Effect of restraint inside the transport vehicle on heart rate and heart rate variability in Thoroughbred horses

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This study aimed to investigate the effect of transportation and restraint in a van on heart rate (HR) and HR variability in Thoroughbreds. Eight healthy Thoroughbreds were exposed to four conditions, each for a duration of 30 min: stall rest (REST), restraint inside a van (VAN), restraint inside a van with the engine running (V + E), and road transportation (TRANS). Electrocardiograms were recorded to determine HR, low-frequency (LF) power, high-frequency (HF) power, and LF/HF ratio. During TRANS, HR was significantly greater than during REST and V + E. There was a significant increase during VAN compared with REST. These results demonstrated that restraint inside the transport vehicle was one of the major stressors that may cause physiological changes during transportation.

Key words: autonomic nervous activity, heart rate, rest, restraint, stress

Horse transportation is required in horse racing and equestrian competitions but has some risk of causing transport-related respiratory disease, or “shipping fever” [13, 15, 18, 19, 24], which is primarily associated with pneumonia and is thought to be due to bacterial infections of the lower respiratory tract. Therefore, deterioration of the environment inside a horse trailer, such as an increase in dust and ammonia, is considered to be one possible cause of respiratory diseases. Another cause, which leads to fever associated with transportation, is the stress caused by transportation. Horses are exposed to various stressors, including vibration, noise, and restraint, during transportation. These stressors may produce a variety of physiological responses, such as decreased body mass, elevated heart rate (HR), and elevated respiratory rate [1, 9, 11, 13–15, 18, 19, 21–24]. It has also been reported that cortisol, a stress hormone, increased during transportation [15, 21].

It has been suggested that HR variability is a noninvasive method of assessing the autonomic nervous functions [2, 5, 16, 17] and has sufficient sensitivity to evaluate stress in horses [4–7, 9, 11]. It has also been reported that the power spectrum, in terms of high-frequency (HF) power as an index of parasympathetic nervous activity, decreased during transportation [9, 11]. Transport-associated fever is observed more as the transport time and transport distance increase [13]; therefore, continuation of the stressful conditions is one of the causes of shipping fever. However, it is not clear which factors during transport are more stressful. We hypothesized that stressors during transport may affect the HR and HR variability responses of Thoroughbred horses. This study aimed to investigate the effect of transportation and restriction in a van on HR and HR variability in Thoroughbred horses.

All experiments were performed in accordance with the ethical and welfare regulations of the Animal Care Committee of the Equine Research Institute, which approved the study protocol.

Eight healthy Thoroughbreds (four males and four females; 3-years-old; 471 ± 38 (SD) kg) were studied. All horses were clinically normal as determined by physical examinations. The horses had been housed in individual stalls (3.6 m × 3.6 m) with straw bedding and turned out in 2-hectare pastures for approximately 7 hr/day. They were fed a concentrate three times a day at 6:00 AM, 4:00 PM, and 7:00 PM and had ad libitum access to hay and water. The horses were subjected to multiple instances of transport within 10 min to acclimate them to the environment inside the van before the experiment.

The horses were randomly exposed to four conditions, each for a duration of 30 min: stall rest (REST), restraint inside a van (VAN), restraint inside a van with the engine running (V + E), and road transportation (TRANS). Different

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conditions were imposed on different days. In VAN, the horses were loaded into a nine-horse diesel-powered van (loaded with two horses for each condition). The horses faced forward and were restricted in horse stocks with their heads cross-tied loosely in the van, with the floor covered by a rubber mat. The horses were confined for 30 min with the van engine off. In V + E, the horses were loaded and restricted in the van for 30 min in the same manner as VAN but with the van engine running; however, the van was not moved. In TRANS, the horses were loaded, restricted in the van in the same manner as VAN, and then transported for 30 min on a general road only. Before loading the van, three electrocardiograph (ECG) electrodes were affixed to the thorax with foam tape to provide the leads, and a Holter-type ECG monitor (SM-60, Fukuda Denshi Co., Ltd., Tokyo, Japan) was placed in a pocket on a blanket placed on the horse. ECGs were recorded for the horses using base-apex leads for 30 min under each condition. During REST, ECGs were recorded by the same method in their stalls. All horses were free of restrictions in their stalls. All experiments, including measurements, were performed from 13:00 to 15:00 so that diurnal variation would not bias the measurements. The recording duration was decided by referring to a previous report [12].

The 30-min ECG recordings were analyzed with the Softron ECG Processor System (Softron Co., Ltd., Tokyo, Japan), which has been previously used to obtain HR and HR variability [5]. The software first detected R waves and calculated the R-R interval tachogram as the raw HR variability in sequence order. Noise that the software identified as R waves was eliminated manually through visual inspection and examination of values that were outside the interval of 75% to 125% of the mean value. From this tachogram, data sets of 512 points were resampled at 200 msec. We applied each set of data to a Hamming window and a fast-Fourier transform to obtain the power spectrum of the fluctuation. We set the low-frequency (LF) power at 0.01–0.07 Hz and the HF power at 0.07–0.6 Hz, and HR, LF power, HF power, and the LF/HF ratio were obtained from each recording. The standard deviation of the R-R intervals (SDRR) and coefficient of variation of the R-R intervals (CVRR) were also calculated for 30-min ECGs.

Data are presented as the mean ± SE. One-factor repeated measures ANOVA and the Holm-Sidak post-hoc pairwise multiple-comparison procedure were used for comparisons among the REST, VAN, V + E, and TRANS data. A P-value ≤0.05 was considered to be indicative of statistical significance. There were no differences due to gender, and therefore it was not used as a comparison factor.

Figure 1 shows the HR, LF power, HF power, and LF/HF ratio under the REST, VAN, V + E, and TRANS conditions. The HR values during REST, VAN, V + E, and TRANS were 35.3 ± 1.5, 43.4 ± 2.9, 41.5 ± 2.9, and 53.9 ± 4.6 beat/min, respectively. HR was significantly greater during TRANS than during REST (P=0.001) or V + E (P=0.01). HR during VAN was also significantly increased relative to that under the REST conditions (P=0.03). The HF power values during REST, VAN, V + E, and TRANS were 528 ± 82, 398 ± 96, 343 ± 71, and 303 ± 50 msec², respectively. HF power was lower during TRANS than during REST (P=0.047). The LF power values during REST, VAN, V + E, and TRANS were 2,472 ± 521, 2,032 ± 628, 1,714 ± 513, and 1,613 ± 340 msec², respectively. The LF/HF ratios during REST, VAN, V + E, and TRANS were 4.6 ± 0.7, 5.2 ± 0.9, 5.4 ± 1.2, and 5.5 ± 0.9, respectively. No significant effects of the transport conditions on LF power or LF/HF ratio were observed. The SDRR values during REST, VAN, V + E, and TRANS were 169 ± 25, 165 ± 34, 165 ± 22, and 157 ± 18 msec, respectively. The CVRR values during REST, VAN, V + E, and TRANS were 9.97 ± 1.34, 12.5 ± 3.21, 11.55 ± 1.70, and 14.41 ± 2.12%, respectively. There were no significant differences in SDRR and CVRR, respectively.

We conducted this study to investigate the effects of transportation and restriction for 30 min in a van on HR and HR variability in Thoroughbred horses. HR was significantly greater during TRANS than during REST or V + E. A significant increase was also observed for VAN compared with REST. HF power, which represents parasympathetic nervous activity, was lower during TRANS than during REST. HR power tended to be lower under the VAN and V + E conditions than under REST (P<0.1), but the differences were not significant. These results demonstrated that parasympathetic nervous activity decreased during transportation of the horses. Restraint inside the transport vehicle caused sufficient stress to affect HR, although the sensitivity to detect HR variability was insufficient to detect further differences in this study. We concluded that transport stress affects autonomic nervous activity during transportation, and this may be due to accumulated effects of stressful conditions that included not only vibration and noise during transportation but also restraint in the transport vehicle.

It has been reported that transportation for 12 hr decreased immune responses to equine herpes virus [3]. Therefore, decreased immunity due to transport is thought to contribute to the development of shipping fever. Confinement with the head elevated reportedly resulted in significant bacterial contamination within the lower respiratory tract [18, 19]. Stress due to restraint may also affect immune responses. It has also been reported that tying horses just outside their holding pens reduced the HR variability in them resulting from anxiety, which is caused by removal from the herd, environmental noise, and restraint [6]. In this study, HR significantly increased during VAN compared with REST. These results suggest that restraint inside the transport
vehicle might be a major stressor that induces physiological changes in horses. Therefore, it is possible that reducing the restrictions during transportation could reduce the stress in horses.

There was no significant difference between VAN and V + E in terms of HR and HR variability in this study. The difference between the VAN and V + E conditions was that the engine was on under the V + E conditions and off under the VAN conditions. Therefore, vibration and the difference in sound between the van’s engine being on or off may not affect the HR and/or autonomic nervous activity of horses. However, stress responses to restraint may have masked any changes in HR variability caused if the switching was not the factor but rather the on or off was. Therefore, there should be no concern about the effect of the engine being on or off on horses while a van is parked.

Power spectral analysis of HR variability is a method used to assess the autonomic nervous functions [5]. In this study, SDRR and CVRR were also calculated, and they did not show any differences between stress conditions. Power spectral analysis may be more sensitive than a time domain analysis with these values. Previous studies reported that it accurately reflected the influence of various environmental changes and stress on autonomic nervous activity, even though HR did not change significantly in the horses [4, 8, 10]. However, those studies were performed under resting conditions. Other studies reported that some adrenergic agents did not increase the LF power of HR variability under various conditions [1, 20, 25, 26]. In the present study, HR was significantly greater during TRANS than during REST or V + E. Additionally, HR also significantly increased during VAN compared with REST. In addition to transportation, restraint inside the transport vehicle was sufficient stress to affect HR, although the sensitivity to detect HR variability was insufficient to detect further differences. These results show a possibility that HR may be more sensitive than an HR variability analysis, depending on the type of stress. However, the decrease in HF power during TRANS may be the main reason for the increase in HR during TRANS. The increase in HR under the VAN and

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**Fig. 1.** Heart rate (HR), high-frequency (HF) power, low-frequency (LF) power, and LF/HF ratio. Data represent mean ± SE values of 8 horses, and asterisks indicate significant differences (P ≤ 0.05). REST, stall rest; VAN, restraint inside a van; V + E, restraint inside a van with the engine running; TRANS, road transportation.
V + E conditions may be due to both a decrease in parasympathetic nervous activity and an increase in sympathetic nervous activity. Further studies are needed to clarify the difference in the stress responses of HR and HR variability to autonomic nervous activity in Thoroughbreds.

In conclusion, the study results demonstrated that parasympathetic nervous activity decreased during transportation in horses. Restraint inside the transport vehicle was sufficient to affect HR, even when the horses were not transported. Therefore, restraint inside the transport vehicle was found to be one of the major stressors that may cause physiological changes in horses. We concluded that transport stress affects autonomic nervous activity during transportation, which may be due to accumulated effects of uncomfortable conditions in the transport vehicle.

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