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Heart rate variability during high-speed treadmill exercise and recovery in Thoroughbred racehorses presented for poor performance

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Abstract

Background: Heart rate variability (HRV) analysis measures the inter-beat interval variation of successive cardiac cycles. Measurement of these indices has been used to assess cardiac autonomic modulation and for arrhythmia identification in exercising horses.

Objectives: To report HRV indices during submaximal exercise, strenuous exercise and recovery, and explore relationships with clinical conditions (arrhythmias, lameness, equine gastric ulcer syndrome [EGUS], lower airway inflammation and upper respiratory tract obstructions [URTOs]) in Thoroughbred racehorses.

Study design: Retrospective, observational cross-sectional study.

Methods: One hundred and eighty Thoroughbred horses underwent a treadmill exercise test with simultaneous electrocardiographic recording. Time-domain HRV indices (standard deviation of the R–R interval [SDRR]; root mean square of successive differences [RMSSD]) were derived for submaximal and strenuous exercise and recovery segments. Clinical conditions (arrhythmia [during each phase of exercise], lameness, EGUS, lower airway inflammation and URTO) were assigned to binary categories for statistical analysis. Relationships between selected HRV indices and the clinical conditions were explored using linear regression models.

Results: During submaximal exercise, lameness was associated with decreased logRMSSD ($B = -0.19$ 95% confidence interval [CI] -0.31 to -0.06, $p = 0.006$) and arrhythmia was associated with increased logRMSSD ($B = 0.31$ 95% CI 0.01–0.608, $p = 0.04$). During strenuous exercise, arrhythmia was associated with increased HRV indices (logSDRR $B = 0.51$ 95% CI 0.40–0.62, $p < 0.001$; RMSSD $B = 0.60$ 95% CI 0.49–0.72, $p < 0.001$). During recovery, arrhythmia was associated with increased HRV indices (logSDRR $B = 0.51$ 95% CI 0.40–0.62, $p < 0.001$, logRMSSD $B = 0.60$ 95% CI 0.49–0.72, $p < 0.001$).

Main limitations: The main limitations of this retrospective study were that not every horse had the full range of clinical testing, therefore some horses may have had undetected abnormalities.

Conclusions: The presence of arrhythmia increased HRV in both phases of exercise and recovery. Lameness decreased HRV during submaximal exercise.

KEYWORDS
arrhythmia, cardiology, electrocardiogram, equine, horse

1 | INTRODUCTION

Analysis of heart rate variability (HRV) measures the inter-beat interval variation of successive cardiac cycles. There are several methods to assess HRV, including time, frequency, and non-linear domain analyses. Time-domain analysis is computationally the simplest and most consistently used across human studies.[1] The two most common measures are the standard deviation of R–R intervals (SDRR), a measure of overall variability, and the root mean square of successive differences of R–R intervals (RMSSD), a measure of beat-to-beat variability.[2] In human studies RMSSD is preferred because of increased sensitivity while being less affected by respiratory and heart rate or recording duration, and because it largely reflects parasympathetic activity.[3]

HRV analysis provides insight into the responses of the autonomic nervous system in a range of conditions.[4] Traditionally, higher HRV indices were associated with health while lower HRV indices were associated with health impairments[5] like chronic pain[6] and asthma,[7] however more recent research has shown this to be an oversimplification: several pathological conditions increase HRV[2] including cardiac conduction abnormalities and atrial fibrillation.[5] Most studies have utilised HRV analysis from recordings obtained in resting patients and while it is known that HRV indices decrease in response to exercise,[1] much less is known about the clinical value of HRV indices obtained during exercise.

A previous study suggested HRV analysis during exercise may be of utility as an assessment of autonomic modulation only at low exercise intensities.[8] More recently, HRV analysis from horses exercising at higher intensities has been used for arrhythmia identification.[9,10] As arrhythmias occurring during strenuous exercise are potentially clinically concerning, if HRV analysis is to be clinically useful for arrhythmia identification, it requires further proof of efficacy during strenuous exercise. It is important to establish what clinically useful information can be gained from HRV analysis during all phases of the exercise and recovery period.

The objectives of this study were to:

1. Investigate relationships between HRV indices and arrhythmia in Thoroughbred racehorses during phases of exercise and recovery. The hypothesis was that the presence of arrhythmia would increase HRV.
2. Investigate relationships between HRV indices and common causes of poor performance in Thoroughbred racehorses during phases of exercise and recovery. The hypothesis was that pain and/or hypoxia would reduce HRV.

2 | MATERIALS AND METHODS

2.1 | Study sample

A retrospective review of the clinical records of Thoroughbred racehorses presented for high-speed treadmill investigation of poor performance between 2007 and 2018 was conducted to identify suitable cases (Figure 1). Inclusion criteria were, a complete electrocardiographic (ECG) recording throughout a treadmill exercise test and at least 5 min of recovery with no loss of connectivity throughout. Horses in which the peak heart rate was <200 bpm were excluded from the study to ensure that all horses exercised strenuously during the exercise test. All horses underwent a full clinical examination, including an orthopaedic evaluation and assignment of lameness grade on the 0–10 scale commonly used in the United Kingdom,[11] performed by sports medicine clinicians. Any horses identified with clinical abnormalities consistent with poor performance, for example, high-grade lameness (>4/10) or persistent atrial fibrillation did not proceed to treadmill exercise. All horses were deemed fit to complete the treadmill test based on clinical judgement and had completed a minimum of three treadmill training sessions before testing. All horses underwent upper airway endoscopy and ECG during the treadmill test. Some horses underwent further clinical investigations, with trainer/owner consent, including lower airway evaluations (post-exercise tracheal endoscopy, tracheal wash [TW], bronchoalveolar lavage [BAL]) and/or gastroscopy based on clinical recommendations (see Figure 1 for experimental design). One hundred and twenty-three of the horses included in this study were included in a previous study.[12]

2.2 | Exercise test

Warmup consisted of 20 min on a mechanical walker plus exercise on the treadmill on a 7.5% incline for 2 min at walk (1.8 m/s), 4 min at trot (3.5 m/s) and 1 min at canter (6 or 7 m/s). For most horses, a standardised incremental exercise test (SIET) was then performed, consisting of 1 min at each of 6, 8 and 10 m/s on a 10% incline, followed by further increments of 1 m/s at 1 min intervals. The exercise test was continued until either a definitive diagnosis of an upper respiratory tract obstruction (URTO) was made, or to the point of fatigue. From 2011, a high-speed exercise test (HSET) was introduced for horses in training for flat racing consisting of 1 min at each of 11 and 12 m/s on a 10% incline.[13] Following strenuous exercise, horses were walked on the treadmill on a 0% incline for a 5-min recovery phase, washed off and returned to the mechanical walker for 20 min

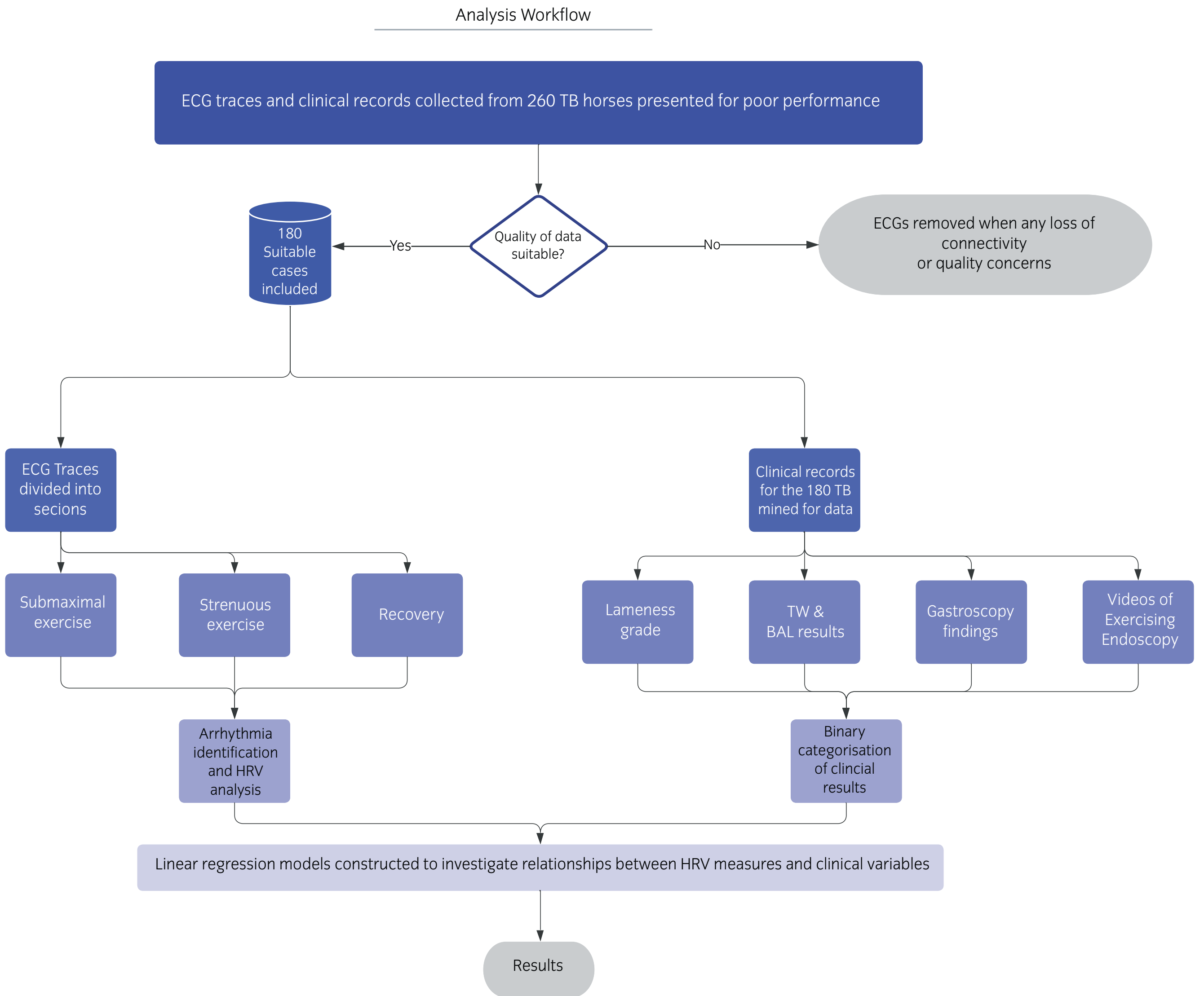

FIGURE 1 Flow diagram of experimental design. BAL, bronchoalveolar lavage; ECG, electrocardiogram; HRV, heart rate variability; TB, Thoroughbred; TW, tracheal wash.

2.3 | Electrocardiogram and HRV analysis

Self-adhesive electrodes (Kruuse ECG electrodes, Kruuse) were placed in a modified base-apex configuration.[14] The recordings were obtained using a telemetric ECG system (Televet 100, Engel Engineering System Gmbh). The ECG was recorded throughout the duration of treadmill exercise and recovery, with the 50 Hz artefact filter on. ECG analysis was performed using standard software (Televet 100, Engel Engineering System Gmbh) which was set to detect the largest deflection, which in a horse is typically the S wave (referred to as R waves for consistency of inter-species nomenclature) and the software set to highlight R–R deviations of 8%. Manual correction of R wave detection, and the ECG findings recorded in the horse's file, were verified by two operators (AH and WS), with any queries on arrhythmia diagnosis being reviewed by a specialist sports medicine clinician (KA). ECG recordings with loss of bluetooth connectivity, or with sub-optimal quality were excluded from the study. No ectopic beats or arrhythmias were deleted from the recording. Raw ECG files were divided into 'submaximal', 'exercise' and 'recovery' segments. Submaximal exercise was defined as the period of relative heart rate stability without steep increases or decreases and the horses were mostly trotting with individual instantaneous heart rates (between sinus beats) ranging between 44 and 148 bpm. Strenuous exercise was the period from the start of the plateau at gallop, after the steep increase in heart rate, to the point at which the heart rate sharply decreased (Figure 2). The duration of peak exercise was derived from this portion of the ECG. Recovery was defined as the 5-min period starting from the onset of the steep decline in heart rate following peak heart rate.

The corrected R–R time file was imported into HRV software (Kubios 3.2 for Windows, Kubios HRV Software) that calculated the time-domain measures: the standard deviation of R–R intervals (SDRR) and the root mean square of successive differences (RMSSD). Kubios results were all calculated from the non-detrended R–R series

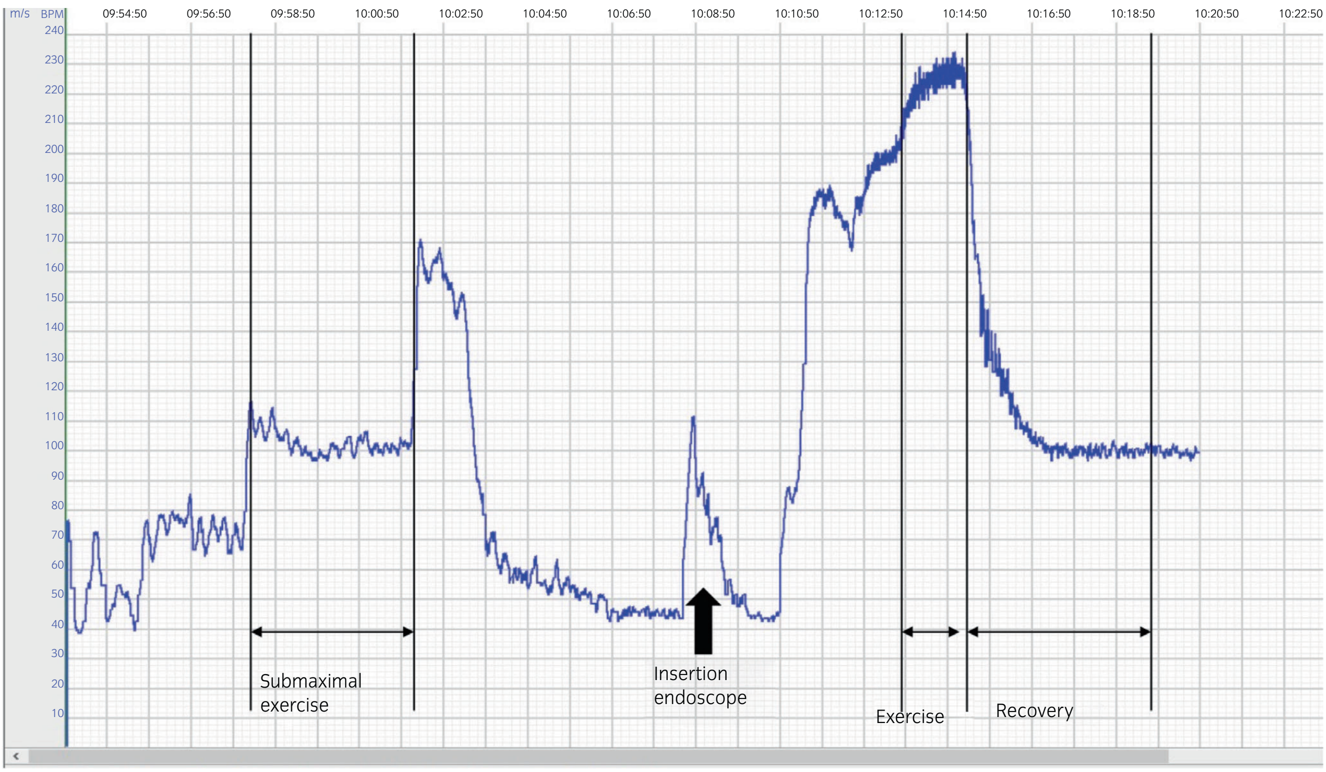

FIGURE 2 Tachogram of the treadmill exercise test showing sections used for HRV analysis. HRV, heart rate variability.

with beat, artefact, noise correction and quality detection turned off. There were no limits applied to the RR intervals for the time domain analysis. The duration of the segments analysed was variable and cut from the complete ECG as described above.

2.4 | Clinical classifications

2.4.1 | Arrhythmia

Arrhythmias during submaximal exercise, exercise and recovery were categorised separately. Horses were assigned to one of two categories for each period of recording: Group 1 (no arrhythmias recorded) or Group 2 (arrhythmias recorded). Horses were assigned to the arrhythmia groups (arrhythmia during submaximal exercise, strenuous exercise, and recovery) if they had any form of arrhythmia, including sinus arrhythmia, atrioventricular block, premature depolarisations or paroxysmal atrial fibrillation. Arrhythmias were classified in accordance with previous descriptions,[15] by the clinicians responsible for the case, these classifications were reviewed and a final classification was made by one researcher (KA).

2.4.2 | Upper airway obstruction

An endoscope was placed after the warmup exercise, allowing examination of the upper respiratory tract (URT) during the exercise test. For this study, clinical reports and endoscopy recordings were reviewed by a single specialist equine sports medicine clinician (KA) to categorise horses with URTO. Horses were assigned to one of two categories; Group 1 (no or minor abnormalities) or Group 2 (URTO deemed likely to be clinically relevant to the poor performance). Examples of URTO categorised as clinically relevant included dorsal displacement of the soft palate and recurrent laryngeal neuropathy. Changes considered unlikely to be clinically relevant included slight flattening of the epiglottis or slight laxity in the aryepiglottic folds.

2.4.3 | Lower airway inflammation

Tracheal endoscopy, TW and BAL were obtained as previously described,[16] 30–60 min following the treadmill test. Horses were assigned to one of two categories; Group 1 (no or minor abnormalities including tracheal mucus 0–1/5, TW neutrophils <20% or BAL fluid with <10% neutrophils, <5% mast cells or <5% eosinophils) or Group 2 (the presence of any of the following: tracheal mucus >1/5,[17] TW neutrophilia greater than 20%,[17] BAL fluid containing >10% neutrophils, >5% mast cells or >5% eosinophils).[18]

2.4.4 | Gastric ulceration

Gastroscopy was performed on sedated horses after treadmill exercise testing at the discretion of the clinician and trainer. Squamous lesions were graded 0–4 and glandular lesions were graded 0–4 along with description.[19] Horses were assigned to one of two categories;

Group 1 (no or minor abnormalities including squamous ulceration graded 0–2 and/or glandular disease graded 0–2) or Group 2 (squamous ulceration graded 3–4 and/or glandular disease graded 3–4). If squamous and glandular categories were discordant, horses were assigned to Group 2.

2.4.5 | Lameness

For the purposes of this study, the lameness grade used was a 10-point scale,[11] based on horses trotting in a straight line, on a hard surface. Horses were assigned to one of two categories: Group 1 (no or minor lameness [graded ≤1/10]) and Group 2 (lameness graded 2–4/10).

2.5 | Data analysis

The data were analysed using IBM SPSS (version 25 for Windows, IBM United Kingdom Limited). Distribution and variance of the HRV measures were tested for normality using Kolmogorov–Smirnov tests and visual inspection of histograms. For the periods of submaximal exercise, strenuous exercise and recovery, univariate linear regression models were constructed to assess associations between each HRV measure as the dependent variable and the following clinical variables: arrhythmia during the same period, HSET versus SIET, lameness, gastric ulceration, upper airway obstruction and airway inflammation, each categorised as described above. For the strenuous exercise and recovery period, the variables explored also included arrhythmia during submaximal exercise and time in exercise, and for the recovery period arrhythmia during exercise was included. Variables associated with the dependent variable at the 20% level in the univariate analysis were then entered into a backwards, stepwise multivariable linear regression model. The residuals were plotted against the predicted values and inspected visually to assess for normality; if this assumption was violated the variables were Log transformed, following which the residuals were re-inspected for normality. A Student's t test was used to compare the ages of horses undertaking a SIET versus an HSET. Receiver operator characteristic (ROC) curve analysis was performed for each HRV measure, in each phase of exercise or recovery, to investigate potential cut-offs to discriminate horses that experienced arrhythmia from those that did not. The area under the curve (AUC) was evaluated to establish the validity of the ROC curve. Examination of the ROC curves to define thresholds to identify the presence of an arrhythmia, with each HRV measure, was attempted using the sensitivity and specificity of each coordinate. The level of significance was set at $p < 0.05$.

3 | RESULTS

3.1 | Clinical data

Data were available for 180 Thoroughbred horses who had an age range from 2 to 10 years (mean 5.4; SD 1.9). Of the 180 horses, 50 raced on the flat, 127 were National Hunt horses and 3 horses raced in both categories. Lameness grade was recorded in 109 horses, 62 (57%) were graded with ≥2/10 lameness. The highest lameness score was 3/10. In 128 /179 (72%) horses, URTO that was considered relevant to the poor performance was identified. Data for URTO was missing for one horse. Post-exercise tracheal endoscopy was performed in 114 horses, BAL was performed in 85 horses with 74 (65%) horses characterised as having lower airway inflammation. Gastroscopic examination results were available for 84 horses, with 58 (69%) having grade 3–4 squamous and/or glandular lesions. Horses undertaking the HSET ($n = 12$) had mean age of 3.5 years (SD 1.34; minimum 2, maximum 5 years), whereas horses undertaking the SIET ($n = 168$) were significantly older (mean age of 5.6 years [SD 1.91; minimum 2, maximum 10 years]; $p < 0.001$).

An arrhythmia was observed in 10/176 (5.7%) horses during submaximal exercise, 71/180 (39.4%) horses during strenuous exercise and 131/180 (72.7%) horses during recovery, the classifications of arrhythmias are presented in Table 1.

3.2 | Heart rate variability

The HRV results grouped by the clinical variables are presented in Figures 3 and 4 and summary statistics are provided in Table S1. Taking a high-speed treadmill test (HSET), or not, was the only

TABLE 1 Arrhythmia data for exercise periods

Submaximal exercise period	Arrhythmia	SVPD isolated	SVPD couplet	SVPD triplet	VPD isolated	VPD couplet	VPD triplet	SA	2nd AVB	VT	PAF	IVR
Frequency ($n =$)	10	6	0	0	3	0	0	1	0	0	0	0
Peak exercise period												
Frequency ($n =$)	71	38	0	1	46	1	0	0	0	0	0	0
Recovery exercise period												
Frequency ($n =$)	131	51	4	2	79	39	8	18	2	26	2	2

Abbreviations: IVR, idioventricular rhythm; PAF, paroxysmal atrial fibrillation; SA, sinus arrhythmia; SVPD, supraventricular premature depolarisation; VPD, ventricular premature depolarisation; VT, ventricular tachycardia; 2nd AVB, second degree atrioventricular block.

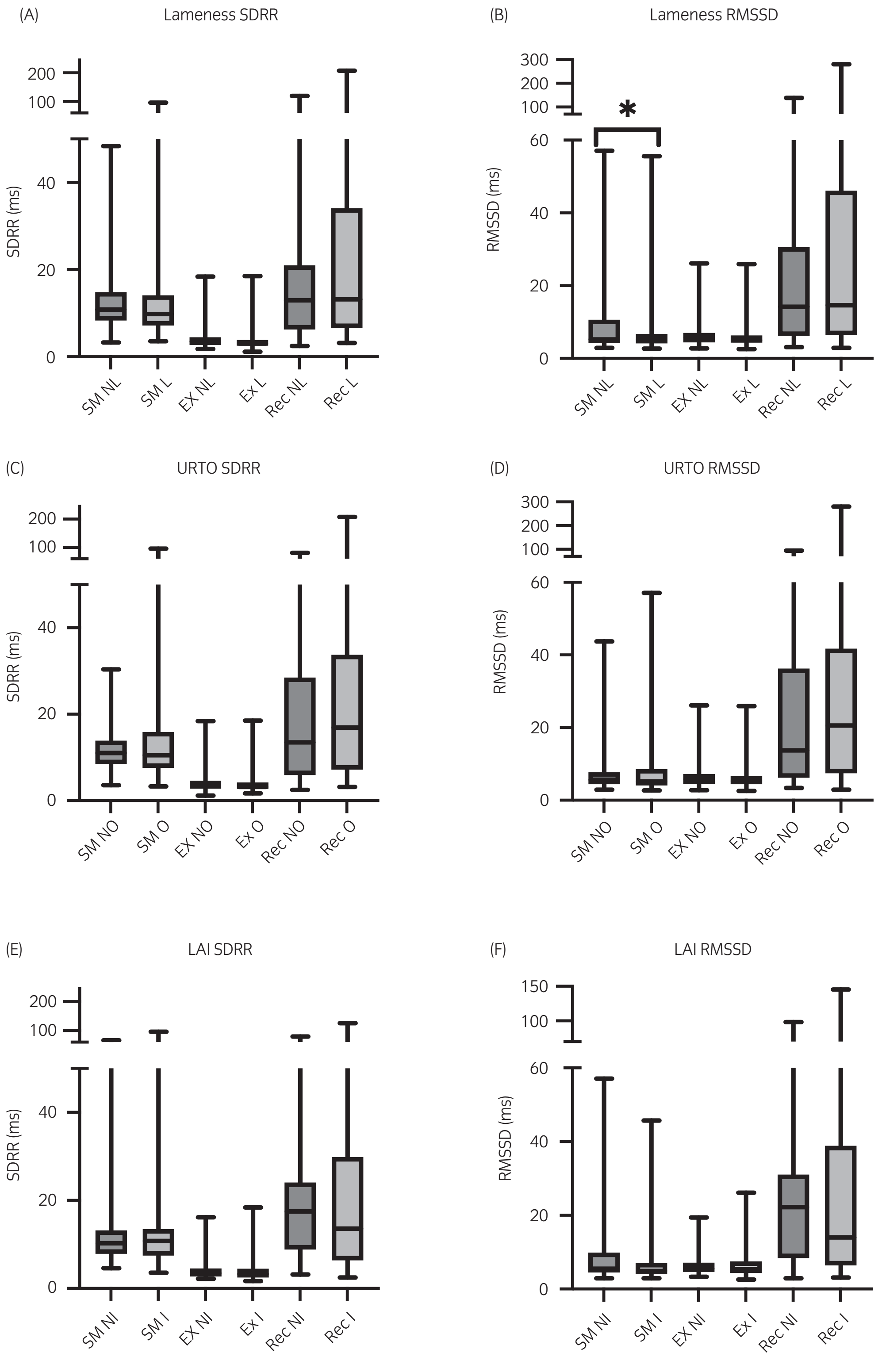

FIGURE 3 Heart rate variability results for each phase of exercise with horses divided into groups according to clinical status. (A) SDRR with horses divided by the presence or absence of lameness, (B) RMSSD with horses divided by the presence or absence of lameness, (C) SDRR with horses divided by the presence or absence of URTO, (D) RMSSD with horses divided by the presence or absence of URTO, (E) SDRR with horses divided by the presence or absence of LAI, (F) RMSSD with horses divided by the presence or absence of LAI. The presence of an asterisk indicating significant difference. EX, strenuous exercise period; I, lower airway inflammation; L, lame; LAI, lower airway inflammation; NI, no/minor lower airway inflammation; NL, not/minor lameness; NO, no/minor upper airway obstruction; O, upper airway obstruction; Rec, recovery period; RMSSD, root mean square of successive differences of the R–R intervals; SDRR, standard deviation of the R–R intervals; SM, submaximal exercise period; URTO, upper respiratory tract obstruction.

variable significant at the 20% level for logSDRR ($B = 0.16$, 95% CI 0.01–0.31, $p = 0.04$) during submaximal exercise and therefore multivariable analysis was not performed (Table S2). For logRMSSD during submaximal exercise, lameness, airway inflammation, arrhythmia and HSET were significant at the 20% level (Table S3) and carried forward into the multivariable analysis; in which lameness was associated with a decrease in logRMSSD, whereas HSET and arrhythmia were independently associated with increased logRMSSD (Table 2).

During strenuous exercise, arrhythmia during strenuous exercise was the only variable significantly associated with an increase in logSDRR ($B = 0.24$, 95% CI 0.18–0.29, $p < 0.001$) and logRMSSD ($B = 0.22$, 95% CI 0.17–0.27, $p < 0.001$) at the 20% level (Tables S4 and S5); multivariable analysis was therefore not performed.

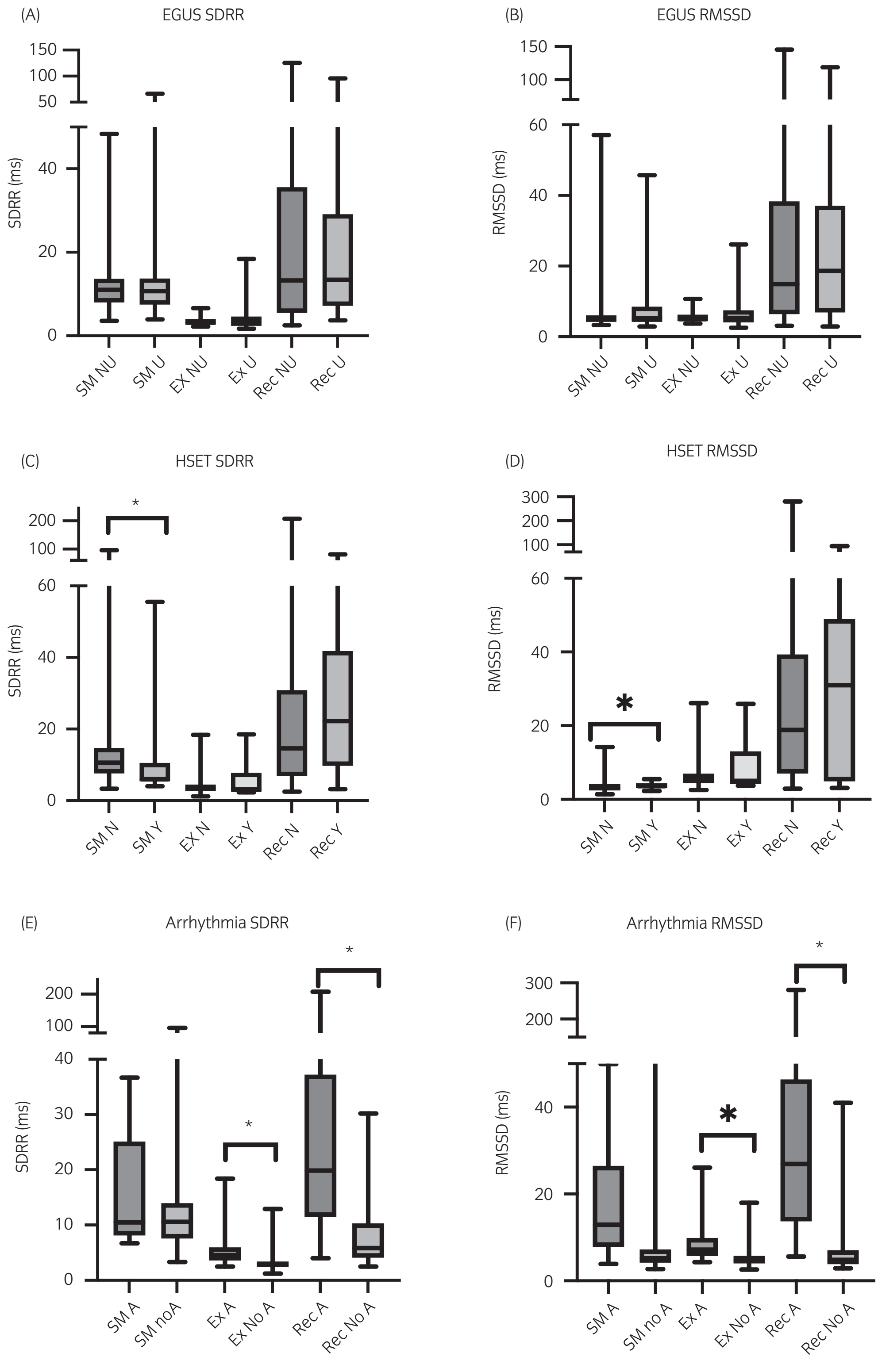

FIGURE 4 Heart rate variability results for each phase of exercise with horses divided into groups according to clinical status. (A) SDRR with horses divided according to presence or absence of EGUS, (B) RMSSD with horses divided according to presence or absence of EGUS, (C) SDRR with horses divided according to the undertaking, or not, of the HSET, (D) RMSSD with horses divided according to the undertaking, or not, of the HSET, (E) SDRR with horses divided according to presence of arrhythmia, (F) RMSSD with horses divided according to presence of arrhythmia. The presence of an asterisk indicates significant differences between groups. A, presence of an arrhythmia; EGUS, equine gastric ulcer syndrome; Ex, strenuous exercise period; HSET, high-speed exercise test; N, high-speed exercise test not undertaken; No A, no arrhythmia present; NU, no/minor gastric disease; Rec A, recovery period; RMSSD, root mean square of successive differences of the R–R intervals; SDRR, standard deviation of the R–R intervals; SM, submaximal exercise period; U, gastric disease; Y, performance of high-speed exercise test.

For logSDRR during recovery, URTO, time in strenuous exercise and arrhythmia during recovery was significant at the 20% level (Table S6) and carried forward into the multivariable analysis, where arrhythmia during recovery was the only variable independently associated with logSDRR (Table 2). Again, an arrhythmia during recovery was associated with an increase in the value of logSDRR. In the univariate analysis for log RMSSSD in recovery (Table S7), the length of time for strenuous exercise and arrhythmia during recovery were the only variables significant at the 20% level and thus carried forward to the multivariable analysis, where increased time in strenuous exercise and arrhythmia in recovery were independently associated with logRMSSD values (Table 2).

TABLE 2 Multivariable analysis for HRV indices during all phases of exercise

Variable	B	95% CI for B	p
LogRMSSD submaximal exercise			
Lameness	−0.18	−0.31 to −0.06	0.006
HSET	0.31	0.02–0.61	0.03
Arrhythmia during submaximal exercise	0.31	0.01–0.61	0.04
LogSDRR recovery			
Arrhythmia during recovery	0.51	0.40–0.62	<0.001
LogRMSSD during recovery			
TE	0.001	0.000–0.002	0.03
Arrhythmia during recovery	0.60	0.49–0.72	<0.001

Abbreviations: B, coefficient; CI, confidence interval; HRV, heart rate variability; HSET, high-speed exercise test; p, p value; TE, time in exercise phase.

TABLE 3 Results of ROC curve analysis and suggested cut-off points suitable to differentiate between horses with or without arrhythmias during each phase of the exercise test

Phase of exercise test HRV variable	Submaximal exercise SDRR	Submaximal exercise RMSSD	Strenuous exercise SDRR	Strenuous exercise RMSSD	Recovery SDRR	Recovery RMSSD
Suggested threshold to predict an arrhythmia (ms)	Not possible to find any clinically useful value	7.55	3.45	5.55	10.5	9.35
Sensitivity		90%	79%	78%	80%	87%
Specificity		76%	79%	73%	78%	82%
AUC	0.559	0.805	0.866	0.854	0.869	0.902
95% CI for AUC						
Upper	0.373	0.663	0.815	0.800	0.810	0.846
Lower	0.745	0.946	0.918	0.908	0.929	0.958

Abbreviations: AUC, area under the curve; CI, confidence interval; HRV, heart rate variability; ROC, receiver operator characteristic; RMSSD, root mean square of the standard deviation of the R–R interval; SDRR, standard deviation of the R–R interval.

3.2.1 | ROC curve analysis

The results of ROC curve analysis are presented in Table 3. Cut-off values to denote the presence of arrhythmia were derived for all HRV measures other than SDRR in submaximal exercise.

4 | DISCUSSION

The value of HRV analysis during exercise as an indicator of disease is an emerging field of investigation. Here, we present data from a group of equine athletes during exercise and subsequent recovery. A previous study with few horses suggested that HRV analysis during exercise may only be informative in the assessment of autonomic modulation at low exercise intensities,[8] therefore in this study, a period of submaximal exercise was analysed as well as strenuous exercise and recovery. What is interesting is the wide variation in HRV during the exercise period in this defined population undergoing a relatively uniform, controlled exercise test. HRV measures during exercise were lower, with a smaller range than the equivalent measures during recovery. This is expected as, during exercise, increases in sympathetic tone increase HR and differences in inter-beat intervals consequently become less apparent. During recovery, sympathetic tone is withdrawn, and parasympathetic tone predominates resulting in an increase in time-domain HRV measures.[20] During this period, there is also an increase in arrhythmogenesis as the transition from sympathetic to parasympathetic dominance results in marked autonomic instability. This is reflected in the increased number of arrhythmias seen in recovery, as observed in this and previous studies.[12,21]

The presence of an arrhythmia increased the time domain HRV indices in all phases of exercise and recovery, with one exception being logSDRR during submaximal exercise. The association between arrhythmia and increased HRV is expected and also found in human subjects.[22] LogSDRR was not influenced by arrhythmia in submaximal exercise in this study, which may be due to the low numbers in the arrhythmia group. During submaximal exercise, horses undertaking the HSET had an increase in time domain indices compared with those undertaking a SIET, a finding that cannot be an effect of the exercise test as this section of the test was the same for HSET and SIET, before strenuous exercise. This may reflect the differing populations: those horses that completed the HSET were mostly flat-racing horses who were younger. Time domain indices have been shown to be higher in younger horses.[23] During the same period, logRMSSD values were lower in lame horses which is an expected response to pain,[6] although there was a large overlap between groups limiting the clinical utility of this measure.

During strenuous exercise, the factor influencing the time domain HRV indices was the presence of arrhythmia. That the effect of lameness did not continue to influence HRV analysis in exercise is probably due to the overriding of low-level pain by the sympathetic drive of intense exercise, or the effect of arrhythmia during this time.

During recovery, the presence of arrhythmia increased the time domain HRV values which concurs with a previous study.[10] Another factor that influenced recovery HRV analysis was time in exercise; since the exercise test was continued until the point of fatigue, the horses that completed a longer test were the fitter horses and this group may be more likely to have experienced a more rapid restoration of parasympathetic tone.

In the current study, SDRR values were lower than previously reported exercising and recovery values, while those for RMSSD, were similar.[10,20,24] These differences may reflect differing populations as the previous studies included predominantly Warmbloods[10] and Arabians.[24] This study follows on from the study in Warmbloods[10] in which the influence of arrhythmia on HRV at exercise was similar to our findings; however, our study also considered the influence of a range of clinical variables not evaluated in that study. The lower SDRR we observed during exercise and recovery could be attributable to the more intense exercise undertaken in our study which mirrors the situation in humans where exercise intensity is the primary determinant of HRV during exercise.[1] However, the SDRR in submaximal exercise was also lower in our population compared with the previous study.

ROC curve analysis suggested values for RMSSD and SDRR that could be used for arrhythmia detection; if the process of generating HRV data becomes automated in the future, these HRV 'cut-offs' could prove clinically useful for arrhythmia screening. These values are only valid in Thoroughbred racehorses undergoing strenuous treadmill exercise; further studies are required to validate the use of HRV for arrhythmia detection in other types of horses performing ridden and less intense exercise.

4.1 | Limitations

The main limitation of this study was the retrospective use of a pre-existing database which represents a convenience sample, this method expedited the data collection. Cases underwent gastroscopy, tracheal wash and/or bronchoalveolar lavage only at the discretion of the owner/trainer and the clinician, therefore, some cases may have undetected abnormalities.

Further limitations include the classification of the clinical variables and defining which horses should be allocated to the arrhythmia group. The categorisation of the clinical variables to facilitate statistical analysis was challenging as there are few studies defining thresholds at which health conditions become clinically relevant. For airway inflammation, the categories were based on the ACVIM consensus statement,[18] with the tracheal wash data being categorised according to a previous publication.[17] URTO was defined as clinically important based on the judgement of a specialist sports medicine clinician. Lameness was categorised as clinically important once it was graded 2/10 as this degree of lameness has been shown to have a metabolic cost.[25] The grading of 3–4/4 for gastric ulceration was used, as it is the stage where many clinicians would initiate treatment.[26] The classification of arrhythmia was defined as any non-regular rhythm. Classification of arrhythmia at exercise is challenging with differing limits used to define a premature depolarisation. In this study, a cut-off of 8% was used[15] whereas other authors have used 10%[27] and more recently lower thresholds have been suggested at 6%[10] or 4%.[14] Although the decision to assign an ECG complex as originating in the atria or ventricles has poor interobserver agreement in other studies,[28] this would not have changed the classification into having, or not, an arrhythmia in our study. Additionally, exercise intensity and arrhythmia prevalence may be higher during strenuous treadmill exercise tests than during exercise tests on the trainer's gallops.[12]

The binary classification of arrhythmia, or not, is simplistic; although occasional premature depolarisations are not considered a clinical risk,[29] what constitutes 'occasional' has not been well defined. There is also little consensus regarding when cardiac arrhythmias become clinically relevant, other than in specific cases, for example, atrial fibrillation.

Another limitation to this study was the lack of a spectrum of severity for all the co-morbidities explored, from no abnormalities to severe changes. However, this is not practically achievable, due to the frequency with which equine gastric ulcer syndrome is documented in Thoroughbred racehorses in training and the inability to strenuously exercise horses with moderate to severe musculoskeletal conditions safely and ethically.

5 | CONCLUSIONS

In conclusion, the presence of arrhythmia influenced the results of HRV analysis in all phases of exercise and recovery. Along with arrhythmia, lameness and HSET influenced HRV in submaximal exercise, time in exercise influenced HRV in recovery.

AUTHOR CONTRIBUTIONS

Anna Hammond, Kate Allen, Sarah Smith and Melanie Hezzell conceived the study. Kate Allen and Samantha Franklin collected the original data. Anna Hammond, Kate Allen, Melanie Hezzell and William Sage collated and interpreted the data. Melanie Hezzell performed the data analysis. All authors contributed to manuscript preparation and approved the final version of the manuscript. Anna Hammond had full access to all the data and took responsibility for the integrity of the data and the accuracy of the data analysis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/evj.13908.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

This study was approved by the University of Bristol Animal Welfare and Ethical Review Body reference VIN/19/021. Explicit owner consent for animals' inclusion in the study was not stated. Horses were enrolled in the study with consent for research in general; the University of Bristol hospital admission form outlines consent for the horse's data to be used in future research studies.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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