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Review

Heart rate variability analysis in horses for the diagnosis of arrhythmias

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ABSTRACT

Heart rate variability (HRV) analysis has been performed on ECG-derived data sets for more than 170 years but is currently undergoing a rapid evolution, thanks to the expansion of the human and veterinary medical technology sector. Traditional HRV analysis was initially performed to identify changes in vago-sympathetic balance, while the most recent focus has expanded to include the use of complex computer algorithms, neural networks and machine learning technology to identify cardiac arrhythmias, particularly atrial fibrillation (AF). Some of these techniques have recently been translated for use in the field of equine cardiology, with particular focus on improving the diagnosis of arrhythmias both at rest and during exercise. This review focuses on understanding the basic HRV variables and important factors to consider when collecting data for use in HRV analysis. In addition, the use of HRV analysis for the diagnosis of arrhythmias is discussed from human, small animal and equine perspectives. Finally, the future of HRV analysis is briefly introduced, including an overview of future developments in this rapidly expanding and exciting field.

Introduction

We are fortunate to live in an era of rapid technology advancement, where our 'smart' wristwatch or phone can detect and alert us to the presence of cardiac arrhythmias, allowing fast and potentially life-saving interventions to be performed. These 'smart' devices are using changes in heart rate variability (HRV) to alert us to the problem (Li et al., 2019).

Heart rate variability (HRV) is the term used to describe oscillations in rate between consecutive cardiac cycles captured on an ECG recording, heart rate monitoring device or other technology (e.g. utilising photoplethysmography). Either instantaneous heart rates or intervals between normal QRS complexes (RR intervals, also referred to as inter-beat intervals or IBI) can be used for analyses. The degree of variability reflects the complex interplay between the autonomic nervous system, blood pressure regulation and reflexes, pulmonary function and gas exchange, gastrointestinal function and other organ system inputs (Shaffer and Ginsberg, 2017). A certain amount of beat-to-beat variability is considered normal or 'healthy' and results from the short term fluctuations in heart rate associated with predominately parasympathetic inputs and regulatory mechanisms. Decreased variability is associated with reduced parasympathetic and increasing sympathetic tone. This can be a normal physiological response to stress, exercise or excitement but also occurs during pathological conditions such as heart failure, following myocardial infarction or with non-cardiac diseases such as stroke or seizures. Increased variability is also possible and can be considered a physiological response to waxing and waning parasympathetic tone (e.g. in dogs with respiratory sinus arrhythmia) (Hamlin et al., 1966) or with increasing parasympathetic tone (i.e. following endurance training) (Mourot et al., 2004). Increased variability also occurs with pathological conditions such as is seen with particular breathing patterns (e.g. Cheyne-stokes breathing) (Ernst, 2017) or arrhythmias (Shaffer and Ginsberg, 2017).

Traditional use of HRV in human medicine has centred around investigating autonomic balance in states of health and disease. In human medicine, task force-created guidelines are available that attempt to standardise nomenclature and specify standard methods of measurement to be able to compare results across studies (Malik et al., 1996). Unfortunately, no such standardised guidelines exist for veterinarians, despite HRV being more widely used in recent times, both in the research setting and in clinical practice.

In human medicine, HRV analysis has been utilised across a wide variety of fields, from detecting foetal distress, predicting heart failure following myocardial infarction, predicting the onset of seizures, assessing the effect of overtraining in athletes or to encourage lifestyle modification (Woo et al., 1992; Perini and Veicsteinas, 2003; Reed et al., 2005; Perkiomaki et al., 2014; Disertori et al., 2016; Singh et al., 2018; Giannakakis et al., 2019; Li et al., 2019).

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Table 1
Indications for performing heart rate variability analyses in horses with arrhythmias.

Indication
When an overall summary of heart rate and heart rate variability is required (graphical representations are particularly useful here)
To compare the response of a therapy or intervention (e.g. anti-arrhythmic therapy)
To detect subtle arrhythmias (e.g. those with only mild prematurity, just slightly exceeding the normal RR variation in a particular horse)
To compare changes in arrhythmia frequency over time
To evaluate the effect of training or rest on heart rate variability and occurrence of arrhythmias
To monitor for evolution of disease (e.g. failing of compensation mechanisms and subsequent onset of heart failure)
To detect recurrence of an arrhythmia (e.g. atrial fibrillation recurrence after cardioversion)

Traditional HRV analysis has been performed on longer-term ECG recordings, with arrhythmic and subsequent complexes being deliberately excluded during post processing (often by using software filters). This allows the focus of any inherent variability in RR intervals to be attributed to sino-atrial node (SAN) function and thus reflect the balance between the parasympathetic and sympathetic nervous system inputs into the SAN as well as intrinsic SAN cellular function. The resulting distance between adjacent sinus-origin QRS complexes (excluding arrhythmias) is referred to as the Normal–Normal (NN) interval.

Studies utilising HRV analysis have been reported for a number of animal species including but not limited to cattle, dogs, small ruminants, pig, poultry and rodents (Calvert, 1998; Calvert and Jacobs, 2000; Spier and Meurs, 2004a; Abbott, 2005; Rowan et al., 2007; von Borell et al., 2007; Moise et al., 2010; Gladuli et al., 2011; Rasmussen et al., 2012, 2014; Blake et al., 2018; Moise et al., 2020).

In horses, HRV analysis has been broadly studied in many contexts, although with little standardisation between the approach of different research groups, making direct comparisons of study conclusions difficult (Thayer et al., 1997; Physick-Sheard et al., 2000; Rietmann et al., 2004; Cottin et al., 2006; Ohmura et al., 2006; Nagel et al., 2010; Munsters et al., 2013; McConachie et al., 2016; van Vollenhoven et al., 2016; Younes et al., 2016; Broux et al., 2017; Eggensperger and Schwarzwald, 2017; Frick et al., 2019).

There is a shortfall of reports in the wider literature regarding the use of HRV analyses when arrhythmias are present. As mentioned earlier, traditional HRV data series have the RR intervals surrounding arrhythmic complexes filtered out. In recent times, HRV analyses have started looking at differences identified between normal and abnormal cardiac rhythms, in particular focusing on common rhythms like atrial fibrillation (AF) or ventricular tachycardia (Esperer et al., 2008; Li et al., 2019; Ringwald et al., 2020). Using some of this recent technology, ECG-linked smart watch (e.g. Apple watch-KardiaBand) or smart phone apps (e.g. AliveCor-Kardia) systems can fairly reliably diagnose AF based on the increased beat-to-beat variability that occurs with this rhythm (Chong et al., 2015; Wegner et al., 2020; Rajakariar et al., 2020).[1]

In the equine context, investigation of HRV analyses to diagnose arrhythmias is an emerging field of research. While recent studies have shown that HRV is higher in horses with AF and in horses with arrhythmias during exercise (Broux et al., 2017, 2018; Frick et al., 2019), further work is ongoing to utilise the available technology to improve the accuracy and ease of arrhythmia diagnosis in the horse.

The aim of this review is to summarise the currently available information about HRV and its use in horses, specifically focusing on the current use of HRV techniques in horses with arrhythmias. The future of HRV analysis with respect to mobile technologies and advanced computer-driven analysis will also be discussed.

Terminology and methodological criteria

The reasons for performing HRV analysis in horses are many and varied and are summarised in Table 1. To understand and interpret studies utilising HRV analyses, it is important to understand the following terminology and methodological criteria.

Heart rate variability terminology

There are three main approaches for HRV analysis – time domain, frequency domain and non-linear methods (Malik et al., 1996). Time domain analysis is the simplest group of calculations to understand and can be further divided into statistical and graphical (e.g. RR times series, histograms as seen in Fig. 1A and C) methods of heart rate or RR interval representation. Frequency domain analysis involves various spectral methods of tachogram transformation to determine the total power, low frequency and high frequency components of HRV (see Fig. 1D). Non-linear analysis is an evolving field in HRV and involves graphical representations of the data (e.g. Poincaré plots, see Fig. 1B), complex mathematical equations and transformation of data. From these three groups, the various HRV parameters can also be described as short-term components (high frequency/vagal inputs), longer-term components (low frequency/sympathetic and parasympathetic inputs) or overall measures of HRV. Terminology and methods for calculation of the commonly reported HRV measures are explained in Table 2.

Factors to consider when collecting data for HRV analysis

The factors to consider when collecting data for HRV analyses are summarised in Table 3.

Type of recording devices

Heart rate variability analysis can be performed on data series recorded from an ECG, heart rate monitor or other devices that record pulse waveform information. The main advantages of collecting data from an ECG recording device (e.g. Televet ECG recorder) is that QRS detection can be manually verified and corrected if necessary and a rhythm diagnosis can also be made in addition to the HRV analysis. Disadvantages include the expense and wearability of ECG recording units, particularly for longer duration recordings, although this is a fast-growing area of medical innovation and newer, more user-friendly devices are constantly being developed.

Heart rate monitors (those devices detecting RR intervals using proprietary algorithms, such as the Polar® heart rate monitor) are also frequently used in HRV data collection. They can be cheaper and easier to operate, although they do not allow flexibility in the manipulation of the data, as the QRS detection cannot be externally

[1] See: Eko Devices, Inc., 2020. Eko AI Validation white paper. https://assets-global.website-files.com/5d43b941a4923b9c4685f98d/5e2f3088842db36d0cdf9e70_Whitepaper_Abridged_final.pdf (Accessed 30 November 2020).

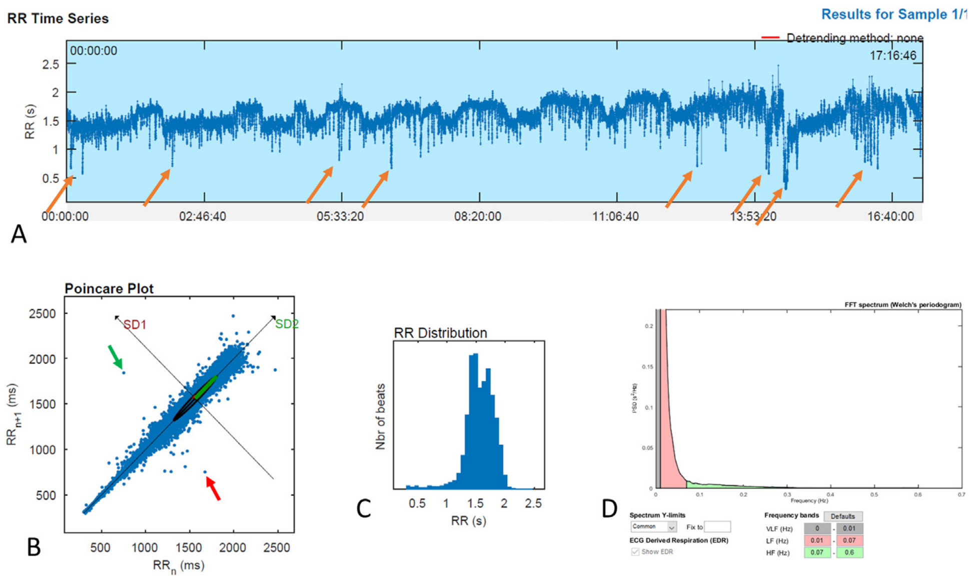

Fig. 1. (A) An RR interval time series plot (also referred to as tachogram) from a healthy horse obtained from a 17 h resting ECG recording. The orange arrows indicate periods of sinus tachycardia occurring during this period, a normal finding in horses. No atrio-ventricular blocks were detected. (B) The same data set as A, presented as a Poincaré plot, with each RR interval (RR_n) plotted against the following RR interval (RR_{n+1}). The majority of the RR interval pairs (RR_n vs. RR_{n+1}) are visualised along the line of identity, indicating relatively little beat-to-beat variation (98% of all beats in this recording fall between −7% and +7% RR deviation). This Poincaré pattern is known as a 'comet' pattern. The axis along which the heart rate variability indices SD1 and SD2 are calculated, are indicated. A single atrial premature complex occurring during this recording is identified by the red arrow (normal RR interval followed by the shorter RR interval of the premature complex) and the green arrow (shorter RR interval of the premature complex followed by a longer RR interval, consistent with a pause occurring as a result of the premature complex). (C) The same data set as A, presented as a histogram, showing the distribution of RR intervals. The largest peaks occur at longer RR intervals (i.e. around 1.5 s, corresponding to a HR of 40 beats per min [bpm]), which is typical of a horse at rest. (D) The same data set as A, presented after Fast Fourier Transformation of the data. The frequency bands were defined as very low frequency 0–0.01 Hz (grey), low frequency 0.01–0.07 Hz (pink) and high frequency 0.07–0.6 Hz (green) bands on the graph (as defined by Marr and Bowen, 2010).

validated. There is no possibility to evaluate the heart rhythm or P-QRS-T morphology independently. This is particularly important when using these devices in horses, as the normal equine ECG shows frequent T wave morphology changes which can be incorrectly detected as QRS complexes (oversensing). Most heart rate monitors utilise proprietary post-processing filters without providing the user any specific details – resulting in a so-called 'black box' for understanding and describing the data handling process (Parker et al., 2009; Lenoir et al., 2017). The use of heart rate monitoring devices is not recommended when investigating horses with arrhythmias because correct identification of the rhythm is not possible and it is likely that these proprietary artefact filters will remove arrhythmic beats from the data set.

The fast-growing area of wearable technology has started to employ photoplethysmography sensors, which detect pulse wave-forms and can calculate inter-beat intervals, and these data can also be used to detect variability within the pulse rate in a similar way to heart rate monitors and ECGs (Castaneda et al., 2018; Cheung et al., 2018). Many of these new technologies also contain a so called 'black-box', with little available knowledge of the complex algorithms under-pinning their analyses. Therefore, caution should always be applied to interpretation of their reported results and where possible, these devices should be validated against currently accepted gold-standard technologies.

It is important to consider all these factors when selecting the kind of device used to collect HRV data, as the type of data output will have downstream effects when it comes to analysis and interpretation of results.

Timing of recordings

There is considerable fluctuation in HRV parameters during a 24 h period (a result of the circadian rhythm), depending on the recording environment and influenced by other animal-related factors (animal husbandry practices like feeding, handling, grooming and pasture turn out) (Eggensperger and Schwarzwald, 2017). It is very important to standardise the timing of data recordings when performing HRV studies (i.e. recordings are obtained at the same time of day, following a similar to normal routine). It is also important to realise that HRV parameters may differ when the animal is in a familiar environment compared to those obtained in a hospital or research facility. A period of acclimation may be required when introducing animals to a new facility before reliable data can be obtained.

Length of recordings

Similarly, it is very important to standardise the length of recordings when collecting data for HRV analyses (Eggensperger and Schwarzwald, 2017). It is considered inappropriate to compare time-domain measures (especially those representing overall HRV) obtained from recordings of different durations. Additionally, some HRV variables can only be calculated on recordings over a certain length (i.e. SDANN – the standard deviation of the averages of NN intervals in all 5 min segments of the entire recording, requires a recording considerably longer than 5 min to achieve a reliable output). Indices like the triangular index (TI) are based on the summation of all intervals and therefore, are affected by shorter duration recordings and by the lower overall heart rate of horses (and subsequent number of observations) compared to other species (Eggensperger and Schwarzwald, 2017).

Resting or exercising recordings

Depending on the question wishing to be answered, the data should be collected either at rest or during an intervention (i.e. therapy or exercise). The conditions should be standardised wherever possible to try and reduce confounding factors into the HRV analysis. Recent evidence provided by Lenoir et al. (2017) indicated that caution should be applied when analysing data

Table 2
Basic terminology explanations: short term, long term, and overall heart rate variability (HRV) indices.

Variable	Units	Type of analysis	Explanation	Calculated by	Notes
Overall HRV assessment					
Mean heart rate	bpm	Time domain	The mean heart rate present in the time series analysed	Sum of instantaneous heart rates/ number of observations	
Mean RR (NN) interval	ms	Time domain	The mean RR (NN) interval present in the time series analysed	Sum of RR intervals/number of observations	
SDNN (SDRR)	ms	Time domain	Standard deviation of NN (RR) intervals in the time series	Square root of the variance	Only compare between similar length recordings
Triangular index		Time domain	Integral of the density of NN (RR) interval histogram, divided by the height of the histogram	Total number of NN (RR) intervals/ number of NN (RR) intervals in the modal bin	Longer recordings provide more stable data
TINN	ms	Time domain	Triangular interpolation of the NN (RR) interval histogram	Baseline width of the NN (RR) interval histogram	Longer recordings provide more stable data
Total power	n.u. ms^2 Hz	Frequency domain	Signal energy found within all frequency bands – represents variance of all NN intervals	Transformation of the data by various algorithms (e.g. FFT)	Validation of the appropriate frequency band cut-offs is lacking in veterinary studies
LF/HF		Frequency domain	Ratio of low to high frequency power	Transformation of the data by various algorithms (e.g. FFT)	Represents complex interplay between parasympathetic and sympathetic nervous systems. Interpretation depends on the study context.
SD1/SD2		Non-linear domain	Ratio of SD1 to SD2	Created from Poincaré plots as described below	Requires a longer data set
Short-term components (high frequency/vagal input)					
RMSSD	ms	Time domain	Square root of mean squared differences between successive NN intervals	Calculate each successive difference between heart beats, square each value and average them, then square root the total.	Mathematically identical to SD1
SD1	ms	Non-linear domain	Standard deviation of Poincaré plot, perpendicular to the line of identity (width)	The width of an ellipse fitted to the plotted points on a Poincaré plot – calculate the standard deviation (square root of the variance) of all the distances from the point $y = x$ axis (line of identity).	Mathematically identical to RMSSD
HF	n.u. ms^2 Hz	Frequency domain	High frequency (rapidly fluctuating) spectral components	Transformation of the data by various algorithms (e.g. FFT).	Validation of the appropriate frequency band cut-offs is lacking in veterinary studies. Variations can relate to parasympathetic nervous system tone and respiratory frequency in humans
Long-term components (low frequency/sympathetic ± vagal inputs)					
SDANN	ms	Time domain	Standard deviation of the average NN intervals in all non-overlapping 5 min segments	For each 5 min segment of recording, the mean NN interval is calculated. The combined mean and standard deviation of all segments is then determined.	Long data sets required
SD2	ms	Non-linear domain	Standard deviation of the Poincaré plot, along the line of identity (length)	The length of an ellipse fitted to the plotted points on a poincare plot – calculate the standard deviation (square root of the variance) of all the distances from the point $y = x$ + average RR interval.	
LF	n.u. ms^2 Hz	Frequency domain	Low frequency spectral components	Transformation of the data by various algorithms (e.g. FFT).	Validation of the appropriate frequency band cut-offs is lacking in veterinary studies. Represents inputs from the parasympathetic and sympathetic nervous system and baroreceptors

bpm, beats per min; FFT, Fast fourier transformation; ms, milliseconds; ms^2, milliseconds squared; Hz, hertz; n.u., normalised units.

Table 3
Factors to consider standardising when collecting and analysing data for heart rate variability (HRV) analysis.

Factor
Type of recording device (e.g. traditional ECG recording vs. heart rate monitor)
Timing of the recording, as there is considerable fluctuation in HRV parameters over a 24 h period
Length of recording – particularly important when looking at indices of long-term HRV
Use of RR interval or PP interval data sets in horses, given the frequent occurrence of 2nd degree atrio-ventricular blocks at rest in some horses
Resting vs. exercising recordings
Post processing, filtering and automated ‘black box’ analysis

obtained from equine heart rate monitors during exercise as there was poor agreement with HRV parameters obtained from a simultaneously recorded ECG.

Post processing/filtering

The recorded RR interval sequence should be free of artefacts and traditionally also excluding rhythm disturbances other than sinus arrhythmias (resulting in the NN interval sequence). Some reports describe using adaptive filtering algorithms to replace the abnormal inter-beat intervals with interpolated 'normal' data points (Lerma et al., 2008). This cleaning of data can be performed manually, automatically performed by proprietary software included in the device or using open access HRV software. Some post processing functions provided by open access HRV software (e.g. Kubios HRV software, University of Eastern Finland) allow for application of different degrees of artefact correction (in Kubios described as very low to very strong thresholds) or a custom threshold can be applied (intervals x ms different is filtered out; Tarvainen et al., 2014).

If too much artefact correction is applied, loss of some 'normal' NN intervals will occur, while if artefacts are not appropriately excluded this could also alter the data analysis and lead to misinterpretation. Fig. 2 shows the effects of artefact filters applied to a manually corrected or uncorrected data set. It is important when reporting on studies that include HRV analysis that specific details about data handling, post processing and filtering are included in a clear and transparent manner.

Use of HRV analysis with arrhythmias: the human perspective

The use of HRV analyses in humans has expanded far beyond that limited to just the field of cardiology. In particular, sports medicine and many areas of lifestyle management have adapted HRV techniques to detect overtraining in elite athletes, predict the development of acute mountain sickness in mountaineers or alert a person with epilepsy to an imminent seizure, among many other uses (Mellor et al., 2018; Singh et al., 2018; Giannakakis et al., 2019).

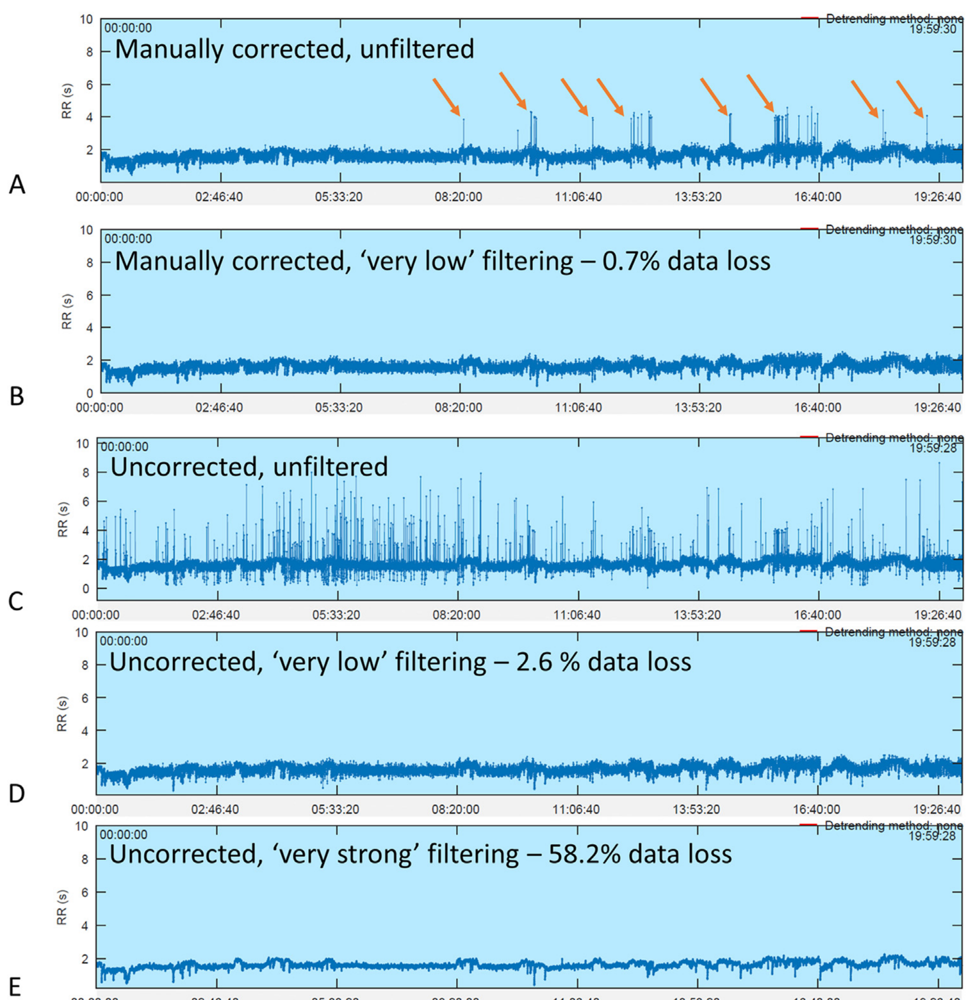

Fig. 2. (A) An RR interval time series plot from a healthy horse obtained from a 20 h resting ECG recording. The orange arrows indicate periods of second-degree atrioventricular (AV) block, a common finding in resting horses. This data set has been manually evaluated for accurate QRS detection and corrected where required. (B) The same data set as in A, with an automated artefact filter applied at 'very low' settings (Kubios HRV v3.1.0, 2017, University of Eastern Finland). The second-degree AV blocks have been removed by the filter, including approximately 0.7% of the RR intervals in the data set. This level of filtering may be appropriate if the investigator wants to automatically remove the influence of AV blocks from the heart rate variability analysis (Eggensperger and Schwarzwald, 2017), but might cause undesired loss of data. (C) The same data set as in A, however this data set has not been manually evaluated for accurate QRS detection and therefore, errors in QRS detection occur frequently (compare this tachogram with that above in A) and are seen as upward and downward spikes on the tachogram. (D) The same data set as C, with a 'very low' artefact filter applied. The artefacts created by the inaccurate QRS detection have been removed, with approximately 2.6% of all the RR intervals removed from the data set. (E) The same data set as C, with a 'very strong' artefact filter applied. Here more than half of the RR intervals have been removed, resulting in a much smaller and less representative data set.

In the field of arrhythmia detection and management, the main area that has been under recent development is that surrounding AF. Atrial fibrillation is the most common sustained tachyarrhythmia described in people, with a large number of challenges and costs associated with its diagnosis and management. This had led to the rapid expansion of methodologies and technology that is focused on improving these areas (Li et al., 2019; Wegner et al., 2020; Rajakariar et al., 2020). Many of these technologies rely on the development of complex algorithms that include data on heart rate, HRV, ECG morphology (if recorded using a device that contains ECG leads) and components of machine learning technology, where the devices 'learn' to differentiate patterns between normal and abnormal (e.g. AF) rhythms (Sansone et al., 2013). These technologies still rely on the fundamental of obtaining good quality data (i.e. free of noise and artefacts) but can provide an accurate evaluation of the rhythm or, if the findings are unclear, can facilitate a more thorough evaluation by a third-party expert (Li et al., 2019; Pereira et al., 2020).

Many of the HRV analyses performed in human medicine have the arrhythmic complexes and surrounding beats removed or replaced by interpolated data. Among other things, these data sets are utilised for prognosticating about the risk of developing different arrhythmias. As one example, a large amount of work has focused on using HRV patterns preceding periods of ventricular tachyarrhythmia, to predict life-threatening cardiac events, particularly using implantable cardioverter defibrillators. Early prediction of a ventricular tachyarrhythmic event can allow a tiered approach to real-time therapy and is less battery intensive, improving both clinical outcomes and the lifespan of the device (Parsi et al., 2019).

Heart rate turbulence is a specific measure of HRV, which looks at the presence of ventriculophasic sinus arrhythmia (short-term heart rate acceleration then deceleration) following a ventricular premature complex (VPC). Loss of this turbulence (i.e. absence of the ventriculophasic sinus arrhythmia) is associated with increased risk of arrhythmia or sudden cardiac death, particularly following myocardial infarction or heart failure (Lombardi et al., 2007; Disertori et al., 2016). This, and many similar HRV measures have shown promise in the development and validation phases of various monitoring devices and are now being brought to clinical practice, particularly through rapid expansion of the wearable medical technology market (Cheung et al., 2018).

The Poincaré plot (sometimes referred to as a Lorenz plot) is a graphical representation of an entire data set, where each RR interval (RR_n) is plotted on the x-axis, against the next following RR interval (RR_{n+1}) on the y-axis. A normal equine example is seen in Fig. 1B. These graphical tools provide a global view of overall HRV, while evaluation of the width (SD1, see Table 2) and length (SD2, see Table 2) of an ellipse fitted to the plot can provide objective measures of short-term and long-term HRV. These plots can be assessed subjectively and classified based on their similarity to familiar shapes – comet pattern, torpedo pattern, fan pattern, double or triple side-lobe pattern or propeller pattern being the most common patterns described in the literature (Esperer et al., 2008; Zhang et al., 2015). When arrhythmic complexes are not excluded (i.e. filtered out) from an RR data set, identification of specific patterns can be used for rhythm diagnosis (Esperer et al., 2008; Zhang et al., 2015; Borracci et al., 2018). In addition, these geometric patterns can be further analysed by complex computer algorithms, which are combined with other HRV variables within classifiers like artificial neural networks and support vector machines, allowing rapid, automated, objective analysis of complex data sets (Zhang et al., 2015; Pereira et al., 2020).

Use of HRV analysis with arrhythmias: the small animal perspective

In the small animal veterinary field, utilising HRV analysis in animals with arrhythmias occurs less commonly than is seen in human medicine.

The main descriptions of HRV analyses in the context of canine arrhythmia appear in the literature when attempting to stratify risk of ventricular tachyarrhythmias and sudden cardiac death in populations of dogs with cardiomyopathy (particularly Boxers and Dobermans) or mitral valve disease (Calvert, 1998; Calvert and Jacobs, 2000; Calvert and Wall, 2001; Spier and Meurs, 2004a; Rasmussen et al., 2012, 2014). Changes in HRV (decreased from control animals) were typically seen in dogs with clinical heart failure; while animals with frequent ventricular arrhythmia unrelated to heart failure were not different (although ventricular arrhythmias were excluded from the dataset in these studies). Heart rate turbulence (as described earlier) calculated in ECG data sets from Dobermans could be found reduced in dogs with preclinical dilated cardiomyopathy as compared with healthy controls, indicating a loss of the short-term changes in sinus rhythm (Harris et al., 2017).

Interestingly, the Poincaré plot patterns of healthy dogs have been described by Blake et al., in 2018 and there is a marked difference in the geometrical patterns of normal dogs when compared with the human literature (Esperer et al., 2008; Moise et al., 2020). In fact, dogs seem to exhibit a predominantly Y shaped fan pattern (shown in Fig. 3), which is more typical of the pattern that is described in people with AF (Esperer et al., 2008; Zhang et al., 2015). This is considered, in part, to be related to the rapid, frequent and dramatic effect that respiratory sinus arrhythmia has on increasing and decreasing the RR interval (Hamlin et al., 1966; Blake et al., 2018; Moise et al., 2020). In addition, a common 'zone of avoidance' was identified, where a lower density of points was observed in the central area of the canine plots. This corresponds to a range of RR intervals that occur with lower frequency than others and is thought to relate to the process of initiation of the cardiac

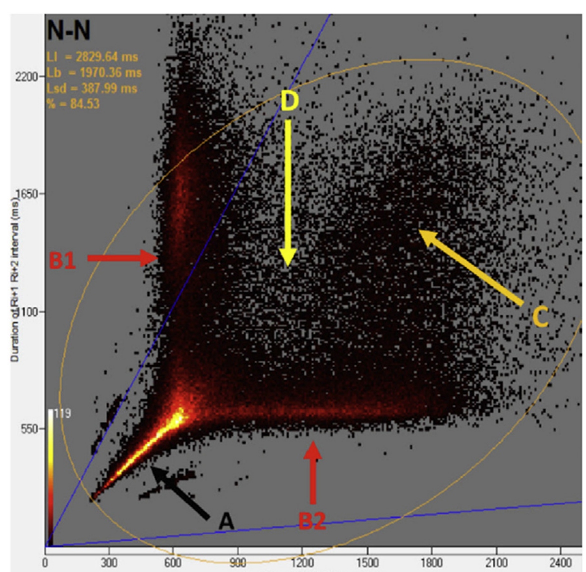

Fig. 3. A representative Poincaré plot created from a 24 h ambulatory ECG recording from a healthy dog. (A) stalk representing short–short RR intervals; (B1) arm representing short–long RR intervals; (B2) arm representing long–short RR intervals; (C) cluster representing long-long RR intervals; (D) zone of lower density representing a range of RR intervals occurring infrequently. Reproduced with permission, Blake et al. (2018).

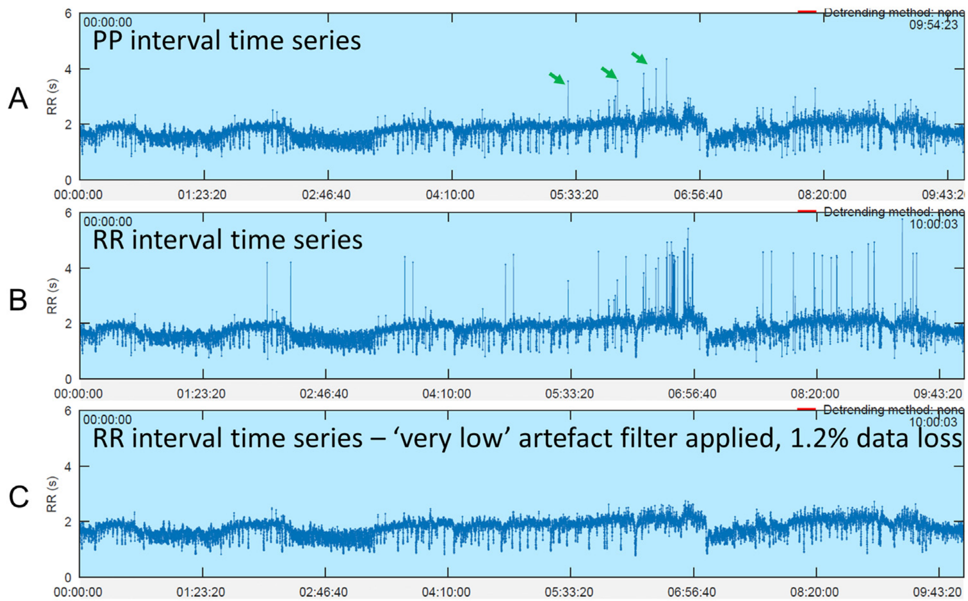

Fig. 4. (A) A PP interval time series plot from a healthy horse over a resting 10 h recording period. This data series was extracted using emka ecgAuto software (emka Technologies). The influence of most of second-degree atrio-ventricular (AV) blocks has been removed using this technique. However, some PP intervals appear to have escaped detection (resulting in PP intervals twice as long as normal – green arrows). (B) The same data set as in A, this time reported as an RR interval time series plot. Here the second-degree AV blocks can be seen as upwards spikes through the time series. (C) The same data as in B, with a 'very low' artefact filter applied, resulting in removal of the second-degree AV blocks and loss of 1.2% of the RR intervals. These data appear similar to that obtained using the PP interval detection seen in A.

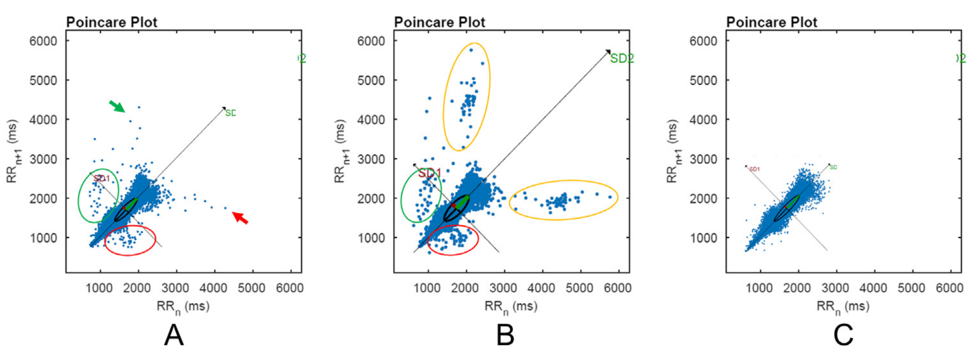

Fig. 5. (A) A Poincaré plot created from the PP time series data seen in Fig. 4A. This horse exhibits a central 'wedge' pattern with small side lobes consistent with atrial premature complexes (approximately 50 in 10 h of recording). Some PP intervals appear to have escaped detection (resulting in PP intervals twice as long as normal – green and red arrows). (B) A Poincaré plot created from the RR time series data seen in Fig. 4B. Here, the central 'wedge' pattern with small side lobes is seen (red and green circles), with additional clusters of second-degree atrio-ventricular blocks present (orange circles). (C) A Poincaré plot created from the filtered RR time series data seen in Fig. 4C. Here, only the central 'wedge' pattern is seen and most of the premature complexes, subsequent pauses and second-degree AV blocks have been filtered out.

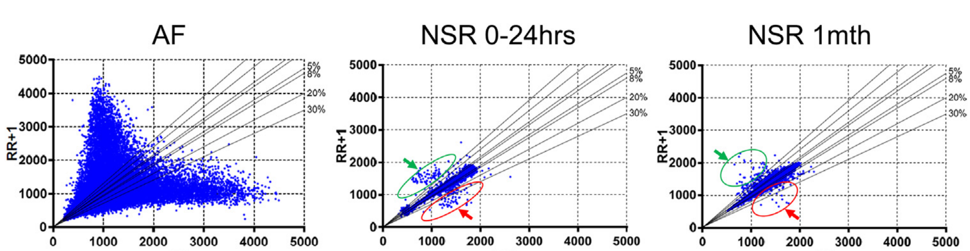

Fig. 6. The first Poincaré plot is created from an RR interval data set recorded from a horse with atrial fibrillation (AF), at rest, over a 24 h period. In contrast with the 'comet' pattern seen in the Poincaré plot of Fig. 1B, this pattern is described as a 'fan' or 'butterfly' pattern, representing the irregularly-irregular nature of AF. The thin black lines indicate RR deviation from the line of identity (which represents 0% RR deviation) by 5%, 8%, 20% and 30%. The second Poincaré plot is from the same horse, immediately following conversion of AF to normal sinus rhythm. A central 'comet' shape is seen, representing the more regular beat-to-beat nature of this ECG. Clouds or lobes are seen to either side of the central 'comet'. The red circled cloud represents normal RR intervals followed by a shorter RR interval (as with an atrial premature complex) while the green circled cloud represents the shorter RR interval, followed by a longer RR interval (as with a pause following the premature complex). The third Poincaré plot is from the same horse, presented for a recheck evaluation 1 month after conversion to sinus rhythm. A similar central 'comet' shape is identified and the clouds to either side of the 'comet' contain fewer dots, consistent with a reduction in the frequency of atrial premature complexes during this recording.

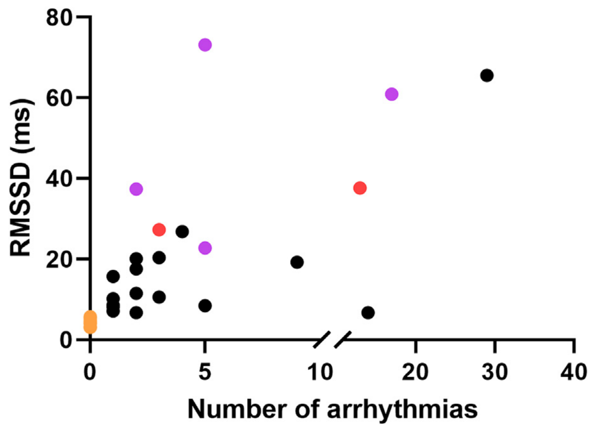

Fig. 7. Data from 29 horses that underwent a high-speed standardised exercise treadmill test with an ECG recorded simultaneously. The number of arrhythmias during the ECG recording were counted, with arrhythmias during the recovery period reported in this figure. The square root of mean squared differences between successive NN intervals (RMSSD; ms) was calculated for the recovery phase of each horse. Orange, horses without arrhythmias during recovery (n = 7); black, horses with atrial or ventricular premature complexes during recovery (n = 16); purple, horses with second-degree atrio-ventricular blocks, sinus pauses or marked sinus arrhythmia during recovery (n = 4); red, horses with second-degree atrio-ventricular blocks, sinus pauses or marked sinus arrhythmia and ventricular premature complexes during recovery (n = 2). The largest variation in RMSSD occurred in horses that showed second-degree atrio-ventricular blocks, sinus pauses or marked sinus arrhythmia during recovery.

rhythm within the SAN and subsequent conduction through the sino-atrial pathways (Moise et al., 2010, 2020).

In the context of AF, no clinical studies utilising HRV in the diagnosis of naturally occurring AF in dogs have been performed. Heart rate variability analysis has been reported in dogs with sick sinus syndrome, where periods of supraventricular tachycardia and varying AV nodal conduction have been described using unique patterns on Poincaré plots and tachograms and in changes to short-term HRV parameters (Gladuli et al., 2011; Bogucki and Noszczyk-Nowak, 2017).

Use of HRV analysis with arrhythmias: the equine perspective

In the equine field, HRV analysis to identify or describe arrhythmias has seen several recent developments. Particularly with the rapidly evolving veterinary medical technology sector and translation from human medicine, several groups have been working to utilise the available technology from an equine perspective.

Normal sinus rhythm

A recent study reporting the use of Poincaré plots to describe patterns of overall HRV in healthy horses has shown that horses have similar patterns to those described in people, commonly called comet or torpedo shaped. This is in contrast to the patterns described in dogs (fan or Y shaped), indicating much less influence

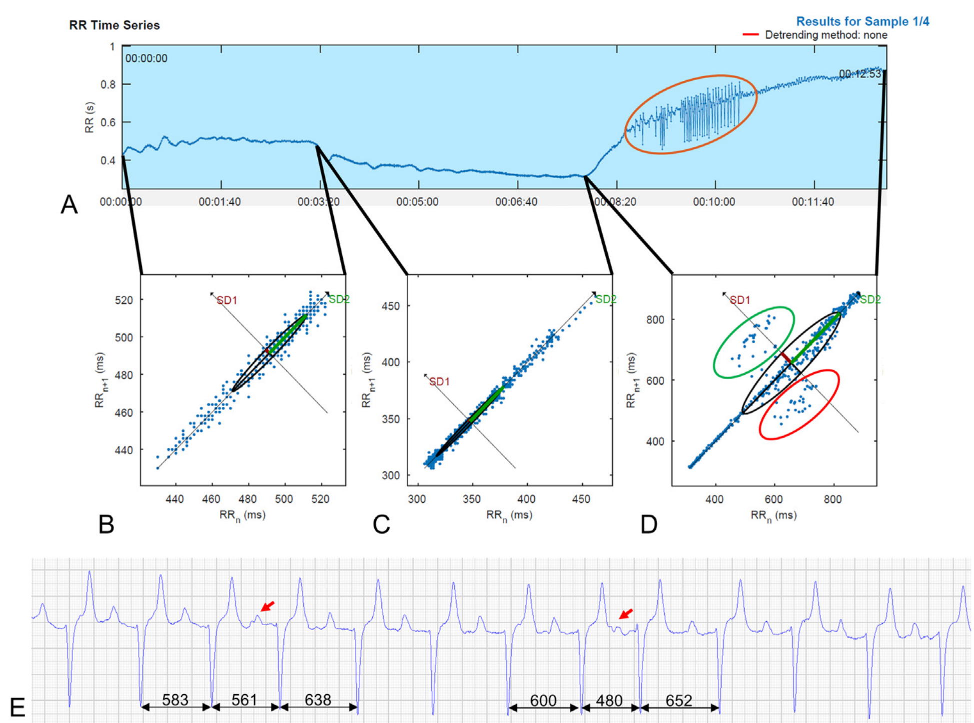

Fig. 8. (A) An RR interval time series plot from a horse during a high-speed standardised exercise treadmill test, with an ECG recorded simultaneously. Very little variation is seen between RR intervals until the recovery phase. The orange circle contains RR intervals occurring as a result of 30 atrial premature complexes, occurring during the recovery phase. A downward spike represents the shorter RR intervals of the premature complex, while an upward spike represents the longer RR intervals occurring after the premature complex and subsequent resetting of the sinus node. (B) A Poincaré plot created from the RR interval time series during the period of trotting exercise. The rhythm is regular and there is very little beat-to-beat variation detected (−2.5% to +2.4% RR deviation). The square root of mean squared differences between successive NN intervals (RMSSD) calculated for this segment is 3.8 ms. (C) A Poincaré plot created from the RR interval time series during the period of cantering exercise. The rhythm is regular and there is very little beat-to-beat variation detected (−3.2% to +3.2% RR deviation). The RMSSD calculated for this segment is 3.1 ms. (D) A Poincaré plot created from the RR interval time series during the period of recovery after exercise. Some of the rhythm is regular but there is a period of irregular rhythm that starts when the heart rate drops below approximately 100 beats per min (bpm) and ends once the heart rate drops below 75 bpm (−28% to +47% RR deviation). The red circled cloud represents normal RR intervals followed by a shorter RR interval (as with an atrial premature complex) while the green circled cloud represents the shorter RR interval, followed by a longer RR interval (as with a pause following the premature complex). The RMSSD calculated for this segment is 65.3 ms. (E) A representative segment of ECG recorded during the recovery phase. The atrial premature complexes are identified (red arrows). The RR interval sequences surrounding the premature complex are displayed (black double-ended arrows, ms).

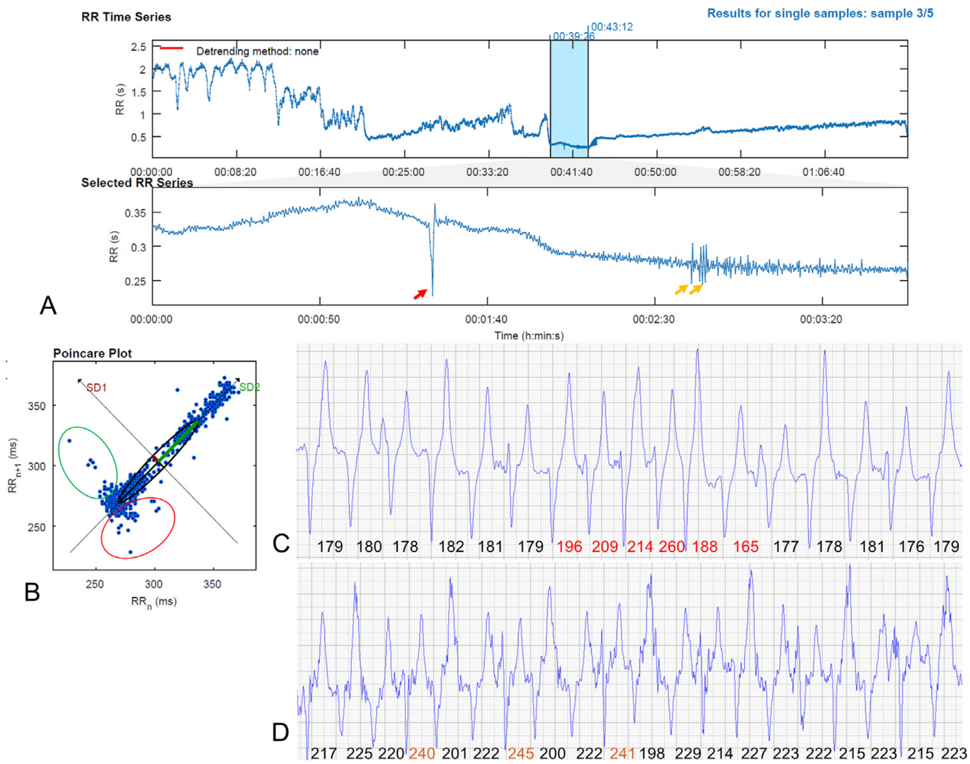

Fig. 9. (A) An RR interval time series plot from a horse during a ridden field exercise test, with an ECG recorded simultaneously. The blue area of the top graph is extended below (selected RR series) and corresponds with a period of fast gallop. The rhythm appears regular until the red arrow, when a period of rapid tachycardia occurs (corresponding with the ECG seen in C). Four complexes with short RR intervals are identified, followed by one more regular and one complex with a longer RR interval. The relationship between the six complexes can clearly be seen in the tachogram. Addition periods of arrhythmia are shown by the orange arrows (corresponding with the ECG seen in D). These are single complexes with short RR intervals followed by longer RR intervals. (B) A Poincaré plot created from the selected RR interval time series described in A (the segment of RR tachogram in blue). Most of the RR interval pairs fall on the line of identity, indicating a regular rhythm with low beat-to-beat variability however, the red circled cloud represents normal RR intervals followed by a shorter RR interval (as with the premature complexes) while the green circled cloud represents the shorter RR interval, followed by a longer RR interval (as with a pause following the premature complex). (C) A representative segment of ECG recorded during the period of arrhythmia highlighted with the red arrow in A. The premature complexes and their respective instantaneous heart rates are highlighted in red text. These QRS complexes show similar morphology to the surrounding sinus beats and are difficult to recognise by simply looking at QRS complex morphology. (D) A representative segment of ECG recorded during the period of arrhythmia highlighted with the orange arrows in A. The premature complexes and their respective instantaneous heart rates are highlighted in orange text. These QRS complexes show similar morphology to the surrounding sinus beats and are difficult to recognise by simply looking at QRS complex morphology.

of respiratory sinus arrhythmia and other, as yet, unknown factors on the pattern of HRV in horses (Esperer et al., 2008; Zhang et al., 2015; Blake et al., 2018; Kuhni et al., 2020; Moise et al., 2020). Healthy horses typically show between −13% to +21% RR interval beat-to-beat variation unless atrioventricular blocks are present (Flethoj et al., 2016; Kuhni et al., 2020). The within-horse, between-day repeatability of Poincaré plot patterns was found to be excellent (Kuhni et al., 2020).

Second-degree atrio-ventricular blocks

The question of whether atrio-ventricular (AV) blocks should be included or excluded in analysis is particularly relevant for the discussion of HRV analysis in horses. Tonic vagal inhibition of sinus impulse generation and conduction occurs at both the sino-atrial and atrio-ventricular level, resulting in sinus arrhythmias and AV blocks (AVBs) in some horses. Second-degree AV blocks are frequently excluded from data analysis by the automatic filtering features of heart rate monitoring devices, resulting in a loss of data about inputs from the parasympathetic nervous system. One way to avoid this bias is to detect and calculate PP intervals rather than RR intervals, which can be performed with specific ECG analysis software (e.g. emka ecgAuto software, emka Technologies, France) (Eggensperger and Schwarzwald, 2017). This study elegantly showed the effects of including or excluding AVB on HRV analysis and indicated that application of an artefact filter set to a ‘very low’ level had good agreement with the PP based sets, thereby simulating the situation where AVB are removed. An example of this is seen in Figs. 4 and 5. This is important for future studies to consider, particularly when investigating parasympathetic-sympathetic nervous system balance.

Sustained arrhythmias

Similar to human medicine, AF is the arrhythmia most frequently studied in the context of equine HRV analysis. Two studies have described the differences in HRV variables between horses in sinus rhythm and in AF (Broux et al., 2017, 2018). These studies found, similar to the human experience, that HRV was markedly increased in horses where AF is present. An example of this is described graphically using Poincaré plots in Fig. 6. An important accessory finding of the study by Broux et al., in 2018 was that overzealous application of artefact filters could lead to a substantial loss of data, reducing the ability to differentiate between sinus rhythm and AF. In itself, these findings of increased HRV in horses with AF are neither new nor unsurprising, as AF is well known as an irregularly irregular rhythm. The importance of these findings is in the context of utilising this detection of increased heart rate variation in home monitoring or training recording devices, where owners and trainers are alerted to the

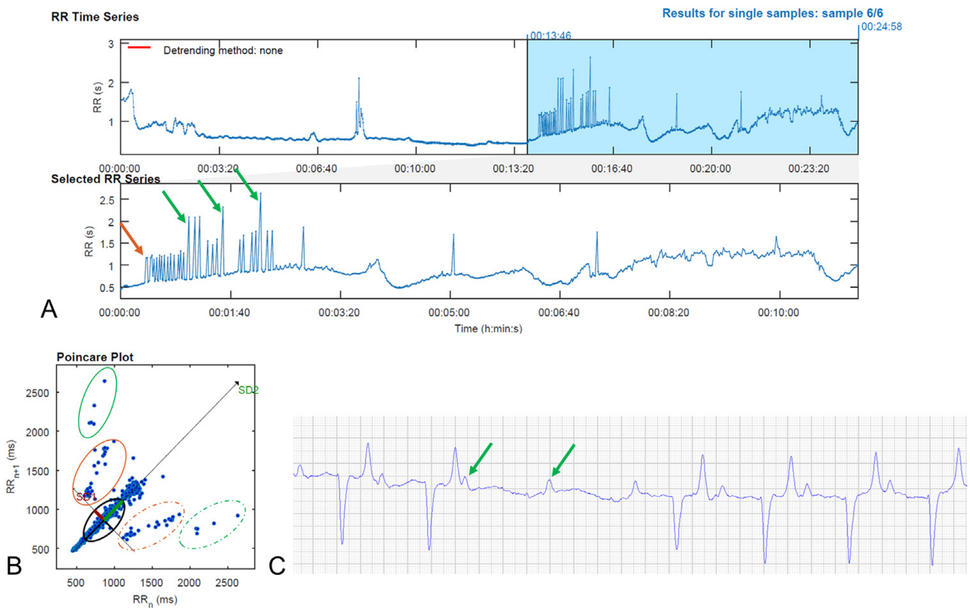

Fig. 10. (A) An RR interval time series plot from a horse during a lunging exercise test, with an ECG recorded simultaneously. The blue area of the top graph is highlighted below (selected RR series) and corresponds with the period of recovery after exercise. The rhythm appears regular until the orange arrow, when a period of sudden deceleration of heart rate occurs (corresponding with the ECG seen in C) once the heart rate drops below approximately 100 beats per min (bpm). These are second-degree atrio-ventricular (AV) blocks mostly blocking one sinus beat at a time, although there are several periods (green arrows) of two sinus beats blocked in a row. The relationship between the sudden decelerations clearly be seen in the tachogram. No premature complexes are noted during this recording period. (B) A Poincaré plot created from the RR interval time series described in A (the selected segment of RR tachogram marked in blue). Most of the RR interval pairs fall on the line of identity, indicating a regular rhythm with low beat-to-beat variability however, the solid orange circled cloud represents normal RR intervals followed by a twice as long RR interval (as with a single second-degree AV block) while the solid green circled cloud represents the normal RR interval, followed by a RR interval that is three times longer (as with two second-degree AV blocks in a row). The dotted orange-circled cloud represents the RR intervals that are twice as long, followed by normal intervals (as after a single second-degree AV block) while the dotted green-circled cloud represents the RR intervals that are three times as long, followed by normal intervals (as after two second-degree AV blocks in a row). (C) A representative segment of ECG recorded during the period of arrhythmia highlighted with the green arrows in A. Two second-degree AV blocks are present in a row (green arrows).

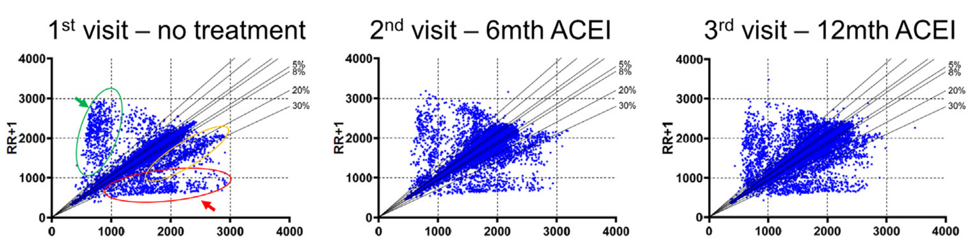

Fig. 11. These three Poincaré plots are created from RR interval data sets recorded from a horse with severe aortic regurgitation and left ventricular (LV) and left atrial enlargement, at rest, each over a 24 h period. In contrast with the normal 'comet' pattern seen in the Poincaré plot of Fig. 1B, this pattern is described as a 'three lobed' pattern, representing a sinus rhythm and frequent ventricular ectopy. The first recording was obtained before any therapy was commenced. The red circled cloud (or 'lobe') represents normal RR intervals followed by a shorter RR interval (as with the ventricular premature complexes) while the green circled cloud (or 'lobe') represents the shorter RR interval, followed by a longer RR interval (as with a pause following the premature complex). The third, orange circled cloud (or 'lobe') represents the longer RR interval followed by a normal RR interval (as with a normal complex following the pause). The thin black lines indicate RR deviation from the line of identity (which would represent 0% RR deviation) by 5%, 8%, 20% and 30%. The second Poincaré plot is from the same horse, 6 months after commencing therapy with an angiotensin-converting enzyme inhibitor (ACEI). The 'three lobed' appearance is still present, however each of the clouds appears to have less density, representing less frequent ventricular ectopy. Echocardiographically, this horse showed reduced left ventricular (LV) and left atrial dimensions and improved LV systolic function. The third Poincaré plot is from the same horse, presented for a recheck evaluation 1 year after the initial examination, still undergoing therapy with the ACEI. The 'three lobed' pattern is still present and there is an increased density to each of the lobes, indicating a higher frequency of ventricular ectopy. Echocardiographically, while the LV systolic function was maintained, the LV dimensions were increased slightly but were still smaller than 12 months previously.

development or reoccurrence of AF, thus allowing the horse to receive appropriate interventions more rapidly.

Intermittent/paroxysmal arrhythmias

Currently, there is only a single publication describing the use of HRV analysis to detect intermittent or paroxysmal arrhythmias in horses. The study by Frick et al. (2019) found that during exercise, where normal beat-to-beat variability should be very low, horses with arrhythmias had far greater variability compared to horses in normal sinus rhythm. This effect was dependent on the number and type of arrhythmias (a larger number of arrhythmias resulted in higher HRV, AV blocks and sinus pauses resulted in larger beat-to-beat variations, this is shown in Fig. 7) and was seen most strikingly during the recovery phase after exercise, where arrhythmias were more prevalent. An example of this is seen in Fig. 8.

In particular, the graphical representations of HRV are useful when examining exercising ECG recordings. The normal low beat-to-beat variability can be clearly seen in both the RR interval time series tachograms and in Poincaré plots (Fig. 8A, B and C). Any premature complexes, subsequent pauses or delayed complexes are clearly identified and the relationship between beats in complex periods of arrhythmia can be examined. Examples of this are seen in Fig. 8D and E and in Figs. 9 and 10. The visual display of a data set allows rapid interpretation and understanding of the number, complexity and timing of any complexes that disturb the normal sinus rhythm. Detailed evaluation of the ECG tracing is still required to detect interpolated or fusion beats that fail to disrupt the underlying rhythm.

The authors also use HRV graphical analysis to provide summary information about arrhythmia frequency during longer-duration Holter recordings. These can provide the clinician a rapid assessment of the overall rhythm for the duration of the recording as can be seen in the example of a horse following conversion from AF (see Fig. 6) where the Poincaré plot clearly shows a reduction in the number of atrial premature complexes 1 month after conversion. Similarly, in Fig. 11, the number of VPCs in a horse with severe aortic regurgitation and left ventricular enlargement can be visually estimated. A decrease in number of VPCs is seen 6 months after commencing therapy with an angiotensin-converting enzyme inhibitor; however, after a further 6 months of therapy there is an increase in the number of VPCs. This fitted with evidence of disease progression detected echocardiographically, although day-to-day variability in the absolute arrhythmia burden of such horses is not yet reported in the literature and could account for some of the change seen in this Poincaré plot (Spier and Meurs, 2004b).

Outlook for equine ECG and HRV analysis: machine learning, support vector machines and artificial neural networks

The detailed analysis of ECGs, heart rate or pulse waveform data sets involves many time-consuming steps to the process. It is of critical importance that good quality recordings are obtained in the first instance, to reduce the interference of artefacts on accurate detection of the inter-beat intervals. The manual analyses of these data sets by individuals such as clinicians is not sustainable in the longer term. Therefore, integration of advanced computer algorithms for pattern recognition, disease surveillance and other clinical utilities will provide faster and hopefully more accurate information to the end-user (Sansone et al., 2013; Tison et al., 2018; Pereira et al., 2020). The first of these approaches applied to equine cardiology has just been reported, describing the use of an artificial neural network to correctly classify isolated premature complexes within equine ECG recordings (Van Steenkiste et al., 2020).

Another group has investigated the use of complexity estimation techniques, which break down the ECG tracing into a series of binary data, to analyse resting ECGs in horses reported to have a history of paroxysmal AF (PAF). While preliminary, their results suggest that horses with a history of PAF have lower complexity in their sinus rhythm recordings when compared with controls, which could aid in the diagnosis of PAF in horses (Alexeenko et al., 2020). These studies open the door for pathways for the future and this rapidly expanding area will provide many exciting findings in the years to come.

Conclusions

Heart rate variability analysis can be extremely useful when examining data sets acquired from individuals with arrhythmias. The rapid expansion of knowledge in interpretation of changes in HRV variables as a consequence of arrhythmia is helping improve our recognition and treatment of arrhythmias in a number of different contexts, particularly in the human field. By translation of these data analysis techniques to our equine patients, we will also be able to improve our understanding of disease mechanisms, predict adverse outcomes and improve our therapeutic strategies for horses with cardiac arrhythmias.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship that could inappropriately influence or bias the content of this paper.

Declaration of Competing Interest

The authors report no declarations of interest.

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References

Abbott, J.A., 2005. Heart rate and heart rate variability of healthy cats in home and hospital environments. Journal of Feline Medicine and Surgery 7, 195–202.
Alexeenko, V., Fraser, J.A., Bowen, M., Huang, C.L., Marr, C.M., Jeevaratnam, K., 2020. The complexity of clinically-normal sinus-rhythm ecgs is decreased in equine athletes with a diagnosis of paroxysmal atrial fibrillation. Science Reports 10, 6822.
Blake, R.R., Shaw, D.J., Culshaw, G.J., Martinez-Pereira, Y., 2018. Poincare plots as a measure of heart rate variability in healthy dogs. Journal of Veterinary Cardiology 20, 20–32.
Bogucki, S., Noszczyk-Nowak, A., 2017. Short-term heart rate variability in dogs with sick sinus syndrome or chronic mitral valve disease as compared to healthy controls. Polish Journal of Veterinary Sciences 20, 167–172.
Borracci, R.A., Montoya Pulvet, J.D., Ingino, C.A., Fitz Maurice, M., Hirschon Prado, A., Domine, E., 2018. Geometric patterns of time-delay plots from different cardiac rhythms and arrhythmias using short-term EKG signals. Clinical Physiology and Functional Imaging 38, 856–863.
Broux, B., De Clercq, D., Decloedt, A., Ven, S., Vera, L., van Steenkiste, G., Mitchell, K., Schwarzwald, C., van Loon, G., 2017. Heart rate variability parameters in horses distinguish atrial fibrillation from sinus rhythm before and after successful electrical cardioversion. Equine Veterinary Journal 49, 723–728.
Broux, B., De Clercq, D., Vera, L., Ven, S., Deprez, P., Decloedt, A., van Loon, G., 2018. Can heart rate variability parameters derived by a heart rate monitor differentiate between atrial fibrillation and sinus rhythm? BMC Veterinary Research 14, 320.
Calvert, C.A., 1998. Heart rate variability. The Veterinary Clinics of North America: Small Animal Practice 28, 1409–1427 viii.
Calvert, C.A., Jacobs, G.J., 2000. Heart rate variability in doberman pinschers with and without echocardiographic evidence of dilated cardiomyopathy. American Journal of Veterinary Research 61, 506–511.
Calvert, C.A., Wall, M., 2001. Effect of severity of myocardial failure on heart rate variability in Doberman pinschers with and without echocardiographic evidence of dilated cardiomyopathy. Journal of the American Veterinary Medical Association 219, 1084–1088.
Castaneda, D., Esparza, A., Ghamari, M., Soltanpur, C., Nazeran, H., 2018. A review on wearable photoplethysmography sensors and their potential future applications in health care. International Journal of Biosensors and Bioelectronics 4, 195–202.
Cheung, C.C., Krahn, A.D., Andrade, J.G., 2018. The emerging role of wearable technologies in detection of arrhythmia. The Canadian Journal of Cardiology 34, 1083–1087.
Chong, J.W., Esa, N., McManus, D.D., Chon, K.H., 2015. Arrhythmia discrimination using a smart phone. IEEE Journal of Biomedical Health and Informatics 19, 815–824.
Cottin, F., Barrey, E., Lopes, P., Billat, V., 2006. Effect of repeated exercise and recovery on heart rate variability in elite trotting horses during high intensity interval training. Equine Veterinary Journal 38, 204–209.
Disertori, M., Mase, M., Rigoni, M., Nollo, G., Ravelli, F., 2016. Heart rate turbulence is a powerful predictor of cardiac death and ventricular arrhythmias in postmyocardial infarction and heart failure patients: a systematic review and meta-analysis. Circulation - Arrhythmia and Electrophysiology 9.
Eggensperger, B.H., Schwarzwald, C.C., 2017. Influence of 2nd-degree AV blocks, ECG recording length, and recording time on heart rate variability analyses in horses. Journal of Veterinary Cardiology 19, 160–174.
Ernst, G., 2017. Heart-rate variability-more than heart beats? Frontiers in Public Health 5, 240.
Esperer, H.D., Esperer, C., Cohen, R.J., 2008. Cardiac arrhythmias imprint specific signatures on Lorenz plots. Annals of Noninvasive Electrocardiology 13, 44–60.
Flethoj, M., Kanters, J.K., Pedersen, P.J., Haugaard, M.M., Carstensen, H., Olsen, L.H., Buhl, R., 2016. Appropriate threshold levels of cardiac beat-to-beat variation in

semi-automatic analysis of equine ECG recordings. BMC Veterinary Research 12, 266.
Frick, L., Schwarzwald, C.C., Mitchell, K.J., 2019. The use of heart rate variability analysis to detect arrhythmias in horses undergoing a standard treadmill exercise test. Journal of Veterinary Internal Medicine 33, 212–224.
Giannakakis, G., Tsiknakis, M., Vorgia, P., 2019. Focal epileptic seizures anticipation based on patterns of heart rate variability parameters. Computer Methods and Programs in Biomedicine 178, 123–133.
Gladuli, A., Moise, N.S., Hemsley, S.A., Otani, N.F., 2011. Poincare plots and tachograms reveal beat patterning in sick sinus syndrome with supraventricular tachycardia and varying AV nodal block. Journal of Veterinary Cardiology 13, 63–70.
Hamlin, R.L., Smith, C.R., Smetzer, D.L., 1966. Sinus arrhythmia in the dog. The American Journal of Physiology 210, 321–328.
Harris, J.D., Little, C.J.L., Dennis, J.M., Patteson, M.W., 2017. Heart rate turbulence after ventricular premature beats in healthy Doberman pinschers and those with dilated cardiomyopathy. Journal of Veterinary Cardiology 19, 421–432.
Kuhni, M., Schwarzwald, C.C., Mitchell, K.J., 2020. Poincaré plots as a visual measure of heart rate variability in healthy horses. Proceedings of the Annual American College of Veterinary Internal Medicine Forum, Virtual Format, June 2020.
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. Frontiers in Veterinary Science 4, 170.
Lerma, C., Wessel, N., Schirdewan, A., Kurths, J., Glass, L., 2008. Ventricular arrhythmias and changes in heart rate preceding ventricular tachycardia in patients with an implantable cardioverter defibrillator. Medical and Biological Engineering and Computing 46, 715–727.
Li, K.H.C., White, F.A., Tipoe, T., Liu, T., Wong, M.C., Jesuthasan, A., Baranchuk, A., Tse, G., Yan, B.P., 2019. The current state of mobile phone apps for monitoring heart rate, heart rate variability, and atrial fibrillation: narrative review. JMIR mHealth and uHealth 7, e11606.
Lombardi, F., Tundo, F., Abukwaik, A., Tarricone, D., 2007. Heart rate turbulence and variability in patients with ventricular arrhythmias. Heart International 3, 51.
Malik, M., Bigger, J.T., Camm, A.J., et al., 1996. Heart rate variability: standards of measurement, physiological interpretation, and clinical use: Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 93, 1043–1065.
Marr, C.M., Bowen, M., 2010. Ambulatory electrocardiography and heart rate variability, Cardiology of the Horse. 2nd edition Saunders, Elsevier, St Louis, MO, USA pp 133.
McConachie, E.L., Giguere, S., Rapoport, G., Barton, M.H., 2016. Heart rate variability in horses with acute gastrointestinal disease requiring exploratory laparotomy. Journal of Veterinary Emergency and Critical Care 26, 269–280.
Mellor, A., Bakker-Dyos, J., O'Hara, J., Woods, D.R., Holdsworth, D.A., Boos, C.J., 2018. Smartphone-enabled heart rate variability and acute mountain sickness. Clinical Journal of Sport Medicine 28, 76–81.
Moise, N.S., Gladuli, A., Hemsley, S.A., Otani, N.F., 2010. Zone of avoidance': RR interval distribution in tachograms, histograms, and Poincare plots of a Boxer dog. Journal of Veterinary Cardiology 12, 191–196.
Moise, N.S., Flanders, W.H., Pariaut, R., 2020. Beat-to-beat patterning of sinus rhythm reveals non-linear rhythm in the dog compared to the human. Frontiers in Physiology 10, 1548.
Mourot, L., Bouhaddi, M., Perrey, S., Rouillon, J.D., Regnard, J., 2004. Quantitative Poincare plot analysis of heart rate variability: effect of endurance training. European Journal of Applied Physiology 91, 79–87.
Munsters, C.C., de Gooijer, J.W., van den Broek, J., van Oldruitenborgh-Oosterbaan, M.M., 2013. Heart rate, heart rate variability and behaviour of horses during air transport. The Veterinary Record 172, 15.
Nagel, C., Aurich, J., Aurich, C., 2010. Determination of heart rate and heart rate variability in the equine fetus by fetomaternal electrocardiography. Theriogenology 73, 973–983.
Ohmura, H., Hiraga, A., Aida, H., Kuwahara, M., Tsubone, H., Jones, J.H., 2006. Changes in heart rate and heart rate variability in Thoroughbreds during prolonged road transportation. American Journal of Veterinary Research 67, 455–462.
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. Comparative Exercise Physiology 6, 137–142.
Parsi, A., O'Loughlin, D., Glavin, M., Jones, E., 2019. Heart rate variability analysis to predict onset of ventricular tachyarrhythmias in implantable cardioverter defibrillators. Conference proceedings: Annual International Conference of the IEEE Engineering in Medicine and Biology Society, July 2019, pp. 6770–6775.
Pereira, T., Tran, N., Gadhoumi, K., Pelter, M.M., Do, D.H., Lee, R.J., Colorado, R., Meisel, K., Hu, X., 2020. Photoplethysmography based atrial fibrillation detection: a review. NPJ Digital Medicine 3, 3.
Perini, R., Veicsteinas, A., 2003. Heart rate variability and autonomic activity at rest and during exercise in various physiological conditions. European Journal of Applied Physiology 90, 317–325.
Perkiomaki, J., Ukkola, O., Kiviniemi, A., Tulppo, M., Ylitalo, A., Kesaniemi, Y.A., Huikuri, H., 2014. Heart rate variability findings as a predictor of atrial fibrillation in middle-aged population. Journal of Cardiovascular Electrophysiology 25, 719–724.
Physick-Sheard, P.W., Marlin, D.J., Thornhill, R., Schroter, R.C., 2000. Frequency domain analysis of heart rate variability in horses at rest and during exercise. Equine Veterinary Journal 32, 253–262.
Rajakariar, K., Koshy, A.N., Sajeev, J.K., Nair, S., Roberts, L., Teh, A.W., 2020. Accuracy of a smartwatch based single-lead electrocardiogram device in detection of atrial fibrillation. Heart 106, 665–670.
Rasmussen, C.E., Falk, T., Zois, N.E., Moesgaard, S.G., Häggström, J., Pedersen, H.D., Ablad, B., Nilsen, H.Y., Olsen, L.H., 2012. Heart rate, heart rate variability, and arrhythmias in dogs with myxomatous mitral valve disease. Journal of Veterinary Internal Medicine 26, 76–84.
Rasmussen, C.E., Falk, T., Domanjko Petric, A., Schaldemose, M., Zois, N.E., Moesgaard, S.G., Ablad, B., Nilsen, H.Y., Ljungvall, I., Hoglund, K., et al., 2014. Holter monitoring of small breed dogs with advanced myxomatous mitral valve disease with and without a history of syncope. Journal of Veterinary Internal Medicine 28, 363–370.
Reed, M.J., Robertson, C.E., Addison, P.S., 2005. Heart rate variability measurements and the prediction of ventricular arrhythmias. Quarterly Journal of Medicine 98, 87–95.
Rietmann, T.R., Stauffacher, M., Bernasconi, P., Auer, J.A., Weishaupt, M.A., 2004. The association between heart rate, heart rate variability, endocrine and behavioural pain measures in horses suffering from laminitis. Journal of Veterinary Medicine 51, 218–225.
Ringwald, M., Crich, A., Beysard, N., 2020. Smart watch recording of ventricular tachycardia: case study. American Journal of Emergency Medicine 38, 849.
Rowan 3rd, W.H., Campen, M.J., Wichers, L.B., Watkinson, W.P., 2007. Heart rate variability in rodents: uses and caveats in toxicological studies. Cardiovascular Toxicology 7, 28–51.
Sansone, M., Fusco, R., Pepino, A., Sansone, C., 2013. Electrocardiogram pattern recognition and analysis based on artificial neural networks and support vector machines: a review. Journal of Healthcare Engineering 4, 465–504.
Shaffer, F., Ginsberg, J.P., 2017. An overview of heart rate variability metrics and norms. Frontiers in Public Health 5, 258.
Singh, N., Moneghetti, K.J., Christle, J.W., Hadley, D., Froelicher, V., Plews, D., 2018. Heart rate variability: an old metric with new meaning in the era of using mHealth technologies for health and exercise training guidance. Part two: Prognosis and training. Arrhythmia and Electrophysiology Review 7, 247–255.
Spier, A.W., Meurs, K.M., 2004a. Assessment of heart rate variability in Boxers with arrhythmogenic right ventricular cardiomyopathy. Journal of the American Veterinary Medical Association 224, 534–537.
Spier, A.W., Meurs, K.M., 2004b. Evaluation of spontaneous variability in the frequency of ventricular arrhythmias in boxers with arrhythmogenic right ventricular cardiomyopathy. Journal of the American Veterinary Medical Association 224, 538–541.
Tarvainen, M.P., Niskanen, J.P., Lipponen, J.A., Ranta-Aho, P.O., Karjalainen, P.A., 2014. Kubios HRV—heart rate variability analysis software. Computer Methods and Programs in Biomedicine 113, 210–220.
Thayer, J.F., Hahn, A.W., Sollers, J.J., van Doornen, L., Johnson, P.J., 1997. Heart rate variability in the horse by ambulatory monitoring. Biomedical Sciences Instrumentation 33, 482–485.
Tison, G.H., Sanchez, J.M., Ballinger, B., Singh, A., Olgin, J.E., Pletcher, M.J., Vittinghoff, E., Lee, E.S., Fan, S.M., Gladstone, R.A., et al., 2018. Passive detection of atrial fibrillation using a commercially available smartwatch. Journal American Medical Association - Cardiology 3, 409–416.
Van Steenkiste, G., van Loon, G., Crevecoeur, G., 2020. Transfer learning in ECG classification from human to horse using a novel parallel neural network architecture. Scientific Reports 10, 186.
van Vollenhoven, E., Grant, C.C., Fletcher, L., Ganswindt, A., Page, P.C., 2016. Repeatability and reliability of heart rate variability in healthy, adult pony mares. Journal of Equine Veterinary Science 46, 73–81.
von Borell, E., Langbein, J., Despres, G., Hansen, S., Leterrier, C., Marchant-Forde, J., Marchant-Forde, R., Minero, M., Mohr, E., Prunier, A., et al., 2007. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals—a review. Physiology and Behavior 92, 293–316.
Wegner, F.K., Kochhauser, S., Ellermann, C., Lange, P.S., Frommeyer, G., Leitz, P., Eckardt, L., Dechering, D.G., 2020. Prospective blinded evaluation of the smartphone-based AliveCor Kardia ECG monitor for atrial fibrillation detection: the PEAK-AF study. European Journal of Internal Medicine 73, 72–75.
Woo, M.A., Stevenson, W.G., Moser, D.K., Trelease, R.B., Harper, R.M., 1992. Patterns of beat-to-beat heart rate variability in advanced heart failure. American Heart Journal 123, 704–710.
Younes, M., Robert, C., Barrey, E., Cottin, F., 2016. Effects of age, exercise duration, and test conditions on heart rate variability in young endurance horses. Frontiers in Physiology 7, 155.
Zhang, L., Guo, T., Xi, B., Fan, Y., Wang, K., Bi, J., Wang, Y., 2015. Automatic recognition of cardiac arrhythmias based on the geometric patterns of Poincare plots. Physiological Measurement 36, 283–301.