

## Evaluation of Heart Rate as Marker of Stress during Road Transport in Horses

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### ABSTRACT

**Background:** Nowadays horse transportation represents an increasingly practice as a greater number of horses are subjected to transport for several purpose including sport competitions, breeding and selling. In the past, horses were usually transported by train and ship however, at the present time, they move mainly by road with trailers or vans. Transport represents a potential stressor that might compromise horse performance therefore, the chance to monitor horse welfare during transportation is of great interest. The autonomic nervous system and its regulation of cardiovascular function have been considered suitable indicators of stress and welfare in humans and animals. Measuring the vagal tone provide a best knowledge about stress vulnerability and the magnitude of a stress response. Considering that heart rate (HR) represents the effect of the vagus, the aim of this study was to evaluate if this parameter is an affordable indicator of stress in horses subjected to different experimental conditions concerning the duration and the time of the day of road transportation.

**Materials, Methods & Results:** Twelve clinically healthy athletic Italian Saddle horses, divided into three equal groups, were transported over two different distances (110 Km and 225 Km) at the same time of the day (5.00 pm) (group A and B), and over the same distance (110 Km) during the evening (5.00 pm) and during the morning (8.00 am) (group A and C). Each journey was divided into 3 parts (T1, T2, T3) on the basis of road characteristics: T1 and T3 periods represented the time spent travelling on secondary roads characterized by several changes of direction and average speed of 35-40 Km/h; T2 was travel duration on nearly completely rectilinear motorways with average speed of 70 Km/h. Mean HR recorded at rest for each horse was  $38 \pm 3$  beats/min. On HR values recorded during transportation one-way ANOVA showed significant statistical differences of HR values in T2 vs T1 and T3 periods within the group A ( $P < 0.0001$ ) and C ( $P = 0.003$ ); no significant statistical difference was found in T1, T2 and T3 periods within group B. T tests showed that mean HR values were lower in group B than in group A, and higher in group C than group A only in T2.

**Discussion:** Even if transport includes a series of potential stressors such as handling, loading, unloading, unfamiliar environments, oscillation and vibration of the mean of transport, noise, social regrouping, poor ventilation, deprivation of both food and water; in the present study our recordings showed that HR changed irrespective of the experimental characteristics of the journey including the different duration of each transportation. We found that neither the average speed of the mean of transport or the characteristics of the road determined significant changes in horse HR. We also found no influence of the time of the day on HR trend during transportation in horses. This might be due to the fact that HR varied when posture and other external stimuli changed during transport masking the biological rhythms that usually affect the cardiovascular activity and the HR diurnal variability that suits the needs of different levels of activity at different time of the day. On the basis of these results, HR seemed to be an extremely variable parameter profoundly affected by the temporary responses of the horses to sudden environmental incidents.

**Keywords:** heart rate, stress, horse, transport.

## INTRODUCTION

Horses are subjected to transport for several purpose including competitions, breeding and selling [7]. In the past, horses were transported by train and ship [14]; at the present time, they move mainly by road with trailers or vans [5,12-14,17,18] but also by air [7,16].

Transport is a stressor in large animals [1,4,14,18], and the magnitude of stress depends on physical factors, as noise and vibrations of the mean, psychological factors, as new environment and social regrouping, and climatic factors, as temperature and humidity [4].

Even if stress response is necessary for animals to overcome adverse situations that disrupt their homeostasis [9], the damage of stress depends on the ability of the animal to control and cope with the stressors [12,18]. Animals often adapt to a stressor and return to homeostasis, resulting in positive effects on health.

Measuring the vagal tone provide a best knowledge about stress vulnerability and the magnitude of a stress response [11-13]. A reduced activity of the vagus (efferent parasympathetic nerve) would limit physiological and behavioural capacity to cope with stressful events. Therefore, the assessment of the autonomic nervous system and its regulation of cardiovascular function have been used as indicators of stress and welfare in humans and animals [3,6]. Heart rate (HR) represents the net effect of the vagus that slows it down by increasing the variability between the consecutive beats and the sympathetic nerves that accelerate it through the production of a more metronome-like heart beating [12]. On the basis of this knowledge the aim of this study was to value if HR is an affordable stress indicator in horses subjected to different experimental conditions concerning the duration and the time of the day of transportation.

## MATERIALS AND METHODS

Twelve clinically healthy athletic Italian Saddle horses, aged between 8 and 16 years, body weight  $510 \pm 50$  kg, were used in this study with the informed owner consents. Horses were divided into three equal groups and were loaded in an 8-horse van, with a tilt angle of about  $75^\circ$  to the long axis of the vehicle, in individual tie stalls of 80 cm x 210 cm each. Group A and C were transported over a distance of 110 Km for 115 min during the evening (5.00 pm)

and during the morning (8.00 am), respectively; group B was transported over a distance of 225 Km for 240 min during the evening (5.00 pm). Each journey was divided into 3 parts (T1, T2, T3) on the basis of road characteristics: T1 and T3 periods represented the time spent travelling on secondary roads characterized by several changes of direction and average speed of 35-40 Km/h; T2 was travel duration on nearly completely rectilinear motorways with average speed of 70 Km/h (Table 1). For the duration of transportation horses could not either drink or feed. All horses were well accustomed to transportation.

All horses were equipped with equine heart rate monitors<sup>1</sup> to collect continuous records of heart rate during transportation. Two electrodes were placed against the horse's coat and fixed by using elastic girths. The positive electrode was first placed on the left side of the withers under the elastic girth; the negative electrode was then fixed to the girth in a ventral position on the left side of thorax. The electrodes were connected to a transmitter (T51H), fixed to a breast strap, which sent data to a watch type data logger (Polar Equine S-610I), placed near the electrodes. Ultrasound transmission gel was also applied to the horse's coat at each electrode contact point.

During transport HR was logged every 5 seconds and the recorded data were downloaded on a personal computer and analyzed using the Polar Equine 4.0 software.

One-way analysis of variance (ANOVA) for repeated measures was applied to determine significant statistical effects of T1, T2 and T3 periods on HR within each group. Unpaired t-tests were also performed to determine statistical significant effects of transport duration (group A vs B) and time of the day (group A vs C) on HR. *P* values < 0.05 were considered statistically significant. All calculations were done using the PRISM package<sup>2</sup>. All treatments, housing and animal care reported above were carried out in accordance with the standards recommended by the EU Directive 2010/63/EU for animal experiments.

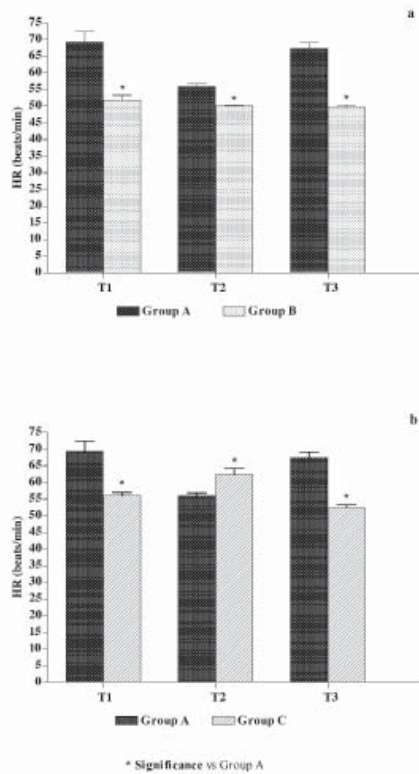
## RESULTS

Mean HR recorded at rest for each horse was  $38 \pm 3$  beats/min. The statistical analysis was applied on the mean values of each min within an hour.

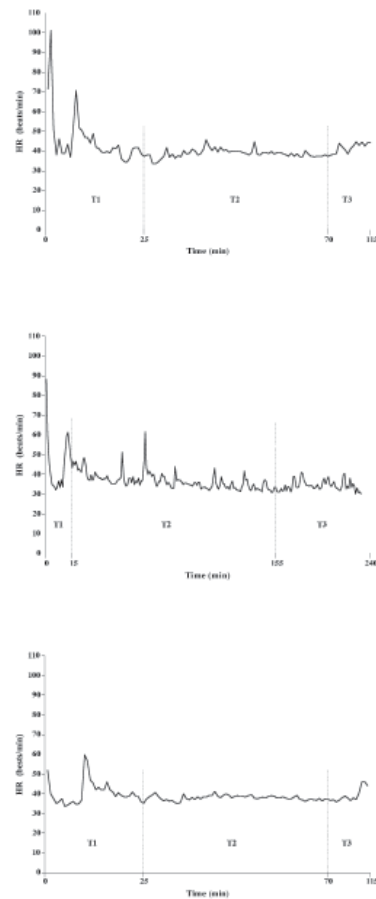
ANOVA showed significant statistical differences of HR values in T2 vs T1 and T3 periods within

the group A ( $P < 0.0001$ ) and C ( $P = 0.003$ ); no significant statistical difference was found in T1, T2 and T3 periods within group B. Unpaired t-test between group A and B showed significant statistical differences of HR values in T1 ( $t_{(55)} = 4.153$ ;  $P < 0.0001$ ), T2 ( $t_{(213)} = 8.786$ ;  $P < 0.0001$ ) and T3 ( $t_{(81)} = 12.55$ ;  $P < 0.0001$ ) periods. Unpaired t-test between group A and C showed

significant statistical differences of mean HR values in T1 ( $t(70) = 4.1$ ;  $P < 0.0001$ ), T2 ( $t(128) = 3.136$ ;  $P < 0.002$ ) and T3 ( $t(28) = 7.799$ ;  $P < 0.0001$ ) periods (Figure 1). Group B showed mean HR values lower than group A during the whole journey, and group C showed mean HR values higher than group A only in T2 (Figure 2).



**Figure 1.** Mean values ( $\pm$ SD) and statistical significances ( $P < 0.0001$ ) of heart rate recorded in group A and B (a), and group A and C (b).



**Figure 2.** Heart rate trend of three subjects belonging to group A, B and C, respectively, during road transport. Each journey was divided into 3 parts (T1, T2, T3) on the basis of road characteristics: T1 and T3 were secondary roads and T2 was motorway.

## DISCUSSION

Horse transport includes a series of potential stressors such as handling, loading, unloading, unfamiliar environments, oscillation and vibration of the mean of transport, noise, regrouping, poor ventilation, deprivation of both food and water [4,17,18]. Therefore, transport stress should not be considered as a single homogeneous stimulus, but as a complex of stress influences where different sources contribute to create a stimulus that presses for an organic physiological adaptive response [5].

Many authors assessed HR as stress expression during horse transportation [5,13,16,18]. In most studies, horses showed higher HR values during transportation rather than at rest in their stall [7]. Smith reported a mean HR of 66 bpm for 8 horses with unknown previous transport experience during 100 min road transport [15]. According to these findings, in our study horses showed a mean HR of  $58 \pm 6$  bpm during transportation in comparison to HR values of  $38 \pm 3$  bpm at rest in the stall. However, in a previous study, during 6 h of road transport, mean HR was only 5 bpm

higher compared to horses kept in pens for the same period of the day [2]. There is also evidence that HR responses to transport can be also influenced by the stage of the journey: HR has been reported to show the greatest elevation during the initial movement of the vehicle, with a gradual decline over the first 10-30 min of transport [7]. However, our data do not show such a trend: in each group HR changes irrespective of the duration of the journey, the mean speed and the characteristics of the road. Since HR is subjected to a diurnal variability that suits the needs of different levels of activity at different time of the day [10], in this study we compared two groups of horses travelled over the same journey during the morning and during the evening in order to assess if biological rhythms [10] affect the cardiovascular activity during transportation. We found no influence of the time of the day on HR

during transportation in horses. This might be due to the fact that HR varies when posture and other external stimuli change during transport [10].

On the basis of obtained results, HR seems to be an extremely variable parameter profoundly affected by the temporary responses of the horses to sudden environmental incidents. Therefore, further studies with different transport characteristics, in different environmental conditions, should be carried out in order to assess HR as a valid marker of transport stress response.

#### SOURCES AND MANUFACTURERS

<sup>1</sup>Polar Equine S-610I, Polar®, Pacific Time, UK.

<sup>2</sup>GraphPad Software Inc., San Diego, CA, USA.

**Declaration of interest.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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