Equine Research

Establishing statistical stability for heart rate variability in horses

Laurie McDuffee [a,*], Molly Mills [a], Mary McNiven [a], William Montelpare [b]

[a] Departments of Health Management, Atlantic Veterinary College, 550 University Ave., Charlottetown, Prince Edward Island C1A 4P3, Canada
[b] Applied Human Sciences, University of Prince Edward Island, 550 University Ave., Charlottetown, Prince Edward Island C1A 4P3, Canada

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ABSTRACT

Heart rate variability (HRV) is used increasingly as a quantitative marker for health in humans and welfare in animals. By evaluating the variability expressed in HRV measures, the information can be used to discern physiological stresses acting on the sympathetic and parasympathetic systems in humans and animals. In equine studies, HRV is used as a noninvasive method to assess stress under various circumstances; however, statistical reliability of HRV measures has not been proven. The purpose of the present study was to establish the statistical stability of HRV measures obtained with a portable heart rate monitor using a test-retest approach. Time and frequency domain measures were obtained for 22 horses during routine activities (free in a box stall, confined on cross ties, and during walking) on two separate days. Within each data set (box stall, n = 19; cross ties, n = 21; walk, n = 7) descriptive statistics were evaluated to determine the group effect between conditions and between days. A comparison of group means on day 1 and day 2 was evaluated using a paired sample t-test. Test-retest reliability estimates were determined with intraclass correlation coefficients (ICCs), which included 95% CI for the ICC estimates. The results indicate that mean values between day 1 and day 2, based on the total group, did not differ across two conditions: box stall and cross ties. When horses were being led at a walk, HRV measurements for mean RR and mean HR were significantly different from day to day; however, no other HRV measurements in either the time or frequency domain were significantly different. ICC estimates of time domain variables were strongest for the box stall condition followed by the cross tie condition and then the walk condition. ICC estimates for the frequency domain variables were strongest for the cross tie condition followed by the walk condition and then the box stall condition. All of the ICCs for frequency domain variables were moderate or strong except for the low frequency:high frequency ratio, which was fair for walking and poor for the stall condition. The results support the hypothesis that HRV measurements from the Polar V800 portable heart rate monitors are statistically reliable and can be easily included in an assessment regimen for horses in different environments. Furthermore, assessment of horses for stress and pain during various circumstances could be reliably conducted using this methodology.

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Introduction

Heart rate variability (HRV) is used increasingly as a quantitative marker for health in humans and welfare in animals. HRV is a comprehensive measure of physiological functioning that is derived from the cardiac cycle and expresses the variability that exists between successive heart beats measured at the R wave of the QRS complex. The time signature of the HRV measure is set by the researcher, and the tempo of the rhythm is a direct reflection of autonomic nervous system contribution. By evaluating the variability expressed in either the time or frequency domains, the information can be used to discern physiological stresses acting on the sympathetic and parasympathetic systems in humans and animals (Stucke et al., 2015; Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology, 1996).

In equine studies, HRV is used as a noninvasive method to assess stress under various circumstances that include, but are not limited to, training protocols and competitions, husbandry procedures, and personality evaluations (Schmidt et al., 2010; Visser et al., 2002;

Vitale et al., 2013; Younes et al., 2016). Similarly, HRV measures are used to assess animal welfare and emotional states (Mengolia et al., 2014; Rietmann et al., 2004; von Borell et al., 2007).

Inexpensive portable heart rate (HR) monitors and accessible software have enhanced the ability to collect HRV measures in horses, both in scientific studies and in daily conditions. In order for HRV measures to be appropriately applicable and comparable, the measures must be valid and reliable (Stucke et al., 2015; van Vollenhoven et al., 2016). Human and equine studies have shown that Polar Heart Rate Monitors (Polar V800 Science Equine, Polar Electro Canada, Lachine, QC Canada) have been validated against portable EKG monitors, which are the gold standard (Board et al., 2016; Giles et al., 2016; Ille et al., 2014; Parker et al., 2009). However, in equine studies, reliability of portable HR monitors is not proven. The paucity of reliability assessments for HRV in horses (Stucke et al., 2015; Houben et al., 2017) is limited to small sample sizes and inconsistency of application protocols. As such, reported results are variable across studies (Eggensperger and Schwarzwald, 2017; Houben et al., 2017; van Vollenhoven et al., 2016). Establishing that the basic measure of HRV is statistically stable (i.e., demonstrates reliability) is a first step in the application of HRV as a clinical estimate for different physiological processes. The purpose of the present study was to establish the statistical stability—reliability—of HRV measures obtained with the Polar Heart Rate monitor using a test-retest approach to estimate the intraclass correlation coefficients (ICCs) for HRV within the time and frequency domains.

Methods

The initial sample consisted of 35 horses comprising 9 Quarter Horses, 20 Standardbreds, and 6 Warmbloods. To maintain measurement consistency, one member of the research team was designated as the individual responsible for attaching the portable heart rate monitor including the heart rate sensor belts on each test day. The heart rate sensor belt was placed around the thorax with the HR sensor positioned halfway between the withers and the elbow on the left side of the thorax as instructed by the manufacturer. Horses were not clipped. However, warm water was applied to the region of the electrodes, and the electrodes were soaked in water. All horses had experienced having a strap/harness placed around their thorax where the HRV recording equipment was placed. The heart rate displayed on the HR monitor was compared with that taken using a stethoscope at the beginning of each recording. Absence of arrhythmias was confirmed with cardiac auscultation.

HRV data were collected from horses in their normal environment (racing stable or riding stable) between 7 am and 12 pm on two days 48 hours apart. Polar® HR monitors were attached to the horse and R-R intervals were recorded for 10 minutes after a 2 minute acclimation period. Data were recorded during box stall and cross tie conditions for all horses, but a third condition—walking—was also conducted for Warmbloods and Quarter Horses but not for Standardbreds.

Horses were allowed to be free in a box stall during recording (condition 1) (n = 35), placed on the cross ties in the barn alley (condition 2) (n = 35), and led at a walk by the researcher (condition 3) (n = 15). Humans were not directly attending the horses during stall rest or cross tie restraint.

Data processing

Data from the heart rate monitor were imported into Kubios® HRV Standard (Kubios HRV software [version 3.0.2], Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) for analysis of time and frequency domain variables to determine HRV measures. As a first step in establishing consistency in signal analysis, a medium artifact correction was applied to reduce error across the sample ensemble (Stucke et al., 2015; Tarvainen et al., 2014; van Vollenhoven et al., 2016). Detrending was not applied to these data. Five minute segments were chosen for analysis of HRV measures in Kubios. All HRV analyses from Kubios were reviewed for percent of artifacts corrected. Only horses in which both days of HRV measures had less than or equal to 15% artifact correction for at least two conditions were used in the subsequent estimation of reliability (Table 1). The final data set was therefore based on 22 horses which met the artifact correction criteria for appropriate signal processing of less than or equal to 15% for each day and across at least two conditions. The calculations of HRV were based on both time domain and autoregressive frequency domain measurements derived from the Kubios software. Time domain measurements included mean RR (MnRR), mean HR (MnHR), standard deviation of the normal R-R (SDNN), and root mean square of the standard deviation (RMSSD). Frequency domain variables included low-frequency power (nu), high-frequency power (nu), and low-frequency to high-frequency ratio. The low frequency (LF) band was set at 0.04-0.15 Hz, whereas the high frequency (HF) band was set at 0.15-0.4 Hz (Rietmann et al., 2004; von Borell et al., 2007).

Within each data set (box stall, n = 19; cross ties, n = 21; walk, n = 7), descriptive statistics, including mean and standard deviation estimates of the HRV variables, were evaluated to determine the group effect between conditions and between days. A comparison of group means for measures collected on day 1 and day 2 was evaluated using a paired sample t-test. Test-retest reliability estimates were conducted based on SAS® University Edition-Proc Mixed (SAS: the Statistical Analysis System, SAS/Studio®. release: 9.04 (2015), Cary, NC: SAS Institute Inc.) for ICC with 95% CI estimates. Confidence intervals for the ICCs were based on the SAS macroformula provided by Lu and Shara (2007). The interpretation of ICC used ≤ 0.2 as indicative of poor agreement, 0.3–0.4 as indicative of fair agreement, 0.5-0.6 as indicative of moderate

Table 1
Organization of horses by condition

Treatment conditions			
Horse ID (N = 22)	Box stall N_1 = 19	Cross ties N_2 = 21	Walk N_3 = 7
4	✔	✔	
6	✔	✔	
7	✔	✔	
10	✔	✔	
11	✔	✔	
12	✔	✔	
15	✔	✔	
16	✔	✔	
17	✔	✔	
18	✔	✔	
20	✔	✔	
23	✔	✔	
24	✔	✔	
25		✔	✔
26	✔		✔
27	✔	✔	✔
28		✔	✔
29	✔	✔	
30	✔	✔	
31	✔	✔	✔
32	✔	✔	✔
34		✔	✔

HRV, Heart rate variability.
Horses where both days of HRV measures had less than or equal to 15% artifact correction for at least two conditions were used to determine statistical stability.

agreement, 0.7-0.8 as indicative of strong agreement, and >0.8 as indicative of almost perfect agreement (Weir, 2005; Koo and Li, 2016).

Results

Table 2 presents the descriptive statistics for the time and frequency domain variables on day 1 and day 2 for horses included in each of the treatment conditions: stall, cross ties, and walk. The results indicate that mean values between day 1 and day 2 for the time and frequency domain variables based on the total group did not differ across two conditions: box stall and cross ties. When horses were being led at a walk, HRV measurements of MnRR and MnHR were significantly different from day to day; however, no other HRV measurements were significantly different. Importantly, there were no frequency domain measurements that were significantly different. Figure 1, a representative graph, also shows minimal day-to-day variability. Day 1 to day 2 variations for the LF to HF normalized measure for power is presented in the bubble plot. Minimal day-to-day variation in each horse's score is indicated by the size of the bubble. Homogeneity of variance within each distribution of the variables is indicated by the proximity of scores to the mean scores for each group. These data represent a subgroup of the total sample as only data for the stall condition were used in this analysis. However, the scores demonstrate that the groups were homogenous and that the day-to-day variation was minimal, regardless of the domain selected.

ICCs with 95% confidence intervals are included in Table 3. The calculation of the intraclass correlation controls for the day-to-day variation of the individual horses that comprise the data set. The results indicate that the ICC estimates were strongest in the box stall condition for the time domain variables except for SDNN that had poor correlation. MnRR and MnHR had almost perfect correlation, and RMSSD had a moderate correlation. ICC estimates for time domain variables in the cross tie condition were stronger than those of the walk condition with MnRR and MnHR having a strong correlation but SDNN and RMSSD having a poor and fair correlation, respectively. Interestingly, the frequency domain variables were strongest for the cross tie condition followed by the walk condition and then the box stall condition. However, all of the ICCs for frequency domain variables were moderate or strong except for the LF/HF ratio, which was fair for walking and poor for the stall condition. Confidence intervals showed only SDNN for the stall condition and MnRR and RMSSD for the walking condition as significantly different from day to day ($P < 0.05$).

Discussion

We evaluated the reliability of HRV measures using a portable HR monitor, Polar V800, and widely used Kubios software. Our results indicated good overall reliability. Time domain variables were most stable during low motion conditions (stall and cross ties confinement). Although time domain measures were not strongly correlated for the walking condition, there was moderate to strong correlation for frequency domain variables, LF power (nu) HF power (nu) and LF/HF during walking. Therefore, the autoregressive frequency domain values may be most useful in further research studies involving horses measured while walking.

Previous studies have shown that portable HR monitors, most commonly the Polar V800, collect valid R-R interval data, comparable to that collected with an ECG, during human and equine studies under specific conditions (Board et al., 2016; Giles et al., 2016; Ille et al., 2014; Lenoir et al., 2017; Parker et al., 2009; Vitale et al., 2013). Although many studies have applied portable HR monitors for HRV estimation in equine studies across several conditions (Christensen et al., 2014; Fenner et al., 2016; McConachie et al., 2016; Schmidt et al., 2010), few studies have been conducted to determine the reliability of HRV measures from day to day for use in horses (Eggensperger and Schwarzwald, 2017; Houben et al., 2017; van Vollenhoven et al., 2016), particularly with sample sizes of sufficient generalizability.

The recent version of Kubios software (Kubios HRV Standard, Version 3.0.2, 2017) indicates the percent corrected beats in a measured segment for analysis. This correction enables the researcher to evaluate the R-R recording segment and adjust the choice of segment for one nearby with a lower percent of artifacts and/or to report the percent of corrected beats (artifact correction). In many research studies, the correction factor that was applied by the researchers is not necessarily stated. This may be due to the use of software that does not provide the information. In this study, we describe the correction factor used and report the percent of corrected beats. As a result, although we started with data from 35 horses for stall and cross tie conditions, limiting the acceptable percent of artifact correction to a maximum of 15% resulted in a sample of 22 horses for final analysis.

Table 2
Descriptive statistics for the time and frequency domain variables on day 1 and day 2 for horses included in each of the treatment conditions: stall, cross ties, and walk

Domain	Variable	Day	Box stall $N_1 = 19$		Cross ties $N_2 = 21$		Walk $N_3 = 7$	
			Mean ± STD	t-test day 1 vs. 2	Mean ± STD	t-test day 1 vs. 2	Mean ± STD	t-test day 1 vs. 2
Time	MnRR	1	1426.2 ± 247.9	t = −0.62	1397.6 ± 306.9	t = −0.47	899.5 ± 131.8	t = −2.01
		2	1478.3 ± 273.4	P = 0.54	1448.1 ± 380.3	P = 0.64	1044.7 ± 138.8	P = 0.07
	SDNN	1	137.0 ± 52.9	t = 0.07	143.4 ± 64.1	t = 0.09	93.3 ± 43.8	t = −0.11
		2	135.6 ± 66.4	P = 0.94	141.8 ± 39.5	P = 0.93	95.7 ± 37.1	P = 0.92
	MnHR	1	43.6 ± 9.8	t = 0.49	45.8 ± 14.8	t = −0.02	68.1 ± 11.0	t = 1.85
		2	42.1 ± 9.7	P = 0.62	45.9 ± 18.8	P = 0.98	58.4 ± 8.35	P = 0.09
	RMSSD	1	63.7 ± 15.9	t = −0.08	56.5 ± 18.1	t = 0.17	55.3 ± 11.3	t = −0.44
		2	64.1 ± 20.0	P = 0.94	55.5 ± 17.4	P = 0.87	59.1 ± 20.6	P = 0.67
Frequency	Low frequency (nu) AR	1	77.4 ± 9.1	t = 0.94	73.7 ± 15.8	t = 0.12	42.9 ± 16.7	t = −0.52
		2	74.4 ± 10.9	P = 0.36	74.3 ± 13.2	P = 0.90	47.3 ± 15.3	P = 0.61
	High frequency (nu) AR	1	22.5 ± 9.1	t = −0.94	26.2 ± 15.8	t = 0.31	57.0 ± 16.6	t = 0.52
		2	25.6 ± 10.9	P = 0.35	24.9 ± 11.5	P = 0.76	52.6 ± 15.2	P = 0.62
	LF:HF ratio	1	4.36 ± 2.91	t = 0.84	4.14 ± 2.73	t = 0.04	0.93 ± 0.73	t = −0.31
		2	3.66 ± 2.07	P = 0.41	4.10 ± 3.04	P = 0.97	1.05 ± 0.61	P = 0.76

MnRR, mean R-R interval; SDNN, standard deviation of the normal R-R interval; MnHR, mean heart rate; RMSSD, root mean square of the standard deviation; low-frequency power (nu) AR, low-frequency power in normalized units analyzed with autoregressive approach; high (HF) (nu) AR, high-frequency power in normalized units analyzed with autoregressive approach; LF:HF, low-frequency to high-frequency power ratio.
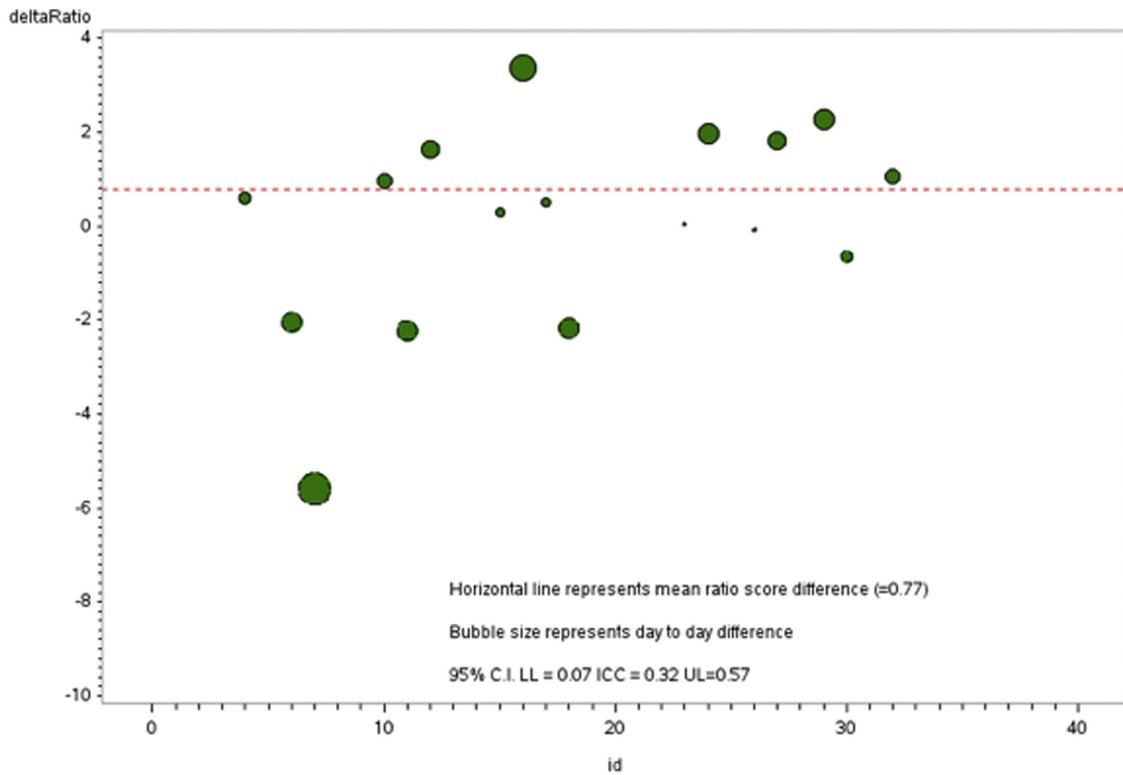
Figure 1. Frequency domain plot of day 1 to day 2 variations in low frequency to high frequency normalized measures for power.

Ten-minute recording segments were used for analysis of short-term 5-minute R-R segments. This allowed some flexibility in choosing segments with the lowest percent of artifacts. Still, when trying to obtain HRV analyses for horses with 5% or fewer artifacts as ideally recommended (von Borell et al., 2007) on two subsequent days during two or more conditions, it was not possible to maintain a large sample size. Statistical analysis used here was based on an artifact correction threshold of 15%. Evaluation of MnHR along with minimum and maximum heart rate data from Kubios output was helpful in identifying segments with valid data as exceptionally high heart rates were considered to indicate erroneous data. Consistency in reporting the correction factor used and percentage of beats corrected would be helpful for comparing studies and determining what is most appropriate for equine research.

Table 3
Intraclass correlation coefficients with 95% confidence intervals for time and frequency domain estimates across conditions

Variables	Condition	Lower limit 95% confidence interval	Intraclass correlation coefficient	Upper limit 95% confidence interval
Time domain variables				
MnRR	Box stall	0.80	0.89	0.99
	Cross ties	0.64	0.80	0.96
	Walk	−0.05	0	0.05
SDNN	Box stall	−.008	0.18	0.37
	Cross ties	0.05	0.28	0.51
	Walk	0.24	0.54	0.84
MnHR	Box stall	0.91	0.95	0.99
	Cross ties	0.63	0.79	0.96
	Walk	−0.15	0	0.15
RMSSD	Box stall	0.39	0.63	0.88
	Cross ties	0.12	0.38	0.64
	Walk	−0.10	0.14	0.39
Frequency domain variables				
Low-frequency power (nu) AR	Box stall	0.32	0.58	0.84
	Cross ties	0.54	0.74	0.94
	Walk	0.31	0.59	0.88
High-frequency power (nu) AR	Box Stall	0.32	0.58	0.84
	Cross ties	0.38	0.63	0.87
	Walk	0.31	0.59	0.88
Low-frequency to high-frequency ratio	Box Stall	0.07	0.32	0.57
	Cross ties	0.39	0.64	0.88
	Walk	0.17	0.48	0.79

MnRR, mean R-R interval; SDNN, standard deviation of the normal R-R interval; MnHR, mean heart rate; RMSSD, root mean square of the standard deviation.

Although an advantage of using portable ECG data includes the ability to manually correct artifacts in interbeat intervals, using portable HR monitors may not allow evaluation of artifacts directly. Kubios software automatically adjusts for artifacts in interbeat intervals from very low to very strong correction techniques where correction is made by replacing the identified artifacts with interpolated values using a cubic spline interpolation (Tarvainen et al., 2014). van Vollenhoven et al. (2016) compared the use of various correction factors in the Kubios analysis and found that setting the software to undergo no correction to strong correction resulted in no significant difference in HRV measures from data collected with the Polar HR monitor from horses. However, when van Vollenhoven et al. (2016) set the software to use the very strong correction factor, the HRV measures were significantly different.

Higher levels of activity are thought to be less reliable due to motion artifacts when using the Polar monitor on horses (Lenoir et al., 2017; Rietmann et al., 2004), and this affects the precision of HRV measurements. Artifacts reportedly occur in human subjects as a result of poor electrode-skin conductance, equipment malfunction, noises from muscle action potentials, and environmental electromagnetic interference (von Borell et al., 2007). Careful attention to artifact correction at all times especially during walking is important for correct interpretation of the HRV data.

Another consideration in consistency of data collection is the frequency ranges used for determining the frequency domain HRV measures. Several frequency ranges have been published as acceptable for horses (Stucke et al., 2015; von Borell et al., 2007). In this study, we used the ranges (0.05-0.15 Hz for LF and 0.15-0.5 for HF) reported previously based on respiratory rate (Rietmann et al., 2004; von Borell et al., 2007). Others have used the frequency ranges reported by Kuwahara et al,. (1996) based on blockade of the autonomic nervous system by pharmacological agents. Reporting frequency ranges used in power spectral density analysis could improve comparability between studies.

Although HRV is a promising marker of autonomic activity (TFESC & NASP, 1996) and many equine studies have used HRV measures as an indicator of stress, close evaluation of HRV analyses should be conducted when choosing HRV measures for evaluation of the autonomic nervous system. In order for accurate comparisons to be made between studies, specific details of the methods which include the HRV measurements evaluated, method of data collection (Polar or ECG,), software used for analysis, artifact correction thresholds, smoothness prior, and frequency ranges used should be reported. Advances in software (Aranda et al., 2017) are providing more of these details on analysis making reporting easier.

Reviews of HRV measures across animal species (Stucke et al., 2015; von Borell et al., 2007) are helpful in establishing standards for HRV measures. These reviews propose standards for use of HRV in horses including frequency ranges, recording time periods, and artifact evaluation. Review of literature discussing HRV measurements in humans also recommends optimal HRV measures for evaluation based on the goal of the study and recording periods (TFESC & NASP, 1996). The present study used four time domain measures (MnRR, MnHR, SDNN, and RMSSD) and three frequency domain measures (LF power [nu] AR, HF power [nu] AR, and LF/HF). SDNN is the simplest variable to calculate; however, it may not be the most appropriate measure for use due to its dependence on the length of recording (TFESC and NASP, 1996). In this study, SDNN was not a highly reliable HRV measurement, whereas other time domain measures were highly reliable. Frequency domain measures are considered most important in evaluating the balance between the parasympathetic and sympathetic nervous system (Marchant-Forde et al., 2004; Stucke et al., 2015; TFESC & NASP, 1996; von Borell et al., 2007). Similar to van Vollenhoven et al., (2016), the present study reported LF and HF power in normalized units, which represent the relative value of each power component in proportion to the total power minus the VLF component, which itself is not well defined (Stucke et al., 2015; TFESC & NASP, 1996). Results of the present study showed robust reliability within the frequency domain variables evaluated, indicating their usefulness in research assessing the autonomic nervous system in horses.

The results presented here provide agreement with previous recommendations and supports evidence of statistical stability when close evaluation of data for analysis is conducted. We also report descriptive data for horses in their normal environment under three common conditions which can be useful for comparison of results across studies. Further studies using larger sample sizes and comparison of breeds, age, and sex would provide further assessment and the establishment of normative data.

Overall, the results support the hypothesis that HRV measurements from the Polar V800 portable heart rate monitor HRV are statistically reliable and can be easily included in an assessment regimen for horses in different environments. Furthermore, assessment of horses for stress and pain during various circumstances could be reliably conducted using this methodology.

Acknowledgments

Ethical considerations

This study received approval from the University of Prince Edward Island Animal Care Committee (AUP #17-019) and complies with all institutional, provincial, and national regulations pertaining to the use of animals in research.

Conflict of interest

The authors declare no conflict of interest.

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