.0001$), from 80.09 to 11.67 ms. No correlation was observed between heart rate and VVTI or SDNN. It was possible to conclude that the physical activity carried out by Mangalarga Marchador horses influenced the autonomic response and consequently heart rate variability indexes, suggesting the possible use of these indices for the evaluation of Mangalarga Marchador performance.

**Keywords**: cardiology; equine; marcha gait; performance

Horses are particularly well suited to intense exercise. Nevertheless, during their athletic careers, sport horses frequently suffer from several problems related to endogenous or exogenous stress (Schmidt et al. 2010). Exercise testing is used to estimate fitness and training status (Courouce et al. 2000), and it is crucial to understand how training and competitions influence biochemical and physiological responses so that the tests can be used correctly. These considerations justify the large amount of research carried out in the field of exercise physiology (Marques et al. 2002).

Heart rate (HR) and its modulation are closed linked to alterations in autonomic activity; therefore, it is useful to examine autonomic fluctuations under different physiological circumstances, to study the effects of training and also to evaluate manifestations of cardiac diseases (Aubert et al. 2003). HR can be used as marker of intensity of exercise with its elevation, which occurs in order to elevate cardiac contraction, stroke volume and cardiac output, correlating with the level of physical effort (Aguera et al. 1995; Pereira Neto et al. 2013).

Alterations of HR, characterised as heart rate variability (HRV), are not unusual as cardiac beats are not regular (Vanderlei et al. 2009). Measurements of HRV are obtained using a high-quality electrocardiograph (ECG) under stationary conditions and

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are recorded with the subject in a stationary position for a sufficient period of time to permit good frequency resolution. Such measurements can be made during many physiological conditions such as sleep, rest, exercise and analysis of HRV can be done through linear methods (time domain or frequency domain) or non-linear methods; the simplest method is the use of the standard deviation of RR intervals, reflecting the variation in heart rate during a period of time (Aubert et al. 2003).

The HRV indicates the ability of the heart to respond to different physiological and ambient stimuli such as breathing, physical exercise, mental stress and haemodynamic and metabolic alterations. HRV is a method of assessing the autonomic response of the sinus node in healthy, athletic people as well as those with cardiac diseases (Aubert et al. 2003; Schmidt et al. 2010). A high HRV means good adaptation and efficient autonomic responses and a low HRV means abnormal and insufficient adaptation of ANS due to excessive sympathetic activity or a vagal tonus reduction (Pumprla et al. 2002).

In human sports medicine, HRV is used to evaluate adaptations related to exercise and resistance training (Javorka et al. 2002; Tulppo et al. 2003). Almeida et al. (2005) described lower values for HRV immediately after the end of exercise when compared to basal values in 100 individuals carrying out high intensity exercises. Recovery time is related to intensity of exercise, level of conditioning and the interaction between vagal and sympathetic activities (Almeida and Araujo 2003).

Research has already shown the effects of physical efforts on cardiac function and the balance between sympathetic-parasympathetic activities in horses; certain reports have demonstrated a lower HR in well-conditioned animals (Thayer et al. 1997; Kuwahara et al. 1999; Visser et al. 2002; Voss et al. 2002) and HRV has been suggested to be useful in the evaluation of physical and psychological stress in horses, as well as in the evaluation of the physical condition of the animal (Cottin et al. 2005; Schmidt et al. 2010).

Mangalarga Marchador is a genuine Brazilian breed that has a special gait called marcha. The marcha gait is an exhausting test, which has no close counterpart anywhere in the world, in which the horse carries out exercise of long duration (sometimes reaching 70 min), on an oval racetrack, without rest and at a constant speed (Rezende 2006; Prates et al. 2009). There is little information about the marcha gait and thus the aim of this study was to evaluate the influence of this strenuous physical exercise on heart rate variability in Mangalarga Marchador horses trained in Espirito Santo, Brazil, as well as to determine patterns of response for this breed. This information will contribute to improvements in exercise evaluation methodologies and to the wellbeing of Marchador horses in tropical weather.

## MATERIAL AND METHODS

This research was carried out on 25 Mangalarga Marchador horses, 14 females and 11 males, weighing 428.3 ± 24.7 kg and aged between three and 11 years (mean age 6.4 ± 2.7 years). All horses were considered healthy based on clinical examination. Animals were maintained in two properties located in Guarapari, Espirito Santo, Brazil (20°39'15.1''S 40°29'55.3''W).

All horses were submitted to the same feed and management conditions. They were fed coastcross grass hay (*Cynodon dactylon*) *ad libitum* and commercial concentrate feed (Corcel Tradicional, Nutrimentos Presence, Paulinia, Brazil), with 12% protein (1.2 kg/100 kg of body weight). Water and mineral salt were given *ad libitum*.

The selected horses possessed the same athletic conditioning and had been trained for at least six months. Training consisted of 60 min of walking without a rider, twice a week, alternating with 30–40 min of Marcha with a rider on the other three days. On weekends, they were subjected to 20 min of marcha gait with a rider on each day.

Animals were evaluated at two time points: T0 (before physical exercise) and T1 (immediately after physical exercise, with animals still on the track). For the present research, horses executed marcha for 40 min, 20 min clockwise and another 20 min counter clockwise, similar to the protocol established by the Associacao Brasileira de Criadores de Cavalos da raca Mangalarga Marchador (ABCCMM – Brazilian Association of Mangalarga Marchador Horse Owners). The training simulation test was performed during the morning period (between 6 a.m. and 11 a.m.); racetrack characteristics were also recorded. Two riders with a mean weight of 70 kg and mean height of 1.73 m were used.

Electrocardiograms (ECG), each of 5-minute duration, were obtained from each horse on T0 and T1 using a 12-channel ECG-PC electrocardiograph

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(Tecnologia Eletronica Brasileira – TEB, Sao Paulo, Brazil), with a sensitivity of 1 mV = 1 cm for bipolar lead II and a speed of 25 mm/s to evaluate rhythm and HR. Alligator clips fixed to the electrocardiographic leads were attached directly to the skin. Electrodes were placed on the caudal aspect of the forelimbs on the level of the olecranon and on the hind limbs, lateral to the stifle joint to record bipolar limb leads I, II and III and unipolar limb leads aVR, aVF and aVL. All recordings were taken with the horse standing near the track; forelimbs were kept parallel to each other and perpendicular to the long axis of the body. A quiet ambience was provided for the examinations and none of the horses were sedated (Robinson 2006).

Determination of HRV was done by extraction of normal RR intervals automatically from the recorded ECG. Time domain analyses included standard deviation of normal intervals (SDNN) and vasovagal tonus index (VVTI) (Bowen 2010).

Additionally, during the exercise horses used a girth containing a heart rate monitoring device with GPS (RS800CX-G3, Polar Electro, Lake Success, USA). Data recorded by the system were later evaluated using the ProTrainer 5 program (Polar Electro, Lake Success, USA) to record speed and distance covered by each animal.

Monitoring of exercise intensity was done by determining plasma lactate and evaluation of HR before (T0) and soon after the end of exercise (T1) and with 30 min (T2) of recovery. After local asepsis, blood samples were obtained through jugular vein puncture using disposable needles (25 mm × 0.8 mm) and negative pressure system, in 2 ml tubes containing the anticoagulant EDTA-sodium fluoride to determine plasma lactate. Samples were transported under refrigeration, in a cooler with ice, to the Clinical Laboratory of the Veterinary Diagnostic Centre (CDV, Vitoria, Brazil), for immediate processing. Plasma lactate was determined using commercial kits (Katal, Sao Paulo, Brazil), in a semi-automatic biochemical analyser (BIO200, Bioplus, Barueri, Brazil).

Results were analysed using the statistical program GraphPad Prism 5 (GraphPad, La Jolla, USA). Data were submitted to the Shapiro-Wilk normality test. Sample values for HR, average RR and VVTI had a normal distribution and data were submitted to a parametric statistical analysis (paired $t$-test) to compare mean results before and after exercise to evaluate the possible influence of marcha gait on both variables. Results were expressed as mean ± SD (confidence interval). For SDNN, analyses were done using the Mann-Whitney test and results were expressed as median and percentiles (25–75%). Additionally, possible associations between HR and VVTI was determined using the Pearson correlation test and associations between HR and SDNN was evaluated by calculating the Spearman coefficient. Values of $P \leq 0.05$ were considered significant.

## RESULTS

Physical examinations performed to select the horses revealed mean values of 45 beats/min for heart rate and 29 breaths/min for respiratory rate. Furthermore, we recorded the presence of normal intestinal sounds on abdominal auscultation, rectal temperature of 37.8 °C, and a pale pink colour of mucous membranes. Auscultation was performed and animals were free of cardiac murmurs.

All animals were evaluated for a total of eight days in May (autumn season) and the high temperatures (29 °C) and high humidity (89%) recorded were typical of the tropical region. The oval sand track (130 m) was dry in all days of evaluation. The cardiac monitor recorded a speed of 11.29 ± 0.51 km/h, a total distance of 7.2 ± 1.1 km and a mean HR of 131.9 ± 31.4 beats/min during the 40 min of marcha gait. The maximum heart rate recorded was 185.7 ± 19.8 beats/min. According to the riders, no signs of discomfort or reduction of performance were observed during the execution of the exercise.

Biochemical analyses showed plasma lactate values of 1.02 ± 0.41 mmol/l at T0, 2.73 ± 2.43 mmol/l at T1 and 1.89 ± 1.24 mmol/l at T2. HR values were 45.7±12.7 beats/min at T0, 131.9 ± 31.4 beats/min at T1 and 47.8 ± 8.3 beats/min at T2.

The mean values of HR, average RR, VVTI and SDNN throughout the experimental period are shown in Table 1. A significant elevation of HR ($P < 0.0001$) and a significant reduction of VVTI and SDNN ($P < 0.0001$) were caused by exercise. However, there was no correlation between HR and VVTI ($P = 0.2691$) and HR and SDNN ($P = 0.4714$ before exercise, and $P = 0.2338$ after exercise).

All ECG records from all animals were carefully examined and no arrhythmias or conduction disturbances were found.

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Table 1. Mean values, standard deviations and intervals of values for heart rate, average RR, VVTI and SDNN recorded on the ECG and plasma lactate of Mangalarga Marchador horses at rest (T0) and immediately after exercise (T1)

| | T0 | T1 | *P* |
|---|---|---|---|
| HR (beats/min) | 45.7 ± 12.7[a] (28–66) | 131.9 ± 31.4[b] (79–165) | < 0.0001 |
| VVTI | 8.66 ± 1.45[b] (8.06–9.26) | 4.65 ± 1.25[a] (4.13–5.16) | < 0.0001 |
| SDNN (ms) | 80.09[b] (40.73–127.1) | 11.67[a] (5.60–17.48) | < 0.0001 |
| Average RR (ms) | 1409.0 ± 321.1[b] (1276–1571) | 782.1 ± 134.5[a] (727–838) | < 0.0001 |
| Plasma lactate (mmol/l)* | 1.02 ± 0.41 (0.44–1.79) | 2.73 ± 2.43 (1.05–10.27) | 0.0117 |

HR = heart rate, RR = R-R intervals, SDNN = standard deviation of normal intervals, VVTI = vasovagal tonus index

[a,b]Different letters in the same line denote significant differences by *t*-test ($P < 0.05$) for HR, VVTI and average RR, and by Mann-Whitney ($P < 0.05$) for SDNN

*Data published from Gama et al. (2012)

## DISCUSSION

All parameters used to select the animals were normal according to Prates et al. (2009) and Coelho et al. (2016).

Cardiovascular responses to physical activity depend on the type and intensity of exercise (Aubert et al. 2003) and on the high metabolic requirements of active muscles (Rumenig et al. 2007). In the Mangalarga Marchador horses used for this research, mean values for plasma lactate levels immediately after exercise (T1) were 2.73 ± 2.43 mmol/l, which characterise a predominantly aerobic, long-term, moderate intensity exercise, as results were inferior to the anaerobic threshold of 4.0 mmol/l (Hinchcliff et al. 2002). Horses used in the present study were considered to be adapted to the imposed level of exercise as HR records and lactate plasma values returned to basal levels on T2 (30 min of recovery) as already described and published by Gama et al. (2012) and Coelho et al. (2016).

According to several studies (Capelleto et al. 2009; Ferraz et al. 2009; Pereira Neto et al. 2013), including those studying Mangalarga Marchador horses (Folador et al. 2014; Coelho et al. 2016), exercise-induced tachycardia is the predominant mechanism by which cardiac output rises (Aubert et al. 2003).

There is little information about HRV in horses and this is the first report studying Mangalarga Marchador horses. No correlations between HR and VVTI or SDNN were observed, in agreement with Alonso et al. (1998). These authors described a progressive reduction of HRV in seventeen men and demonstrated a negative, but non-linear, correlation with HR. Also in agreement with this study, Thayer et al. (1997) observed a decrease of HRV with physical effort, but the authors described large individual differences in parasympathetic activity during exercise for the horses used in their experiments. The study of Visser et al. (2002) reinforced the role of individual response and horse temperament in determining HRV. All Mangalarga Marchador horses in the present study showed a good and calm temperament, which is an important aspect when analysing HRV.

Exercise intensity is routinely evaluated through plasma lactate and HR (Ferraz et al. 2009), as was also carried out in this study. Some researchers have also studied the influence of exercise intensity on HRV for potential use as a marker of exercise-induced changes (Voss et al. 2002). Physick-Sheard et al. (2000) reported equine HRV analysis during exercise (rest, walk, trot and gallop on a treadmill) with the purpose of gaining insight into the methodological issues and patterns of response. The authors concluded that analysis of HRV appears to be only useful for assessing autonomic modulation of HR at low exercise intensities. At exercise intensities associated with HR values of above 120–130 beats/min, humoral and other non-neural mechanisms such as locomotion energy and respiratory efforts may become more important in influencing HR and HRV than autonomic modulation (Cottin et al. 2005). As marcha gait was classified as a moderate intensity exercise, it can be concluded that for those animals HRV analysis must be better understood before it can be validated as a method to evaluate autonomic response during exercise according to Physick-Sheard et al. (2000).

The results obtained in these experiments show that Mangalarga Marchador horses undergoing

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physical activity exhibit alterations in the autonomic response and HRV indices.

These results highlight the importance of understanding how different intensities of physical exercise influence heart rate variability, and contribute to validating the potential use of this parameter as an index of heart condition and evaluation of fitness of athletic horses.

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