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RESEARCH ARTICLE

Continuous Electrocardiography (Holter) in Horses Submitted to Endurance Test of 4, 8 and 20km

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Abstract

Fifteen conditioned Mangalarga machador and Arabian purebred horses were used to verify continuous electrocardiography (Holter) variables, through different endurance tests. Animals were divided into three groups of five animals: G1, 4km endurance`s test with an average speed of 15km/h; G2, 8km endurance`s test with an average speed of 12km/h; G3, 20km endurance`s test and an average speed of 12km/h. Holter was implanted as standardized in previous studies. Samples were taken before the test (T0), half of the competition route (T1), at the end of the test (T2), beginning of the rest period (T3) and every five minutes throughout the resting time (T4, T5 and T6), which lasted 15 minutes. Data were considered significant if $p<0.005$. Results showed sinus tachycardia during physical activity and distances of 4km at 12 or 15km/h, as well as distances of 8km and 20km at 12km/h did not produce heart rate changes. In conclusion, the proposed activities were well tolerated in terms of heart rate and frequency in conditioned horses.

Keywords: Equine; Holter; Endurance; Heart hate

Introduction

Electrocardiography (EKG) is considered the gold standard exam to evaluate frequency and cardiac rhythm throughout different species. The EKG equipment have been improved for a long time and its miniaturization allowed its application to the field, specially in horses [1-6]. Athletic horses, at resting time, should be evaluated by EKG exams before any competition, to find cardiac arrhythmias. Arrhythmias can potently interfere in animals performance or can aggravate during competitions, putting animals life in danger [3]. However, the use of EKG machines, during physical activities, can be a big challenging, which makes the use of other equipment's necessary [7]. Thus, continuous electrocardiographic monitoring (holter) is possible the main tool, that provides the evaluation of cardiac electrical activity of these animals under those different types of physical activities, equestrian tests and training periods, periods in which the use of a typical EKG is not viable [8-12]. Equestrian sports need high performance horses, animals that are capable to tolerate physical effort demanded by the test [13]. Endurance tests, are performed at distances up to 160km and in different environmental conditions (different types of climate, terrain and altitude) imposing a significant effort on the horse, requiring a high level of conditioning [14]. Therefore, animals must have no changes in musculoskeletal, respiratory or cardiovascular systems [13]. Although some authors have written about holter in horses, undergoing different types of physical activity [8-11,15], none of those studies have performed a continuous electrocardiography (holter) in horses through tests of 4, 8 and 20km and controlled speed.

Materials and Methods

The Animal Ethics Committee of the Northern Fluminense State University Darcy Ribeiro evaluated this study, and considered it exempt from the need for a specific protocol, because it is not an experiment that violates ethics code and animal welfare.

Animals and experimental procedure

Fifteen horses were used, 7 of Mangalarga Marchador breed and 8 of Arabian Purebred, all animals came from specific horse`s stalls from the northern and southern regions of Rio de Janeiro`s state, and from the southern region of Minas Gerais state, Brazil. Animals were not selected by sex or age, but they needed to be conditioned to the performance of endurance tests. All tests were performed at the same time of the day and with an average temperature between 20ºC and 24ºC. Inclusion criteria were based on a clinical evaluation to select healthy animals, and included body temperature measurement,

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cardiac and respiratory rate measurement, performed through cardiac and respiratory auscultation at rest, capillary perfusion time (CPT), mucosal staining, and examination of the device locomotive. If no organic dysfunction were perceptible animals were included in the study.

Animals were than randomly assigned into three groups according to the test distance to be performed (Table 1):

A Global Positioning System (GPS) was used, intermittently, to control speed during tests. Holter continuous recording was performed before, during and after tests, each evaluation timing was performed in accordance to the distance previously established. To record continuous electrocardiography, Holter CardioLight (Cardios®) was placed in each animal 10 minutes before the start of the test. For its implantation, electrodes (2223 - 3M® Cardiac Monitoring Electrode) were fixed to the skin of the animal with the aid of cyanoacrylate glue (Superbonder Loctite®) on the hair. The apparatus cables were connected to the electrodes and covered by an adhesive tape to ensure electrode cable contact throughout the test. On each side of the electrode, a drop of cyanoacrylate glue was also added, to ensure the attachment of the adhesive tape to the electrode.

The electrodes were fixed following the pattern described in the literature [12,15]: Negative and neutral electrode placed in the prominence of manubrium, positive electrode of channel 1 placed in the left ventral midline, close to the xiphoid cartilage and approximately 10cm behind the gut, positive electrode of channel 2, placed in the left ventral midline, 10cm above the olecranon and 10cm behind the gut and positive electrode of channel 3 placed on the left side of the withers in front of the cranial angle of the scapula. The tape recorder was packed in a leather pouch fixed to the saddle at a height close to the withers. The data recorded on the recorder's memory card were analyzed in software provided by the manufacturer (CardioManager 530, Cardios®).

	Distance (km)	Average speed (km/h)
Group 1 (n=5)	4	15
Group 2 (n=5)	8	12
Group 3 (n=5)	20	12

Table 1: Experimental design of horses submitted to continuous electrocardiographic evaluation (holter) during endurance tests of 4, 8 and 20km. Veterinary hospital/ UENF, 2017.

Calculation and statistical analysis

The heart rate data were collected according to a previously established schedule (Figure 1). The results were analyzed using GraphPad Prism software version 4.03 for Windows (GraphPad Software). The Shapiro-Wilk test verified the normality of the quantitative data. Simple means, variance, standard deviation, ANOVA with Tukey test, T-test for unpaired data, index of adhesion (r^2) and acceleration of trend curves were performed. For all tests a $p<0.05$ was considered (Figure 1).

Results and Discussion

Average Exam Time

Examination average time for each group is showed in Table 2.

Although pre-tests and post-tests times were standardized (5 and 15 minutes, respectively), the differences between the total execution times of each group happened because the different distances and mean speed stipulated in each group.

P wave morphology

The distribution of the P-wave morphology, in each group, is presented in table 3, and by analyzing it, presents a similar incidence of animals with P-wave in M-format for both G1 and G2. In both groups, 2 horses presented a P wave in a conventional format and 3 horses presented a P wave in M format. For group 3 the distribution was inverted in relation to the other groups, being 3 animals with P wave in M format and 2 animals with P wave in conventional format.

Prevalent heart rhythm and Cardiac Arrhythmias

The prevalent heart rhythm is presented in table 3. The predominant rhythm in G1 was sinus tachycardia, presented during 86% of the examination time and only 14% of the time showed sinus rhythm. G2 presented sinus tachycardia during 92% of the examination time and only 8% of the time was

	Pre-tests (min)	Test (min)	Resting time (min)	Total time (min)
Group 1	5.20	16.00	15.00	36.20
Group 2	4.75	40.00	15.00	59.75
Group 3	74.00	100.00	15.00	122.40

Table 2: Mean duration of continuous electrocardiography in horses submitted to endurance tests of 4, 8 and 20km. Veterinary Hospital/ UENF, 2017.

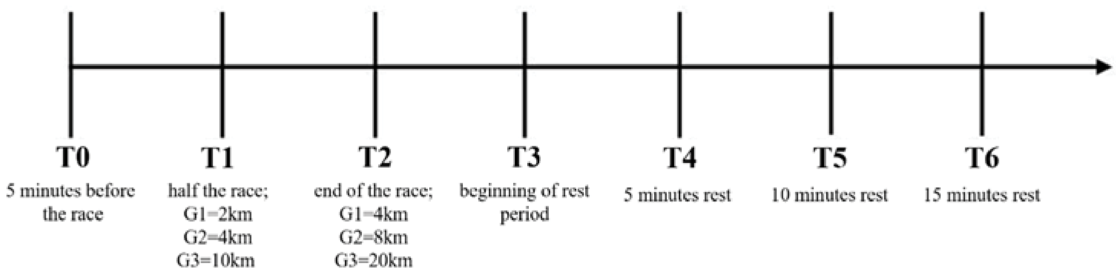

Figure 1: Analysis schedule for each group of animals, monitored with holter, during endurance test. Veterinary hospital/ UENF, 2017.

	Group 1 (n=5)	Group 2 (n=5)	Group 3 (n=5)
		P Morphology	
Normal	2 (40%)	2 (40%)	3 (60%)
Bifid	3 (60%)	3 (60%)	2 (40%)
		Rhythm/Arrhythmia	
Sinus	5,2 min (14,4%)	4,75 min (7,95%)	7,4 min (6,05%)
Tachycardia	31 min (85,6%)	55 min (92,05%)	115 min (93,95%)
Sinus arrest	6 episodes	6 episodes	15 episodes

Table 3: Morphologic distribution of P waves and cardiac rhythm, per group, in horses submitted to endurance tests and monitored continuously by Holter. Veterinary Hospital/ UENF, 2017.

Group/ Time*	T0	T1	T2
G1	34,6 ± 2,70	125,8^{a} ± 11,86	127,6^{a} ± 15,17
G2	32,2 ± 3,83	118^{a} ± 11,51	111,6^{a} ± 9,31
G3	35,6 ± 5,89	118,2^{a} ± 6,41	114,4^{a} ± 12,56
Lines: ANOVA with Tukey test. Values presented with the same letter have no difference considering p<0.05. Blocks with same colors (blue and green): T test for unpaired data. In case of difference for p>0.005, standard variation are superscripted by an asterisk.			

Table 4: Means, standard variation, and statistical analysis of heart rate in horses continuously monitored by Holter during endurance test. Veterinary hospital/UENF, 2017.

Group/ Times*	T2	T3	T4	T5	T6
G1	127,6 ± 15,17	104,8 ± 18,32	54,0 ± 9,64	48,2 ± 5,26	42,0 ± 4,24
G2	111,6 ± 9,31	91,6 ± 7,16	52,0 ± 6,20	48 ± 8,67	43,25 ± 10,56
G3	114,4 ± 12,56	83,8 ± 8,28	54,4 ± 5,59	46,2 ± 6,83	43,0 ± 8,18
Lines: Descriptive behavior of heart rate during resting period by group.					

Table 5: Means, standard variation, and statistical analysis of heart rate in horses continuously monitored by Holter during period time after endurance test. Veterinary Hospital/UENF, 2017.

sinus rhythm. Finally, G3 presented sinus tachycardia during 94% of the test time and only 6% of the time was sinus rhythm.

The only cardiac arrhythmia observed in the animals studied was sinus arrest. Quantification of those arrhythmias and distribution for each group are presented in table 3.

Heart rate behavior during endurance test

Behavior of HR between groups 1, 2 and 3 during physical exercise is shown in table 4. A mean HR during exercise in G1 increased from 34.6 bpm at T0 to 125.8 at T1 and to 127.6 bpm at T2. However, G2 HR presented a mean of 32.2bpm at T0, rising to 118 bpm at T1 and decreased to 11.6 bpm at T2. Finally, the mean HR at T0 was 35.6 bpm, rising to 118.2 bpm at T1 and finishing by 114.4 bpm at T2. The statistical analysis of Table 4 shows that there were significant variations between T0 and T1 times, and between T0 and T2, and no significant variations between T1 and T2, common to all three groups.

Effect of distance and speed on heart rate

Distance effect to HR is presented in table 4 (green cells). In order to evaluate the effect of the distance on the HR, we confront G2 and G3. As they performed tests with equal velocities (12km/h), but different distances, T2 of groups 2 and 3, 8 and 20km, respectively, were compared. Statistical analysis showed no significance for distance.

The effect of mean velocity on mean HR is shown in table 4 (blue cells). In order to evaluate the effects of velocity on HR, it was observed that G1 and G2 performed tests with different speeds (15km/h and 12km/h, respectively). However, G1 at time T2, and G2 at T1, walked equal distances (4km), making it possible to evaluate the effect of velocity on HR. Statistical analysis was not significant for the effect of velocity between G1 at time T2 and G2 at time T1.

Heart rate during post-test rest

Heart rate during post-test between the groups are presented in table 5. Note that, for all groups, HR have reduced to baseline after 15 minutes. However, these recoveries differ among themselves by the tendency velocity to reach the initial values (Figure 2).

Figure 2 shows that the most negative slope (acceleration) curve was seen in G1 ($y = -22.78x + 143.66$, $r^2 = 0,8792$), followed by G2 ($y = -18.03x + 123.38$, $r^2 = 0,8743$) and G3 ($y = -18.04x + 122.48$, $r^2 =0,8826$).

Discussion

The endurance race require effort predominantly aerobic and of moderate-intensity for long duration exercises [16]. in this sense, some electrocardiographic changes could be observed in case of monitoring during the test period. in this study, some results were obtained and discussed with the specialized literature.

First, about the morphology of the P wave, some authors [17] studied horses of the Andalusian breed and found 34.78% of bifid and positive P wave, 60.86% had a single positive P wave and 4.34% showed biphasic P wave. However, the

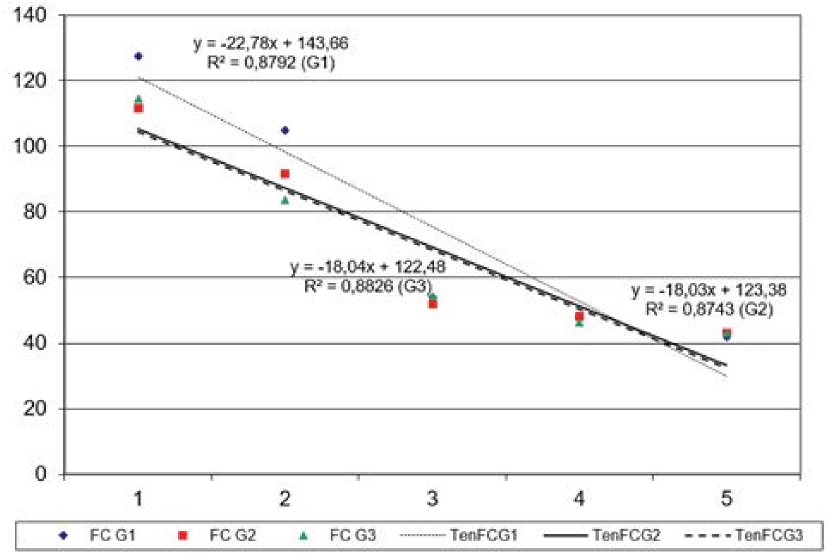

Figure 2: Recovery rate (straight acceleration equation), and homogeneity (adhesion index) of the heart rate, per group, during resting period.

fact that they used 170 animals may justify such distribution differences, in addition to the racial factor studied.

However, others authors [6] found bifida P wave in 7.32% of the cases when comparing electrocardiographic records performed simultaneously by the conventional and computerized techniques. However, this incidence rises to 35.58% when analyzed by the conventional method alone and to 41.46% when analyzed by the computerized ECG. The results of these authors are closer to those of this study (40-60%) when using the computerized technique, probably because it is a digitized method of analysis, as well as the holter.

The M format of P wave is commonly seen in healthy horses [18], and in accordance to literature [19], it happens because of atrial chambers large size, which takes much time for the electrical conduction to pass through intermodal fibers, forming, then, two distinct superimposed P waves, not totally superimposed as in others species. The literature [18] also explain that in the bifid wave, the first peak refers to the depolarization of the medial and caudal third of the right atrium, while the second peak represents the activation of the atrial septum and the medial surface of the left atrium.

In the present study, it was observed that during exercise, the P wave in M format was converted to conventional P wave in all animals. The literature [20] reports that changes in P-wave morphology can occur in animals subjected to loads of physical exercise or excitation. According to others authors [19], this occurs due to the increase in the speed of conduction in the two internodal bundles, right and left, when the heart rate rises.

The results obtained for the cardiac rhythm are common to those described in the literature. According to the literature, sinus tachycardia is considered a frequent rhythm, which may be physiological when associated with pain, fright, excitement or exercise [21]. A several authors [7,19], emphasize that tachycardia is a physiological response to the increase of organism`s oxygen demands when submitted to physical exercise.

Hypothetically, the possibility that G3 may have been the group that spent the largest percentage of time in tachycardia because it was submitted to a more prolonged physical effort due to the greater distance covered, hypothesized that this hypothesis does not apply because the heart rate data during the recovery phase (shown below) are not different from each other.

However, our previously published results indicate that, even though there are differences between them in the actual time of physical activity and time in tachycardia per group, this longer period did not prove to be deleterious to the animals, since the integrity of the myocardial cell did not present important differences among them when analyzed through biomarkers [16].

About cardiac arrhythmias, although horses are very susceptible to various cardiac arrhythmias, such as first and second degree atrioventricular block, sinoatrial block and sinus bradycardia [22], which are attributed to high vagal tone variation [23], those were not abundantly observed in this study.

In the present study, 20% of the animals (1 belonging to each group) had sinus arrest. The animals in groups 1 and 2 presented 6 episodes each, and one of the horses from group 3 presented 15 episodes of sinus arrest. All events were observed at the pre-test time. Some authors [24] found 90% of sinus rhythm during rest in conditioned Arabian Purebred horses, before being submitted to the endurance test, results that were in accordance to literature [13,25]. Also based on those studies, in our study we have encountered similar results, in which the major cardiac rhythm before the tests was also sinus, with eventual episodes of sinus arrest.

According to literature [21], sinus arrest is a manifestation of high vagal tonus, which is less frequent than atrioventricular blocks. Horses with these arrhythmias have slow heart rhythms, with pauses that are similar to or greater than two normal R-R ranges.

According to others authors [19], the sinus arrest is stimulated by a strong parasympathetic action in the horse, for this reason the events of this arrhythmia observed in this study were concentrated in the pre-test rest period (T0), and animals were free of sympathetic activation and catecholamine effects of as a result of physical exercise.

In relation to the behavior of the heart rate observed in this study, during T0 the animals had a mean HR of 34.1 bpm, a value within the normal range for horses (between 26 and 40 bpm). In a past study [26], a mean of 39.3 bpm was observed in Arabian endurance test horses during this phase, information corroborated by the present study. It should be noted that, although previous studies [5] reported a higher HR of Mangalarga Marchador being 52bpm at rest, the standard deviation observed by the authors may represent a degree of equivalence between the data obtained in the present study and other authors mentioned above.

When T1 is observed, all animals presented sinus tachycardia. At this moment the heart rate presents average of 125, 118 and 118bpm in G1, G2 and G3, respectively. However, in T2, while the HR in G1 rises to 127bpm, in other groups, G2 and G3, they decrease to 111 and 114bpm, respectively.

The general causes of sinus tachycardia include elevated body temperature, cardiac stimulation by sympathetic nerves, and toxic conditions to the heart. During exercise there are stimulation of the sympathetic nerves and release of norepinephrine at nerve endings. This olecule is a sympatomimetic neurotransmitter, released by adrenal gland, has positive inotropic and chronotropic effects, and aims to increase cardiac output [19,27-28].

In this study, the effect of velocity and distance on heart rate was also verified. It is believed that the absence of significant differences in HR, based on distance or mean velocity, may be related to the good physical condition of the animals, which are prepared with similar training loads (velocity/distance) before competitions. Also, efforts required in those trainings are normally superior to those analyzed in the present study.

Finally, the data presented in Figure 2 of the heart rate at rest period after test indicate that the higher the slope of the curve, the faster the rate of recovery of the HR from each group. Therefore, G1 was the group that most quickly reduced the heart rate to resting levels, followed by G2 and G3.

The adhesion indexes r^2, shown in figure 2, are considered high for the three groups studied, and similar to each other, with values of 0.8792, 0.8743 and 0.8826 for G1, G2 and G3, respectively. These findings allow inferring that each group, individually, is quite homogeneous in its composition.

This information also suggests that the recovery time of the animal is directly related the distance traveled, even in conditioned animals, in other words, smaller distances are related to faster recovery of HR. the literature [19] argue that this post-exercise recovery rate is independent of the animal's fitness level. In studies previously published by present authors, hemogasometry and cardiac biomarkers were evaluated, and in 4km test, animals showed least impact on electrolytic changes (K^+ and Cl^- depletion, and Na^+ retention), least hematocrit changes [28], as well as the least changes in lactate levels, CK-MB and cTnI [16], physiological factors that can corroborate to the best recovery rate at resting period.

In conclusion, continuous electrocardiography recording (holter) in horses submitted to endurance test of 4. 8 and 20km allowed identification of important data about heart rate and cardiac rhythm, noting that conditioned animals are able to tolerate the proposed activities in this study, without loss of rhythm and/or heart rate.

It is believed that more holter studies in horses submitted to longer endurance tests should be conducted in order to verify the behavior of the cardiac electrical function under situations of stress and additional demand, since there is currently endurance tests of up to 160km.

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