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Use of the Polar V800 and Actiheart 5 heart rate monitors for the assessment of heart rate variability (HRV) in horses.

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Abstract

Heart rate variability (HRV) is a commonly used metric in animal science to quantify an aspect of the physiological response to stressors. HRV analysis relies upon accurate detection of the electrical activity of the heart to define inter-beat interval (IBI). In horses, this can be problematic due to specific features of the equine ECG waveform and a high incidence of 2nd-degree atrioventricular (AV) block in clinically normal horses at rest.

The current study validates the collection and interpretation of IBI data from two heart rate monitors (HRMs) principally designed for human personal monitoring (Polar V800 and Actiheart 5). 30 min of synchronous IBI data was collected using the two HRMs and a 4-lead telemetric ECG that is widely used in equine veterinary clinical practice (Televet 100). The study was conducted using a mixed sample (n=17) of general riding horses, with (n=4) and without (n=13) cardiac dysrhythmias. HRV data was derived from each IBI data set and compared following application of a series of correction factors.

There was a very strong inter-class correlation of HRV parameters from both the Actiheart 5 and Polar V800 with the Televet 100 data. Both HRMs providing an acceptable level of agreement with both time and frequency domain HRV parameters. The data from the Actiheart 5 were found to be interchangeable with the Televet 100 in respect of beat detection whereas the Polar V800 data required a 0.4 s artefact correction to give best agreement. Both HRMs have utility in equine research for demonstrating the direction and magnitude of change in HRV parameters, although users must apply appropriate artefact correction to maximise data validity.

Key Words

HRV, Equine, Polar, Actiheart, ECG

1. Introduction

For behavioural scientists, a key criterion in validating any enrichment protocol or intervention is measurement of the animals' behavioural and psychological responses to its environment (Broom and Johnson, 1993). This is often achieved by quantification of cardiovascular, neurophysiological and/or biochemical parameters (humans, Vogel et al. (2019); horses, Borstel et al. (2017)). Many such parameters require collection of samples which can be invasive (blood) and/or require handling and/or restraint (saliva, urine), which is in itself a stressor (dependent on species) (Vitale et al., 2013; Borstel et al., 2017). Heart rate variability (HRV), the measure of variation in cardiac inter-beat interval (IBI), reflects autonomic nervous system activity (Von Borell et al., 2007) and is a key element of the body's response to stressors. For welfare assessment (Von Borell *et al.*, 2007), HRV has the advantage that it can be measured non-invasively and has been used in a range of species including dogs (Jonckheer-Sheehy et al., 2012; Katayama et al., 2016; Bowman et al., 2017), production animals (Marchant-Forde et al., 2004 ; Erdmann et al., 2018) and horses (Rietmann et al., 2004; Squibb et al., 2018).

Heart rate is influenced by the dynamic interplay between the activity of the sympathetic (SNS) and parasympathetic (PNS) branches of the autonomic nervous system (ANS) (Saul, 1990; Ottaviani, 2018). In response to a stressor, heart rate typically increases and there is a reduction in HRV, primarily reflecting the reduction in PNS activity and/or increased SNS activity (Quintana et al., 2016). HRV can be quantified with regard to i) time-domain parameters such as the Root Mean Square of the Successive Differences (RMSSD) or the Standard Deviation of the IBIs (SDNN) (Allen et al., 2007). ii) frequency-domain parameters that quantify what proportion of the signal can be accounted for by pure sine waves from very low, low and high frequency (VLF/LF/HF) bands (Kuss et al., 2008). iii) Non-linear parameters (SD1 and SD2), derived from Poincare plots, which give a measure of the complexity and unpredictability of the signal (Shaffer and Ginsberg, 2017). As HRV is calculated, as well as the accuracy of the raw IBI data, it is important to evaluate the *post-*

hoc processing of the IBI data to ensure that appropriate metrics are selected, and the accuracy of reported results.

The 'Gold Standard' for recording IBI data in any species is a 4, 6 or 12 lead electrocardiogram (ECG), however, for reasons of practicality, the use of 2 lead heart rate monitors (HRMs) is more popular within animal science, biology, exercise physiology and medical and veterinary science. The use of HRV to assess physiological stress has been driven by the advent of these wearable HRMs, primarily designed for human athletics training. While HRMs have been validated against ECGs for the collection of IBI data in humans (Giles et al., 2016), in animals, data errors are identifiable in HRM generated IBI data (Marchant-Forde et al., 2004). These errors may be attributable to interspecies differences in ECG profiles or body shape, which can affect data recordings. Horses present additional issues for IBI recording using HRMs, as the ECG of a horse exhibits a particularly labile T-wave, periodic changes in P-wave configuration, sinoatrial block and non-respiratory sinus arrhythmia (Durham, 2017). Furthermore, as horses exhibit a high vagal tone, up to 73% of clinically normal horses exhibit physiological 2nd-degree atrioventricular (AV) block (Eggensperger and Schwarzwald, 2017). During 2nd-degree AV block, the SA node fires and a normal P-wave can be observed, but the electrical impulse does not pass beyond the AV node, therefore there is no corresponding R-wave. As cardiac monitoring records the R-R interval, these missed beats effectively double the IBI where the block occurred and data must be corrected for missed beats if the R-R interval is to be used in HRV analysis as a metric of PNS activity.

The Polar S810 HRM (Polar Electro Oy, Kempele, Finland) has been validated for use in static horses (Parker et al., 2009; Ille et al., 2014) but discrepancies within IBI data have been reported when used with moving animals (Parker et al., 2009). While recent technological improvements (i.e., the newer Polar V800) could improve IBI data reliability (Randle et al., 2017) as demonstrated in humans (Giles et al., 2016), the V800 has not been validated for use with horses. An alternative HRM is the Actiheart 5 monitor (CamNtech

Ltd., Fenstanton, UK). This 2-lead system generates a full ECG trace, has been validated for use in humans (Barreira et al., 2009) has been used in some animal studies (Bouthegeourd et al., 2009; Sun et al., 2017), but has not been reported to have been validated for, or used with, horses.

The aims of the current study were, to 1) validate the use of two HRMs, (Polar V800 and Actiheart 5) in horses and 2) to establish which user-adjustable settings associated with HRM data analysis give the best agreement with Televet data to improve HRV accuracy and aid the reproducibility of equine studies.

2. Method

2.1. Subjects Test subjects were 17 horses (8 geldings, 9 mares) mean age 16 ± 1.7 years, mean height 164 ± 1.9 cm. Breed composition of the sample was 5 Irish Sport Horses, 3 Dutch Warmbloods, 4 Cobs, 4 Thoroughbreds and 1 Irish Draft. All were in light to moderate work and stabled at 1 of 4 yards in Northern Ireland or were teaching horses at the Weipers Centre Equine Hospital, University of Glasgow. The IBI recordings were all made in the horses' own stable, with horses at rest.

Following initial data analysis, the sample was sub-divided into those that displayed normal sinus rhythm (2 or less 2nd degree AV blocks over 30 min, Group S) ($n=13$, 6 geldings, 7 mares, mean age 15 ± 2.0 years, mean height 163 ± 2.3 cm) and those that displayed $>2\%$ cardiac dysrhythmias (Group D) ($n=4$, 2 geldings, 2 mares, mean age 20 ± 2.9 years, mean height 167 ± 2.4 cm). Sample size and power calculations can be found in supplementary file S1.

This study was approved by the University of Glasgow School of Veterinary Medicine Ethics and Welfare Committee and conducted in line with the International Society for Applied Ethology's ethical guidelines (Sherwin et al., 2017).

2.2. Data Collection Each horse was fitted with a 4-lead telemetric ECG (Televet 100, Jørgen Kruuse, Marslev, Denmark) and 2 HRMs (Polar V800 with H7 sensor, and Actiheart

5). The 4 leads of the Televet 100 were attached to Kruuse aqua-wet Ag/AgCl ECG electrodes and configured as per the Dubois method (da Costa et al., 2017) (selected to keep the electrodes away from the Polar belts). Two Polar equine science belts were fitted next to each other, with the cranial belt located as close to the elbow as possible. ECG conductive gel (Henry Schein Medical, Gillingham, UK) was liberally applied to the electrodes of the Polar belts to ensure uniform contact. One belt had a Polar H7 sensor fitted, the other the Actiheart 5. As there can be a slight delay from starting the Polar V800 and signal recording, the Televet and Actiheart were set to begin recording synchronously a few minutes after the Polar monitor. Recordings were made for 30 min whilst the horse remained at rest in their stable. Data from all three devices were up-loaded to a laptop for later analysis.

2.3. Data Analysis Televet data were processed using the Televet 100 5.1 software with the beat identification function enabled. Mis-identified beats were identified visually, and manually corrected, before the IBI data were exported as a .txt file. Actiheart data were processed using the Actiheart 5 software. Where an AV block occurred, and the IBI > 2800ms, the software does not recognise this as correct data and 'NA' is reported. Where this occurred, the NA was manually replaced by insertion of an IBI that was twice the mean of the IBI on either side of the reported NA. The resultant data file was then exported as a .txt file. The Polar data was uploaded into the Polar Flow software and exported as a .txt file. The three .txt files were compared. As the data are time-stamped, the Polar data collected prior to the initiation of recording with the other two devices was deleted to ensure it started on the same beat as the Televet and Actiheart.

HRV analysis was conducted on the raw data from each device using Kubios software (Kubios HRV Standard 3.4.1, Kubios Oy, Finland). The Kubios software allows the user to correct for artefacts such as missing, extra, or misaligned beats or dysrhythmias such as AV block or premature ventricular/atrial complexes (PVC/PAC) within the data. This is achieved using a threshold-based artefact correction algorithm which compares every IBI value

against the local median interval calculated from 30 successive beats. Where an IBI differs by more than the threshold value specified by the user (between 0.01 s and 0.99 s) it is replaced with an interpolated value using a cubic spline interpolation (Tarvainen et al., 2021). The user specified threshold value is referred to as the artefact correction threshold and can be reported in either seconds or as a percentage. For human studies, 0.25 s is specified by the software developers (Tarvainen et al., 2021) as a 'medium' artefact correction at 60 bpm. 0.3 s has previously been used for artefact correction in equine studies (Ille et al., 2014; Squibb et al., 2018) and was therefore selected as the first correction option for this study. While 0.3 s has been considered to represent a 30% threshold it should be noted that 0.3 s is only 30% when the IBI is exactly 1000 ms (i.e., HR is 60 bpm). For horses with a resting HR of c. 30 bpm, a 30% threshold loosely equates to a 0.6 s correction, so this was selected as the second correction option for this study. As AV block gives an IBI of double the local average interval (c.4 s versus c.2 s @ 30 bpm), a correction of 0.9 s is sufficient to pick up and correct for missed beats but not impact the rest of the data. Therefore, to determine the best agreement with Televet data to which a 0.9 s correction had been applied (and 2nd degree AV blocks removed), the Actiheart and Polar data had zero, 0.9 s, 0.6 s and 0.3 s corrections applied, and the results compared. Additional corrections of 0.45 s and 0.4 s were subsequently applied to the Polar data to identify the smallest possible bias.

Frequency bands of VLF 0 – 0.01 Hz, LF 0.01 – 0.07 Hz and HF 0.07 – 0.6 Hz were specified for equine-specific frequency domain analysis (Stucke et al., 2015) and the fast Fourier transform (FFT) output was used. Each 30 min recording was analyzed in 1 min epochs, and the results exported as a .csv file for statistical analysis in R Studio (running R version 4.0.2).

Reference ranges (mean \pm 1.96 x SD) for all the HRV parameters generated by the Kubios software were calculated from a log transform of the 30 min 0.9 s corrected Televet values for the 13 horses that had 2 or less 2nd degree AV blocks over 30 min (Group S).

2.4. Statistics The data for HR, RMSSD (time domain analysis) and HF Power (frequency domain analysis) were compared for each device and each correction level in R Studio, performing inter class correlations using the “psych” package, in which each device/correction level was treated as a single fixed rater (ICC3). Based on the estimated relationships between the three devices obtained using the ICC, an agreement interval was determined using the Bland Altman test of agreement (Giavarina, 2015). Acceptable limits were defined *a priori* as a bias within 5% of the sample median for that parameter (Lenoir et al., 2017) and with the 95% CI of that bias encompassing 0. HR acceptable bias < ± 2 bpm, RMSSD acceptable bias < ± 4 ms, HF Power acceptable bias < ± 128 ms².

3. Results

3.1. Artefact Correction. Table 1 shows the percentage and number of “beats” identified as erroneous for each horse, from all 3 devices, when a 0.9 s artefact correction threshold was applied in Kubios i.e., the IBI is >0.9 s longer than the local median interval. There was parity between the devices, as to the number of beats identified as artefact and corrected, but there was wide variance between horses. A high % of erroneous beats as seen in Horses 1,2,3 and 15 is indicative of dysrhythmia. Examination of individual ECG traces (data not shown) for horses 1,2 and 3 indicated that the majority of artefacts were due to 2nd degree AV block whereas horse 15 had previously been diagnosed with atrial fibrillation (AF) and displayed an irregular HR. The studied horses were therefore subdivided into groups S and D for subsequent analysis.

Table 1 –The number of “beats” identified as erroneous and the % of data that those beats represented for each horse from the S and D groups and for each device for the entire 30 min recording period when a 0.9 s artefact correction is applied in Kubios.

Horses in Group S	ECG		AH		Polar	
	%	Beats	%	Beats	%	Beats
4	0.11	1	0.11	1	0.23	2
5	0.07	1	0.07	1	0.08	1
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0.09	1
9	0	0	0	0	0.50	6
10	0.06	1	0.06	1	0	0
11	0	0	0	0	0.67	8
12	0.07	1	0.07	1	0.08	1
13	0	0	0	0	0.06	1
14	0.18	2	0.18	2	1.56	17
16	0.17	2	0.08	1	0	0
17	0.18	2	0	0	0	0
Horses in Group D						
1	2.14	17	2.16	18	2.05	17
2	8.19	61	8.17	61	9.80	74
3	17.21	126	17.19	129	18.36	121
15	7.88	106	7.60	103	7.68	104

Reference ranges were derived for all HRV parameters from the Kubios analysis of the Televet IBI data (with 0.9 s correction threshold), of the horse in Group S (Table 2).

Table 2 – HRV Reference Ranges for 13 resting healthy adult horses showing 2 or less 2nd degree AV blocks over 30 min (Group S). The range is the mean±1.96 x SD and uses equine-specific frequency bands for the analysis.

HRV Parameter	Reference range	Units
Mean RR	1,115 – 1,998	ms
Mean HR	30 - 54	beats/min
RMSSD	31 - 216	ms
NN50	165 – 1,244	beats
pNN50	13 - 100	%
SDANN	12 – 257	ms
SDNN index	73 - 222	ms
RR tri index	9 – 28	
TINN	227 – 1,095	ms
LF Power	604 – 3,529	ms ²
HF Power	547 – 12,191	ms ²
LF/HF Ratio	0.1 – 2.5	
SD1	22 - 152	ms
SD2	47 - 123	ms
SD2/SD1 ratio	0.6 – 2.7	
Approximate entropy	1.3 – 1.7	
Sample entropy	1.4 – 2.2	
Detrended fluctuations α 1	0.5 – 1.6	
Detrended fluctuations α 2	0.1 – 0.6	

When Kubios HRV analysis was applied to the uncorrected IBI data obtained from the horses in Group D (Table 3), both RMSSD and HF power estimates consistently fell outside the range seen in horses from Group S. Application of a 0.9 s correction threshold resulted in both the mean RMSSD and HF Power being brought within the reference range for the three horses with clinically normal dysrhythmias, but not for the horse with the severe pathological dysrhythmia.

Table 3- Mean RMSSD and HF power, for the horses in Group D (n=4) when the Televet IBI data were analysed with no artefact correction or following a 0.9 s threshold correction

Horse	RMSSD (ms)		HF Power (ms ²)	
	Normal range 31 – 216 ms		Normal range 547 – 12,191 ms ²	
	No Correction	0.9 s Correction	No Correction	0.9 s Correction
1	355	108	25612	3081
2	1024	162	269101	6691
3	1200	85	338023	1844
15	770	393	212620	60917

3.2. Inter-device correlation and agreement. The results of the interclass correlations for RMSSD and HF Power for each device and at each correction level tested are shown in Table 4. For the Actiheart (AH), the best correlation was seen for both parameters when a 0.9 s threshold for correction was applied. For the Polar data, the best correlation, was obtained when a correction of 0.4 s was applied.

Table 4 – ICC3 for data from Group S (n=13) relative to the 0.9 s corrected ECG (Televet) data.

Device/Correction	RMSSD	HF Power
AH 0	0.83	0.59
AH 0.9	0.97	0.99
AH0.6	0.94	0.8
AH 0.3	0.84	0.52
Polar.0	0.24	0.02
Polar.0.9	0.76	0.52
Polar.0.6	0.87	0.56
Polar 0.45	0.87	0.69
Polar 0.4	0.87	0.73
Polar.0.3	0.82	0.48

The results of the Bland-Altman tests of agreement for RMSSD and HF Power for Group S are shown in Table 5. The full agreement tables for HR, RMSSD and HF Power can be found in supplemental file S2. Only the Actiheart data with a 0.9 s correction and the Polar data with a 0.4 s correction gave an acceptable level of agreement with the 0.9 s corrected Televet data for both parameters, having a bias of < 5% (< 4 ms and < 128 ms²) and having the 95% CI encompassing 0.

Table 5 – Bland-Altman test of agreement on RMSSD and HF Power data against 0.9 s corrected ECG, Group S (n=13). Acceptable limits were defined as a bias within 5% of the sample median for that parameter and with the 95% CI of that bias encompassing 0. RMSSD acceptable bias < ± 4 ms, HF Power acceptable bias < ± 128 ms².

Device / Correction	RMSSD (ms)			HF Power (ms ²)		
	Bias (< ± 4 ms)	95% CI (Lower)	95% CI (Upper)	Bias (< ± 128 ms ²)	95% CI (Lower)	95% CI (Upper)
AH.0.9	0	-2	1	45	-51	141
Polar 0.4	0	-2	3	-90	-505	325
AH.0.6	1	-1	3	209	-123	542
Polar 0.45	-1	-4	1	-371	-855	112
Polar.0.3	5	2	8	471	-32	975
Polar.0.6	-4	-7	-1	-525	-1137	87
AH.0	-4	-7	0	-619	-1349	111
AH.0.3	6	3	9	683	232	1133
Polar.0.9	-11	-15	-7	-1681	-2642	-721
Polar.0	-31	-47	-14	-11096	-20151	-2042

The output from the Actiheart closely mirrored the Televet in that the closest agreement in time domain and frequency domain came when a 0.9 s artefact correction was applied. For the Polar data, a 0.9 s correction gave an underestimation well outside the acceptable levels of agreement for both time and frequency domain parameters. A stronger correction of 0.6 s also gave an underestimation in both parameters and a 0.3 s correction gave an over estimation. Further corrections of 0.45 s and 0.4 s were trialled, with 0.4 s providing an acceptable level of agreement in both parameters. This agrees with the ICC results in Table 4. HR data for both HRMs, regardless of the correction factor used, exhibited good agreement with the Televet data (ICC >0.99, bias < 0.2 bpm in every case, full agreement tables available in Supplementary File S2).

Figure 1 shows the Bland-Altman plots for the cardiac data from Group S derived from the Polar V800 (figures 1a-c) with a 0.4 s correction applied and the Actiheart (figures 1d-f) with a 0.9 s correction applied and show that, although both devices have a small bias when

compared with corrected (0.9 s) Televet data, the Actiheart provides narrower limits of agreement in all 3 parameters.

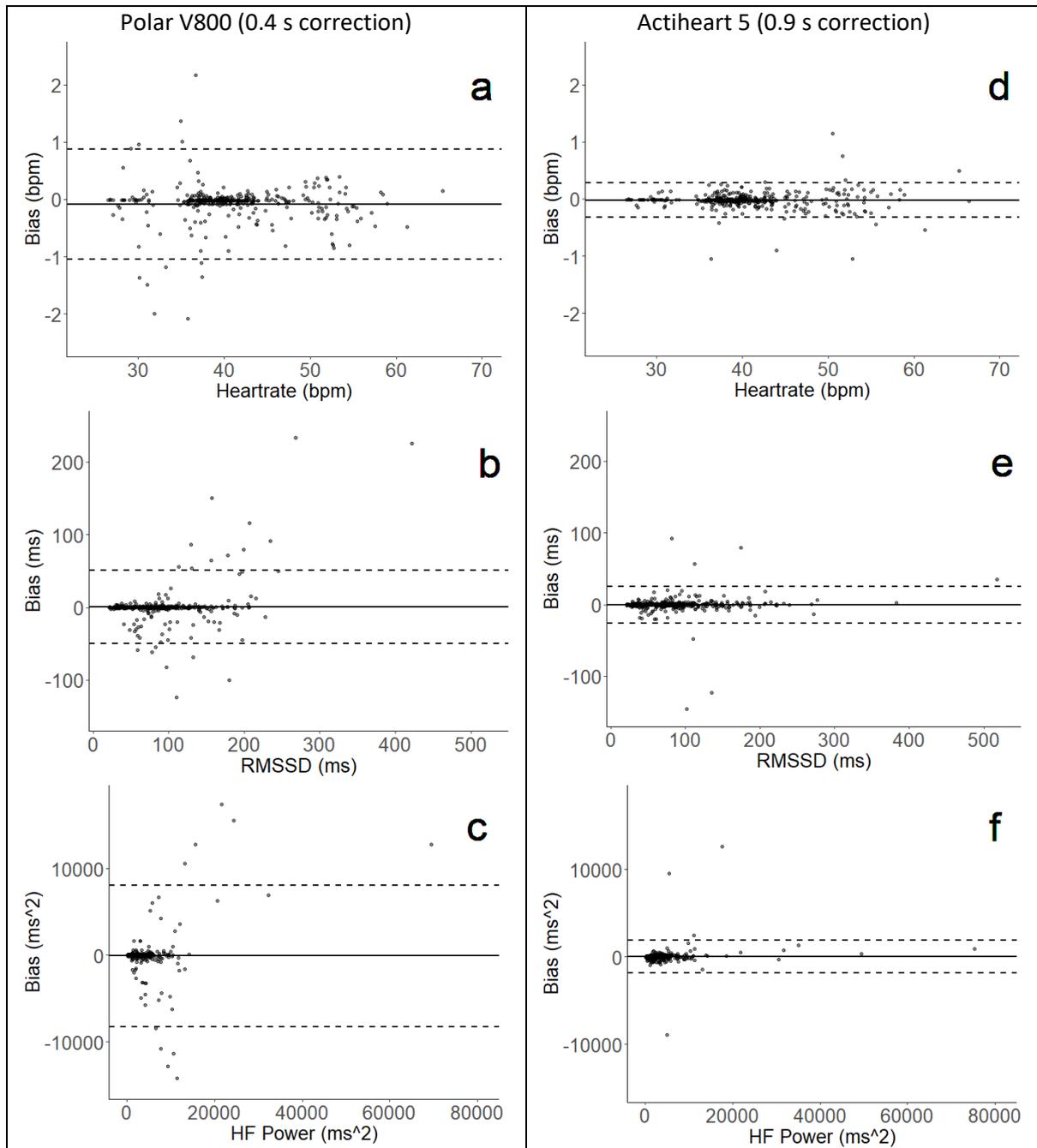


Figure 1 - Bland-Altman plots for cardiac data derived from the Polar V800 with a 0.4 s correction applied (figures 2a-c) and the Actiheart 5 (figures 2d-f) with a 0.9 s correction applied. The bias from corrected (0.9 s) Televet data is indicated by the solid line and the upper and lower levels of agreement are shown by the dashed lines.

4. Discussion

The assessment of physiological stress using HRV relies upon accurate collection and recording of IBI data. In horses, artefact correction is especially important in HRV analysis as a high proportion of horses (up to 73% (Eggensperger and Schwarzwald (2017)) express 2nd degree AV block, which can result in missed beats and the R-R interval not being a true reflection of vagal tone. The results of this study demonstrated that HRV data generated from the Actiheart 5 ECG (2-lead) is almost interchangeable with that obtained using a Televet 100 ECG (4-lead), and the best agreement between the IBI data sets was obtained when the same (0.9 s) artefact correction was applied to the data from both devices. The HRV data derived from the Polar V800 HRM also was also strongly correlated with, and showed acceptable agreement with the Televet 100 output, when a 0.4 s artefact correction was applied.

The most complete recordings of cardiac electrical activity are achieved using a 4, 6 or 12 lead ECG, however, due to their convenience and practicality HRM are often used to collect IBI data in animal studies. The high prevalence of 2nd degree AV block in a significant number of clinically normal horses at rest (Eggensperger and Schwarzwald, 2017) poses specific problems with regard to IBI recordings for equine HRV analysis, as 'missed beats' appear as intermittent double (or greater) IBIs which adds variance to, and compromises the accuracy of, the IBI data. HRV analysis software packages, such as Kubios, typically attempt to correct for 2nd Degree AV blocks by artefact correction, which effectively adds in the missing beat(s). When HRV analysis is being used to assess physiological stress, it is dependent on assessing the impact of the autonomic nervous system on SA node activity. As such while artefact correction could be considered a manipulation of the data, it should be noted that in horses with 2nd degree AV block, electrical activity is initiated as normal in the SA node, but is not transmitted to the ventricles, as such the use of IBI data based on the R-R recordings would not be representative of the direct effects of vagal tone on the SA node (Gordan et al., 2015). If an alternative metric, such as the P-P interval, which is

unaffected in 2nd degree AV block were to be used, a more complete record of SA node activity would be achieved (Eggensperger and Schwarzwald 2017), however, this is not recorded by typical HRMs which rely on the R-R interval. Furthermore, the missed beats added during artifact correction should not skew the overall data as the use of a cubic spline interpolation means that added beats are representative of the surrounding beats. Kubios software reports how many beats and what percentage of the data have been corrected, which also allows the user to decide whether the data set is compromised. Indeed, it is common practice to discard data that has been corrected by more than 5% (Von Borell et al., 2007; Schöberl et al., 2015). As ECGs provide the complete electrical profile of cardiac activity, this allowed for the visual correction of the Televet data for misidentified beats prior to HRV analysis in the current study, again by insertion of IBIs based on the surrounding recorded IBI data.

When subjected to HRV analysis, with a 0.9 s artefact correction, the number of errors in the Actiheart data of the Group S horses (very low incidence of 2nd degree AV block) almost mirrored that of the Televet 4 lead ECG. Given that it is not possible to view the electronic profile to allow data correction when using Polar HRMs, it was reassuring to see good agreement between the Polar IBI data for the Group S horses, with regard to percentage of beats identified as artefact (with a 0.9 s correction) and so it is likely that the Kubios software is also able to identify and correct for 2nd degree AV block within Polar IBI data. The validity of the 0.9 s correction to identify and remove 2nd degree AV block artefacts from equine IBI data is seen when this correction was applied to the IBI data from horses with cardiac dysrhythmias, namely horses 1, 2 and 3. Without application of the correction their RMSSD and HF Power were outside the range determined from the Televet 4 lead ECG data but their HRV parameters were brought within the range of the horses without dysrhythmia when a 0.9 s artefact correction was applied. It is also notable that a 0.9 s correction was not able to bring the HRV results from horse 15 back within the reference range. This horse had previously been diagnosed with atrial fibrillation as opposed to 2nd degree AV block.

Therefore, a 0.9 s artefact correction can be applied to clinically normal, but not to clinically abnormal, horse data to eliminate any erroneous effects of 2nd degree AV block on HRV analysis.

To determine the most appropriate correction thresholds to apply with the HRM generated data we compared the effects of different levels of artefact correction across the range 0.3-0.9 s, reported previously to have been used in HRV analysis (Ille et al., 2014; Squibb et al., 2018) against the data generated using the Televet 4 lead ECG. The results indicated that the Actiheart data, with 0.9 s artefact correction was comparable to the Televet data. The use of a 0.9 s correction with the Polar data resulted in both time and frequency domain HRV parameters falling outside acceptable levels of agreement with the Televet data.

Application of a 0.4 s correction, however, did bring the data in-line with that generated with the Televet. It is likely that this stronger level of correction is required to remove artefacts from the Polar data that were manually removed from both the Actiheart and Televet data, a step which is not possible without access to the full ECG waveform (Quintana et al., 2016).

It is of note that between the three devices for recording IBI data the level of agreement with regard to heartrate was good regardless of the correction applied. When considering the agreement with regard to RMSSD and HF power, the observed decrease in agreement with regard to HF Power is to be expected, as frequency domain data parameters are highly sensitive to missing or misclassified beats, so even small inconsistencies will be magnified leading to a reduction in agreement (Parker et al., 2009; Sheridan et al., 2020). The statistical weakness of the spectral estimation of frequency domain data when compared to time domain data (Kuss et al., 2008) may also explain why closer agreement was achieved with RMSSD than with HF Power.

It should be noted that the above use and validation study was only conducted on horses at rest. If used on horses during exercise, while the incidence of 2nd degree AV block would be expected to decrease, the 0.9 s correction to Actiheart IBI data and a 0.4 s artefact correction with Polar IBI data should still be applied, although it would not be expected to be

required as much as there would be fewer missed beats. Of course, exercise would bring additional challenges for IBI data recording, for instance the electrodes would require more robust attachment to ensure all electrical data is collected. Indeed, it has been hypothesised that movement of the Polar belt and consequent loss of signal and data may account for the additional artefacts and the reduced agreement with ECG recordings reported in equine (Parker et al., 2009; Lenoir et al., 2017) and human studies (See Georgiou et al. (2018) for review). Similar considerations would also apply to the Actiheart which can use conventional ECG electrodes instead of the Polar belt, but further work is required to validate this method of attachment of electrodes for exercise assessment in horses.

5. Conclusion

The Polar V800 provides a strong ICC with a Televet 100, 4 lead ECG, in static horses, and an acceptable agreement for both time and frequency domain HRV parameters. Second degree AV block can be removed from Polar IBI data using a 0.9 s correction within Kubios but a more stringent correction of 0.4 s gives the best agreement with the Televet data corrected for 2nd degree AV block. While this can be acceptable, it must be noted that stronger artefact correction risks greater proportional rejection of data which may alter data validity, and a predetermined cut-off should be instigated after which data is rejected. The Actiheart 5 produces a full ECG waveform which allows for inspection of the raw electrical data as opposed to just the IBI data output from HRMs such as Polar. Availability of the full ECG allows for manual correction of beat misidentification/errors and thus Actiheart IBI data can have a better agreement with the Televet data (when both had a 0.9 s artefact correction applied) than the Polar V800 data. In static horses, the beat detection of the Actiheart 5 is interchangeable with the Televet 100 for the purpose of HRV analysis, but further work is required to validate the use of the Actiheart 5 with exercising horses.

Conflict of Interest

None.

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References

- Allen, J.J., Chambers, A.S., Towers, D.N., 2007. The many metrics of cardiac chronotropy: A pragmatic primer and a brief comparison of metrics. *Biol Psychol* 74, 243-262. <https://doi.org/10.1016/j.biopsycho.2006.08.005>
- Barreira, T.V., Kang, M., Caputo, J.L., Farley, R.S., Renfrow, M.S., 2009. Validation of the Actiheart monitor for the measurement of physical activity. *Int J Exerc Sci* 2, 7. <https://digitalcommons.wku.edu/ijes/vol2/iss1/7/>
- Borstel, U.K.v., Visser, E., Hall, C., 2017. Indicators of stress in equitation. *Appl Anim Behav Sci* 190, 43-56. <https://doi.org/10.1016/j.applanim.2017.02.018>
- Bouthegourd, J.-C., Kelly, M., Clety, N., Tardif, S., Smeets, D., 2009. Effects of weight loss on heart rate normalization and increase in spontaneous activity in moderately exercised overweight dogs. *Int J Appl Res Vet Med* 7, 153. <http://www.jarvm.com/articles/Vol7Iss4/Kelly.pdf>
- Bowman, A., Scottish, S., Dowell, F., Evans, N., 2017. The effect of different genres of music on the stress levels of kennelled dogs. *Physiol Behav* 171, 207-215. <https://doi.org/10.1016/j.physbeh.2017.01.024>
- Broom, D.M., Johnson, K.G., 1993. Stress and strain, welfare and suffering, in: Broom, D.M., Johnson, K.G. (Eds.), *Stress and Animal Welfare*, Chapman and Hall, London, pp. 57-86. <https://doi.org/10.1007%2F978-3-030-32153-6>
- da Costa, C.F., Samesima, N., Pastore, C.A., 2017. Cardiac Mean Electrical Axis in Thoroughbreds—Standardization by the Dubois Lead Positioning System. *PLoS One* 12 (1). <https://doi.org/10.1371/journal.pone.0169619>
- Durham, H.E., 2017. Equine Cardiology, in: Durham, H.E. (Ed.), *Cardiology for Veterinary Technicians and Nurses*. John Wiley and Sons, Hoboken, NJ 07030, USA, pp. 407-442. <https://doi.org/10.1002/9781119357407>
- Eggensperger, B., Schwarzwald, C.C., 2017. Influence of 2nd-degree AV blocks, ECG recording length, and recording time on heart rate variability analyses in horses. *J Vet Cardiol* 19, 160-174. <https://doi.org/10.1016/j.jvc.2016.10.006>

Erdmann, S., Mohr, E., Derno, M., Tuchscherer, A., Schäff, C., Börner, S., Kautzsch, U., Kuhla, B., Hammon, H., Röntgen, M., 2018. Indices of heart rate variability as potential early markers of metabolic stress and compromised regulatory capacity in dried-off high-yielding dairy cows. *Animal* 12, 1451-1461. <https://doi.org/10.1017/S1751731117002725>

Georgiou, K., Larentzakis, A.V., Khamis, N.N., Alsuhaibani, G.I., Alaska, Y.A., Giallafos, E.J., 2018. Can wearable devices accurately measure heart rate variability? A systematic review. *Folia Med* 60, 7-20. <https://doi.org/10.2478/foimed-2018-0012>

Giavarina, D., 2015. Understanding bland altman analysis. *Biochemia Medica: Biochemia Medica* 25, 141-151. <https://doi.org/10.11613/BM.2015.015>

Giles, D., Draper, N., Neil, W., 2016. Validity of the Polar V800 heart rate monitor to measure RR intervals at rest. *Eur J Appl Physiol* 116, 563-571. <https://doi.org/10.1007/s00421-015-3303-9>

Gordan, R., Gwathmey, J.K., Xie, L.-H., 2015. Autonomic and endocrine control of cardiovascular function. *World J Cardiol* 7, 204. <https://dx.doi.org/10.4330%2Fwjcv7.i4.204>

Ille, N., Erber, R., Aurich, C., Aurich, J., 2014. Comparison of heart rate and heart rate variability obtained by heart rate monitors and simultaneously recorded electrocardiogram signals in nonexercising horses. *J Vet Behav* 9, 341-346. <https://doi.org/10.1016/j.jveb.2014.07.006>

Jonckheer-Sheehy, V.S.M., Vinke, C.M., Ortolani, A., 2012. Validation of a Polar® human heart rate monitor for measuring heart rate and heart rate variability in adult dogs under stationary conditions. *Journal of Veterinary Behavior* 7, 205-212. <https://doi.org/10.1016/j.jveb.2011.10.006>

Katayama, M., Kubo, T., Mogi, K., Ikeda, K., Nagasawa, M., Kikusui, T., 2016. Heart rate variability predicts the emotional state in dogs. *Behav Processes* 128, 108-112. <https://doi.org/10.1016/j.beproc.2016.04.015>

Kuss, O., Schumann, B., Kluttig, A., Greiser, K.H., Haerting, J., 2008. Time domain parameters can be estimated with less statistical error than frequency domain parameters in the analysis of heart rate variability. *J Electrocardiol* 41, 287-291. <https://doi.org/10.1016/j.jelectrocard.2008.02.014>

Lenoir, A., Trachsel, D.S., Younes, M., Barrey, E., Robert, C., 2017. Agreement between electrocardiogram and heart rate meter is low for the measurement of heart rate variability during exercise in young endurance horses. *Front Vet Sci* 4, 170. <https://doi.org/10.3389/fvets.2017.00170>

Marchant-Forde, R., Marlin, D., Marchant-Forde, J., 2004. Validation of a cardiac monitor for measuring heart rate variability in adult female pigs: accuracy, artefacts and editing. *Physiol Behav* 80, 449-458. <https://doi.org/10.1016/j.physbeh.2003.09.007>

Ottaviani, C., 2018. Brain-heart interaction in perseverative cognition. *Psychophysiology* 55. <https://doi.org/10.1111/psyp.13082>

Parker, M., Goodwin, D., Eager, R.A., Redhead, E.S., Marlin, D.J., 2009. Comparison of Polar® heart rate interval data with simultaneously recorded ECG signals in horses. *Comp Exerc Physiol* 6, 137-142. <https://doi.org/10.1017/S1755254010000024>

Quintana, D., Alvares, G.A., Heathers, J., 2016. Guidelines for Reporting Articles on Psychiatry and Heart rate variability (GRAPH): recommendations to advance research communication. *Transl Psychiatry* 6, e803-e803. <https://doi.org/10.1038/tp.2016.73>

Randle, H., Steenbergen, M., Roberts, K., Hemmings, A., 2017. The use of the technology in equitation science: A panacea or abductive science? *Appl Anim Behav Sci* 190, 57-73. <https://doi.org/10.1016/j.applanim.2017.02.017>

Rietmann, T.R., Stauffacher, M., Bernasconi, P., Auer, J.A. and Weishaupt, M.A., 2004. The association between heart rate, heart rate variability, endocrine and behavioural pain measures in horses suffering from laminitis. *J Vet Med A*, 51(5), 218-225. <https://doi.org/10.1111/j.1439-0442.2004.00627.x>

Saul, J.P., 1990. Beat-to-beat variations of heart rate reflect modulation of cardiac autonomic outflow. *Physiol* 5, 32-37. <https://doi.org/10.1152/physiologyonline.1990.5.1.32>

Schöberl, I., Kortekaas, K., Schöberl, F.F., Kotrschal, K., 2015. Algorithm-supported visual error correction (AVEC) of heart rate measurements in dogs, *Canis lupus familiaris*. *Behav Res Methods* 47, 1356-1364. <https://doi.org/10.3758/s13428-014-0546-z>

Shaffer, F., Ginsberg, J.P., 2017. An Overview of Heart Rate Variability Metrics and Norms. *Front Public Health* 5, 258-258. <https://doi.org/10.3389/fpubh.2017.00258>

Sheridan, D.C., Dehart, R., Lin, A., Sabbaj, M., Baker, S.D., 2020. Heart Rate Variability Analysis: How Much Artifact Can We Remove? *Psychiatry Investig* 17, 960. <https://dx.doi.org/10.30773%2Fpi.2020.0168>

Sherwin, C., Christiansen, S., Duncan, I., Erhard, H., Lay, D., Mench, J., O'Connor, C., 2017. Ethical Treatment of Animals in Applied Animal Behaviour Research. <https://dx.doi.org/10.30773%2Fpi.2020.0168>

Squibb, K., Griffin, K., Favier, R., Ijichi, C., 2018. Poker Face: Discrepancies in behaviour and affective states in horses during stressful handling procedures. *Appl Anim Behav Sci* 202, 34-38. <https://doi.org/10.1016/j.applanim.2018.02.003>

Stucke, D., Ruse, M.G., Lebelt, D., 2015. Measuring heart rate variability in horses to investigate the autonomic nervous system activity—Pros and cons of different methods. *Appl Anim Behav Sci* 166, 1-10. <https://doi.org/10.1016/j.applanim.2015.02.007>

Sun, Y., Lian, X., Bo, Y., Guo, Y., Yan, P., 2017. Effects of 20-day litter weight on weaned piglets' fighting behavior after group mixing and on heart rate variability in an isolation test. *Asian-Australas J Anim Sci* 30, 267. <https://dx.doi.org/10.5713%2Fajas.16.0215>

Tarvainen, M., Lipponen, J.A., Niskanen, J.-P., Ranta-Aho, P.O., 2021. Kubios HRV Software User's Guide V3.5. https://www.kubios.com/downloads/Kubios_HRV_Users_Guide.pdf (accessed 13/07/2021)

Vitale, V., Balocchi, R., Varanini, M., Sgorbini, M., Macerata, A., Sighieri, C., Baragli, P., 2013. The effects of restriction of movement on the reliability of heart rate variability measurements in the horse (*Equus caballus*). *J Vet Behav* 8, 400-403. <https://doi.org/10.1016/j.jveb.2013.02.003>

Vogel, J., Auinger, A., Riedl, R., 2019. Cardiovascular, Neurophysiological, and Biochemical Stress Indicators: A Short Review for Information Systems Researchers, *Information Systems and Neuroscience*, Springer, pp. 259-273. <https://doi.org/10.1007/978-3-030-01087-4>

Von Borell, E., Langbein, J., Després, G., Hansen, S., Leterrier, C., Marchant-Forde, J., Marchant-Forde, R., Minero, M., Mohr, E., Prunier, A., 2007. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals—a review. *Physiol Behav* 92, 293-316. <https://doi.org/10.1016/j.physbeh.2007.01.007>

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