Abstract

VON BORELL, E., J. LANGBEIN, G. DESPRES, S. HANSEN, C. LETERRIER, J. MARCHANT-FORDE, R. MARCHANT-FORDE, M. MINERO, E. MOHR, A. PRUNIER, D. VALANCE, AND I. VEISSIER. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals – a review. PHYSIOL BEHAV xx (x) 000-000, 2006.-Measurement of heart rate variability (HRV) is a non-invasive technique that can be used to investigate the functioning of the autonomic nervous system, especially the balance between sympathetic and vagal activity. It has been proven to be very useful in humans for both research and clinical studies concerned with cardiovascular diseases, diabetic autonomic dysfunction, hypertension and psychiatric and psychological disorders. In the last 10 years, HRV has been used increasingly in animal research to analyse changes in sympathovagal balance related to diseases, psychological and environmental stressors or individual characteristics such as temperament and coping strategies. This paper summarizes the state of the art of HRV research in farm animals. First, it describes how cardiac activity is regulated and the relationships between HRV, sympathovagal balance and stress and well-being. Then it outlines the types of equipment and methodological approaches that have been adapted and developed to measure inter-beats intervals (IBI) and estimate HRV in farm animals. Finally, it briefly describes experiments and conclusions derived from the measurement of HRV in pigs, cattle, horses, sheep, goats and poultry. Emphasis has been placed on deriving recommendations for future research investigating HRV, including approaches for measuring and analysing IBI data. On the whole, data from published research demonstrate that HRV is indeed a useful tool for evaluating stress and emotional states and has the potential to contribute much to our understanding and assessment of the underlying neurophysiological processes of stress responses and welfare in farm animals.

Indexing terms: Cardiac activity, heart rate variability, stress, welfare, farm animals,

Title: Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals – a review

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1. Introduction

The "Measuring welfare" working group of the EU concerted action on '***Measuring and Monitoring Welfare'*** (COST Action 846) has identified key areas of research that have the potential to contribute to our understanding and interpretation of stress and welfare status in farm animals. The ***"Heart Rate and Heart Rate Variability Task Force"*** has brought together experts in the fields of animal and veterinary sciences who are concerned with research on cardiac activity and heart rate variability (HRV) in farm animals. Their principle goal is to write a report on the state of the art of HRV in farm animals, similar to a task force that had been previously set up by the European Society of Cardiology (ESC) and the North American Society of Pacing and Electrophysiology [NASPE, 1]. The purpose of this review is to: 1) Collate information on the current status of HRV measurement techniques, data handling, analysis and interpretation, 2) Outline appropriate methodology for different farm animal species, and; 3) Identify areas of HRV research that warrant future attention and may improve our ability to evaluate stress and welfare status in farm animals.

Some of the most remarkable non-invasive measures of the functioning of the autonomic nervous system (ANS) are indices of HRV. Detailed and sophisticated analysis of short-term fluctuations in instantaneous heart period has been widely used to indirectly assess ANS regulation of cardiovascular function [1, 2]. In humans, the last three decades have witnessed the recognition of a significant relationship between autonomic function and various diseases and mental states [3, 4], including cardiac dysfunction, sudden cardiac death [5-7], diabetic autonomic dysfunction [8, 9], hypertension [10-12] and psychiatric disorders [13-16]. Analysis of HRV has also been used as an indicator of acute and chronic stress [17, 18], mental challenges and emotional states [15, 19-23]. In the last ten years, HRV has also been applied increasingly in veterinary and behavioural research to investigate changes in

sympathovagal balance related to pathological conditions [24-26], stress [27-31], behavioural dysfunction [32, 33], management practices [34-36], training régimes [37-39] as well as temperament and emotional states [40-42] in various farm and companion animal species.

2. Regulation, interpretation and significance of heart rate variability (HRV) in relation to stress and well-being

2.1. Regulation of heart beat activity

The sinus node or sinoatrial node (SN) acts as the main pulse generator for heart beats [43, 44] although other parts of the cardiac nervous system such as artrioventricular (AV) nodes are also capable of exhibiting autonomous heart beat stimulation properties. The SN, as the primary pacemaker, has the highest discharge frequency and excites other cardiac centres (such as the AV node) before they themselves initiate their own electrical impulses [43]. The SN contains two types of cells, elongated and round. The round, or pacemaker, cells are capable of spontaneous depolarisation that initiates electrical activation within the heart itself. In the absence of autonomic innervations, or under complete autonomic blockage, the discharge rate of pacemaker cells represents intrinsic heart rate (HR). In humans, the SN generates an intrinsic HR between 100 and 120 beats per minute (bpm) in the absence of any neural and hormonal influences [45].

The SN is under the control of the parasympathetic (vagal, PNS) and sympathetic nervous system (SNS) [46]. Both left and right vagus nerves stimulate the SN (the right nerve is dominant and reduces HR), the AV node (left nerve is dominant and prolongs AV conductor) as well as the atrium muscle, whereas efferent control of the ventricle muscle is unclear [43, 47]. In general, activity within the vagal nerves decreases HR when the stimulatory effect of

the right nerve dominates [48]. Postganglionic sympathetic fibres innervate almost all centres of the heart (AV, heterotrophic centres, atrium and ventricle myocardium [43, 47]). Furnival, Linden et al. [49] demonstrated that activity within the right Ansa subclavia (right sympathetic nerve) mostly influences HR, whereas left Ansa subclavia activity increases stroke volume. In resting conditions, both branches of the ANS are tonically active in regulating cardiac activity and there is generally a dominance of vagal regulation [47].

Rapid changes in HR are always caused by shifts in vagal regulation [47, 50-52]. The SN responds to vagal activity within one or two heart beats, but its overall effects are relatively short-lived. Vagal induced changes in HR typically occur within five seconds [47] whereas cardiac responses to SNS regulation occur more slowly with initial response delays of up to five seconds, followed by a progressive change and a maximum response after 20 to 30 seconds [47, 53]. These differences in response times are due in part to the relatively slow exocytotic release of noradrenaline from sympathetic nerve terminal through which the SNS regulates cardiac activity. Also, unlike PNS acetylcholine mechanisms, a secondary messenger (adenylyl cyclase) is involved in SNS regulation which further slows the process. Other anatomical disparities between the autonomic branches may also contribute to the slower response rate to SNS regulation. For instance, the preganglionic cell bodies of the PNS neurons are located within the heart itself, whereas those of the sympathetic neurons are comparatively isolated in the paravertebral ganglia. Furthermore, preganglionic fibres are also myelinated contributing to faster electrical transmission of vagal regulatory signals compared to transmission rates in unmyelinated SNS fibres.

2.2. Heart rate variability (HRV)

Healthy cardiac activity is characterized by irregular intervals between consecutive heart beats [54]. This variability is a result of rhythmic oscillation of the regulatory components of cardiac activity that function to maintain cardiovascular homeostasis within a defined range [46, 55]. They also serve to orchestrate and facilitate appropriate responses to intrinsic and extrinsic challenges [55].

HRV mainly emerges primarily through the non-additive activities of the two branches of the ANS [56-59] (Fig. 1), which in turn is influenced by neuronal, humoral and other physiological control and feedback mechanisms [60, 55]. The central nervous system (CNS), in particular the formatio reticularis of the medulla oblongata (medullar circulation centre), the hypothalamus, and neocortical and paleocortical areas, participate in all levels of cardiovascular regulation (Fig. 2) [61-64].

An oscillatory curve (tachogram) is produced when consecutive IBIs are plotted on a time scale [55]. The "mixed oscillation" of this curve results from the rhythmic undulations of the different regulatory components, where rhythmic activities originating from the PNS exhibit greater frequency than those of the SNS (see Fig. 1). Fluctuations in vagal tone are linked to variations in activity of the vagal nuclei which are influenced by baroreceptors since vagal receptivity for baroreceptor input varies with the breathing cycle [52].

Fig. 1

Fig. 2

HRV was first documented in the 18th century by Hales who did the first quantitative measurements on arterial blood pressure (BP) [cited by 65]. This work demonstrated a relationship between breathing cycles, BP and the interval between two consecutive heart beats. Since then, indices of HRV have been reported in a large body of research assessing different physical, pathologcial and psychological conditions and have been applied very

successfully to the understanding and diagnosis of cardiovascular diseases and autonomic dysfunction in humans and animal [66]. In comparison to the many publications in human research, the application of HRV in animal studies is still very much in its infancy and published work is mainly very basic in nature [60, 67]. Nevertheless, HRV in applied farm animal research is progressively emerging as an indicator of stress and welfare under different housing and management conditions in a variety of species.

2.3. Interpretation and significance

HR, at any point in time in healthy subjects, represents the net interactions between vagal (which reduces HR) and SNS (which increases HR) regulatory activity [47, 68]. At rest, vagal regulation dominates but a change in physical activity is sometimes characterised by decreasing vagal and increasing sympathetic influences. An increase in HR generated by the SN is mainly caused by an increase in sympathetic activity [47] but it may also result from a decrease in vagal regulation or simultaneous changes in both branches.

Separate effects of the two branches of the nervous systems cannot be determined by simple addition or subtraction of the relative components [47]. The branches do not necessarily function on a continuum when regulating cardiac activity, where an increase in one branch results in a decrease in the other. Rather, they have the ability to behave either synchronously or independently of each other, giving rise to the potential of multiple patterns of activation [see 69]. It is therefore difficult to assess the functional regulatory characteristics of the ANS with simple HR measurements as both systems may influence HR differently [70]. The PNS and SNS interact continuously at both central and peripheral levels as well as on pre- and postsynaptic neurotransmission [53]. An increase in HR, therefore, could result from reduced vagal activity as well as from increased sympathetic activity or, in most cases, from a

combination of changes in activity within both branches. In reality, however, the interplay between the branches is quite complex and not yet fully appreciated. Mean HR parameters provide information on the net effects of all components inputting into cardiac activity and are of limited use for accurately assessing sympathovagal regulation [71].

HRV analysis, on the other hand, allow a much more accurate and detailed determination of the functional regulatory characteristics of the ANS. HRV is a particularly good indicator for the non-invasive assessment of ANS activity in response to psychophysiological stress [55]. Psychological states may have an impact on sympathovagal balance in the absence of any palpable changes in heart and/or respiration rates [72]. For example, Sleigh, Henderson [73] failed to demonstrate any relationship between mean HR and anxiety during pre-surgery stress but found a reduction in the relative intensity of the HF-component in the power spectrum of the HRV (representing a decrease in vagal activity) which was positively correlated with pre-surgery anxiety levels. Furthermore, McCraty, Atkinson et al. [74] found a significant decrease in HRV in relation to reduced well-being, particularly in the frequencies of the power spectrum that are sensitive to PNS modulations, whereas no effect was seen on overall mean HR. Catipovic-Veselica et al. [75] reported interactions between basic emotions, such as fear and aggression, and HRV that were not evident in HR responses. Numerous studies on social behaviour in children have employed measures of HR and HRV. Porges, Doussard-Roosevelt et al. [76] compared HR and HRV in 9-month-old children with their social behaviour at 3 years of age and found a negative relationship between HRV (vagal tone, both basal levels and magnitude of change) and behavioural problems, contact problems, aggressiveness, depression, and sleep disorders when no significant correlations with HR parameters were evident.

Although most papers in the published literature on emotional states and HRV relate to humans, there are strong arguments that the same principles can be applied to non-human mammals since: (1) the phylogenetic 'old' limbic system is considered as the neural substrate for emotions and is similarly present in both humans and other mammals [77]; (2) electrical stimulation of the hypothalamus and the limbic system in animal models leads to similar emotional responses to those seen in humans [63], (3) the endogenous impact of emotions is transmitted via the vegetative nervous system in both humans and other mammals, and; (4) the functional control of vagal tone is similar in all mammals [78]. For a broader discussion of this topic see also the review on positive emotions in farm animal in this issue.

2.4. Vagal tone as a stress indicator

Since the time of researchers such as Walter Cannon [79], stress research has mainly focused on the role the SNS plays in orchestrating stress responses. Studies involving pharmacological activation or blockade of ANS activity suggest that sympathetic tone may not be simply or directly derived from HRV parameters [80, 81]. There are, however, many studies that have demonstrated the usefulness of the LF/HF ratio of the power spectrum as an indicator of sympathetic activity during a number of physical and psychological stresses [e.g. 82] with an increase in the LF/HF ratio being interpreted as a regulatory shift towards sympathetic dominance (see details in 3.3). Although there is ongoing debate regarding the suitability of using HRV parameters to estimate SNS activity, it has however proven to be a reliable measure of vagal tone during conditions of stress and homeostasis [83, 84]. Porges defines homeostasis as an autonomous state which enhances visceral functions and is characterised by increased vagal activity. Stress responses can therefore be quantified on this physiological level with relative changes in vagal activity serving as the measurable parameter. In addition, basal autonomic states could also be considered as an index for an individual's susceptibility

to stress with individuals with low vagal tone potentially being more vulnerable to stress. High vagal tone has been linked to efficient autonomic regulatory activity which enables an organism to increase its sensitivity and responsivity to physiological and environmental challenges [37, 59, 76]. For instance, Doussard-Roosevelt et al. [85] reported that high vagal tone in newborn humans was related to greater mental, motor and social abilities at 3 years of age.

Positive emotions may significantly increase the HF-component of a power spectrum [72, 74] whereas the opposite occurs with negative emotions. Indeed, Friedman and Thayer [86] examined HRV (time and frequency analysis) in people who suffered from panic attacks or blood phobia and found that subjects who experienced panic attacks has lower vagal tone than those with a blood phobia, whereas normal controls exhibited the highest tone. In another study, the extent to which the vagal tone of small children changes in test situations 86 has been correlated to their subsequent behaviour. Those that exhibited greater flexibility in vagal tone in response to social and attention tasks also had fewer behavioural problems at a later age [76]. It seems apparent, therefore, that vagal tone is a useful indicator for determining the physiological and psychological flexibility of an organism and for measuring their susceptibility to, and ability to respond, to stress [86].

2.5. HRV and behaviour

Changes in cardiac activity are strongly influenced by behaviour, in particular behaviours that are related to physical activity [39, 40, 87-92]. This motor or physical component is often inappropriately compared with non-motor or psychological components [87, 89, 93]. For comparisons of non-motor 17 components of cardiac activity, only measures made during similar behavioural patterns should be compared [67]. This highlights a potential

methodological difficulty in stress and welfare research since treatments often induce behavioural reactions that are not seen under normal control conditions.

Another potential problem is the fact that change in cardiac function often occurs outside of the interval during which particular behaviour patterns are expressed. Anticipatory changes in cardiac activity has been observed in various species immediately before a change in behaviour which can, for example, induce tachycardia before a flight reaction occurs [93-96]. On the other hand, cardio-physiological responses linked to specific behaviour patterns can persist even when another behaviour pattern has been initiated. In order to accurately analyse the complex oscillatory characteristics of HRV, longer measurement periods are needed than for simpler HR indices for which, theoretically, a single IBI may be sufficient. The TASK FORCE of the ESC and NASPE [1] recommends that IBI dataset undergoing HRV analyses should contain at least 5-min of consecutive IBIs measured during stationary conditions.

3. Methodology of measurement and analysis of HRV in farm animals

3.1. Equipment for recording cardiac activity

Various portable equipment is commercially available for detecting and storing the whole electrocardiogram (ECG) for later detection of IBIs. Some of these systems are designed for ambulatory long-term recording of ECG (mostly up to 24 hours), like Holter systems (Del Mar Reynolds Medical, Hertford, UK; Schiller, Switzerland; Rozinn Electronics, Inc. USA) and are widely used in human medicine. They are often combined with specific algorithms for the detection of IBIs and analysis of HRV (Biopac, Po-Ne-Mah or Cardiopro). However, these systems are very expensive and especially adapted for the study of human cardiac activity. An affordable alternative is to use commercially available monitors that detect the R-

peaks of the ECG during recording and then store IBI data in digital form. To our knowledge, there is currently only one commercial manufacturer (Polar Electro Öy, Finland) who has developed such devices that are primarily marketed for sport and research in sport medicine. There are two different models available on the market that record cardiac activity and detect IBIs at a sampling rate 1000 Hz. The storage capacity of the first model (S810i) can continuously record up to 16,000 IBIs. Postulating a mean HR of around 70 beats per minute (bpm), its maximum recording time is therefore about 4 hours. The S810i, and its predecessor the Vantage NV (stores 4,000 IBIs), have been widely applied in veterinary and behavioural research [30, 35, 36, 67, 97]. Another model, the Polar R-R Recorder, is a digital 24-h ambulatory monitor that can record IBI data over much longer periods [98, 99]. Moreover, this device can also store short (20 sec) epochs of ECG which is not possible with the other model. These devices use an electrode belt containing two coated electrodes that fits around the thorax of the wearer. Detection of the IBIs is carried out during recording and the resulting IBI data are transmitted wirelessly and stored in a data logger. These data may then be downloaded onto a PC for later analysis of HRV. There are two different types of electrode chest belts available to use with these monitors. For smaller animals like goats, sheep, pigs, and calves, a standard belt with an elastic strap can easily be adapted to fix around the thorax of the animal. For large animals, like cattle or horses, a specific transmitter with two separated electrodes should be used to optimise electrode positioning. All can transmit detected IBIs as coded signals to avoid any cross talk between different devices recording at the same time within a given area. Beside the Polar system, various non-commercial mobile systems have also been developed to record not only IBI data but also the ECG in unrestrained animals [28, 100].

A general concern with cardiac monitors that only record IBI data is testing their reliability and identifying true errors in the data. As the ECG signal itself is not recorded, there is no

absolute way to identify errors in IBI measurements after data collection [99, 100]. Several studies have been carried out in cattle and horses, investigating the reliability of these monitors for measuring HR relative to a standard ECG [101, 102]. In humans, Kingsley and colleagues [103] recently compared the Polar S 810i to an ambulatory ECG system (Reynolds, UK) and did not find any differences in IBI data series measured by the two systems. Similarly, good reproducibility was observed both in time- and in frequency-domain measures of HRV in healthy subjects comparing the 24h R-R recorder with a high-quality Holter recorder [104].

Various approaches, from simple visual correction to more sophisticated algorithms, have been developed to correct IBI data for artefact that occur due to the misidentification of R-peaks and ectopic beats. Artefacts may occur as a result of poor electrode-skin conductance, equipment malfunction, noise from muscle action potentials and environmental electromagnetic interference [105-110]. In any case, the editing of the IBI data should be performed to a very high standard ensuring correct identification and correction of IBIs. Marchant-Forde et al. [99] recently identified five (Type 1 – 5) distinct types of errors in IBI data series recorded by the Polar R-R Recorder in pigs (see Fig 3). Type 1 errors were characterised as single point discrepancies, either positive or negative, between the Polar data and IBIs derived conventional ECG data. Type 2 errors were identified as a long IBI immediately followed by a short IBI. In contrast, with type 3 errors, where a short IBI was followed by a compensatory long IBI. Type 4 errors were characterized by a large peak representing more than one IBI, and in type 5 errors, the recorder generated two or more short IBIs in the place of a single IBI. As one can see in Fig. 3, none of these artefacts had ectopic origins, as they were not present in the IBIs derived from the conventional ECG. Type 2 – 5 errors were successfully corrected using an algorithm for recovering IBIs from the information available within the anomalous IBIs themselves [108]. In type 1 errors, the

anomalous IBI could reliably be replaced with the mean of the nearest normal neighbouring IBIs.

Fig. 3

The importance of identifying and correcting artefacts and ectopic beats in IBI data has been demonstrated by a number of authors in both humans and animals alike. The computation of many HRV indices is based upon the amount and type of variability within the data and the presence of even a single error in short duration recordings can significantly bias the outcome of time, and especially, frequency domain analysis [99, 105, 106, 111]. Geometric and non-linear analytical methods seem more resistance to the present of spurious beats and these are discussed in greater detail later on.

3.2. General recommendations for recording of IBIs

When using HRV to measure changes in the sympathovagal balance a general concern is the amount of IBI data necessary for informative analysis of the different indices that accurately represent autonomic function. For a number of rather simple parameters in the time domain (e.g. $SDNN_{index}$, HRV_{index}, see 3.3.), 24-h recording are certainly adequate for determine overall variability. These parameters are useful for detecting tendencies in HRV related to autonomic dysfunction [112], but cannot be reliably used to quantify specific changes in sympathetic or vagal activity. Similarly, frequency-domain analysis has also been applied to human, pig and heifer 24-h ambulatory cardiac data. Although the information obtained has value in general risk assessment, it can be impaired by the occurrence of high numbers of artefacts, ectopic beats and influences of physical activity as well as a lack of stationarity in the data making results difficult to interpret or reproduce at times [2, 113]. Multiple studies have demonstrated that short-term measures of HRV rapidly return to baseline after transient perturbations induced by manipulations such as mild exercise, administration of short acting vasodilators, transient coronary occlusion, etc. For reasons of

standardisation across different studies incorporating HRV, 5-min recordings have been suggested as a recommended recording length unless the nature of the study dictates another design [82]. When analysing longer IBI sequences, averaging the results obtained from 5-min overlapping periods can sometimes minimise some of the difficulties (e.g., non-stationary data) encountered with longer segments of data [1]. This is especially important in the case of spectral analysis of HRV by FFT which is strongly influenced by any non-stationarity inherent in biological data such as cardiac activity. Various studies have demonstrated that analysing 5-min segments of IBI data in the time-, frequency- and non-linear domains deliver results comparable to, or even better than, analysing 24 h of data [1, 68, 114, 115].

As HRV in humans and animals, like many other physiological parameters, is influenced by a variety of factors like sex, age, diurnal rhythms, respiration, fitness levels, posture and physical activity, beside others, it is very important to standardise and control the circumstances under which data are recorded [39, 98, 116-118]. When investigating chronic states, it is recommended that only data relating to time periods when the subjects are supine and calm and undisturbed are analysed to minimise the effects of changing physical activity or arousal on HRV parameters [35, 36]. However, in studies where this is not achievable, any physical activity should be taken into account when analysing and interpreting HRV [40].

IBI data that contain more than 5% anomalies, or segments of IBI data containing 3 or more consecutive error IBIs, should not be included in HRV analysis. Finally, splicing different of segments of data together is also not recommended as it interrupts the underlying time series of the data upon which frequency-domain analysis is based.

3.3. Methods of HRV analysis

Early on, HRV analysis primarily focused on time and frequency domain analysis [1, 55, 68, 119]. However, cardiac activity is an integrated signal that is influenced not only by the two branches of the ANS, but also by a number of other underlying physiological mechanisms and various extrinsic factors. More recent research reports that cardiac signals also contain non-linear components in the dimension of deterministic or non-deterministic chaos [120, 121]. Nowadays, investigation of non-linear components of HRV has been established as a further important field of analysis of HRV [122-124].

Time Domain Analysis

Time-domain measures are the simplest parameters used to analyse HRV. All these measures reflect various aspects of statistical variability in the IBI data series. They are broadly divided into two classes: (a) measures of variability derived from IBI data themselves; (b) measures of variability derived from differences between adjacent IBIs. Prominent indices within each class are described in Table 1.

In the first subclass of time-domain measures, mean IBI and HR are the easiest to calculate but are also the least informative. The standard deviation of all IBIs over a 24-h period or the standard deviation of IBIs of a single 5-min period (SDNN, msec) are good predictors of overall variability present at the time of recording. As the total variance of HRV increases with the length of analysed recording, in practice, it is inappropriate to compare SDNN measures obtained from IBI data series of varying durations. If the SDNN is reported for a 24-h recording it is sometimes referred to as cycle length variability (CLV). The SDANN is the standard deviation of the mean IBI of all 5-min segments in the data. It is also measured and reported in milliseconds and is highly correlated with SDNN. Finally, $SDNN_{index}$ is the mean of the standard deviation of all 5-min segments of a 24-h recording. All these

parameters reflect long-term variability of cardiac activity and are influenced by both sympathetic and parasympathetic nervous activity.

In the second subclass of time-domain measurements, the most informative parameter is undoubtedly the RMSSD (root mean square of successive differences). This is determined by calculating the difference between consecutive IBIs before squaring and summing them, the values are then averaged and the square root obtained. The RMSSD reflects the short-term variability in overall cardiac activity and is the primary time-domain measure used to estimate the high frequency (fast) beat-to-beat variations that represent vagal regulatory activity. Other parameters used to assess beat-to-beat variations include the NN50, the number of neighbouring IBIs that differ by greater than 50 ms, and the pNN50, the proportion of beats differing by 50 ms (NN50/total number of IBIs). As these parameters are highly correlated with RMSSD, they too are also good estimators of vagal activity.

Geometric analyses

Geometric measures metamorphoses sequences of IBIs into geometrical forms and the assessment of HRV is extracted from these forms. Some geometric measures are based on the density distribution (histogram) of IBIs where IBIs are converted into a discrete scale. Most previously published studies have used a scale with bins of approximately 8 ms long (1/128 sec). By deriving information from this density distribution, the effects of anomalous data points are reduced since they are usually substantially shorter or longer than normal IBIs and fall way outside the normal range of the density distribution of the normal data. The most prominent geometrical measures of this type are the HRV_{index} and the $TINN_{index}$ (Table 1). These measures have been preferentially used in commercial systems for analysing 24-h ECG data [1].

Table 1

The Poincaré plot, also referred to as the Lorenz or Scatter plot, is a map of dots in a XY-diagram (Fig. 4). Each dot represents the duration of an IBI plotted against the duration of its preceding IBI. Poincaré plots can be analysed qualitatively by visual inspection whereby the shape of the plot is classified into functional classes that can then be used to interpret the nature of the cardiac signal from which the plot was generated [125-127]. Woo [128] constructed Poincaré plots from 24-hour Holter recordings in healthy subjects and patients with heart failure and found that healthy subjects exhibited comet shaped plots whereas in plots from the heart failure group, three distinctive patterns were identified: (a) a torpedo shaped pattern; (b) a fan-shaped pattern and; (c) a complex, almost erratic, pattern. Visual inspection of Poincaré plots reveals a complexity in cardiac patterns that are not otherwise detected in other HRV measures.

Quantitative analyses of a Poincaré plot entails fitting an ellipse to the plot, with the centre of the ellipse coinciding with the centre point of the scatter plot itself (Fig. 4). In order to do this, the plot is first turned 45° clockwise, and the standard deviation (SD) of the scatter-plot is computed around the horizontal axis, which passes through the data (SD1). This SD1 represents the instantaneous, short-term HRV. The plot is then rotated 45° counter-clockwise, and again the SD of the scatter-plot is computed around the horizontal axis to get the SD of long-term variability of the IBI data (SD2). The absolute values of SD1 and SD2 can then be normalised (SD1n and SD2n, respectively), by dividing them by the average IBI value and then multiplying this by 1,000 [71, 129-132]. Quantitative Poincaré measures have been found to provide useful information on the vagal regulation of cardiac dynamics that is not easily detected by other domains of HRV analysis [133].

Fig. 4

Spectral analysis of HRV by FFT – basic requirements and limits

Fast Fourier Transformation (FFT) is a widespread approach used decrypt and analyse dynamical changes in signals in general. The fundamental principle of this method is based on the fact that every curve can be described by a set of harmonic waves which when added to one another make up the complete waveform. FFT can be used to 'decompose' a waveform into its sine and cosine constituents. There are some prerequisite factors which should be taken into account before using FFT, namely: (a) the time difference between the values of the time series has to be equidistant. In the case of cardiac signals, this means that before analysing IBI data, the tachogram has to be converted into an equidistant time-series by interpolating (preferably using cubic spline functions) and resampling the data. In other words, an instantaneous IBI data series (e.g. for every second of the measuring time) has to be constructed; (b) the FFT-function works on data sets of 2^n numbers. If the data set is not 2^n in length, some programs pad "0" at the beginning and end of the data range to reach the length 2^n. This leads to substantial alterations of the power-spectrum (see Fig. 5.) so it is strongly recommended to extend, or shorten, the data sets by interpolation to get a data set size of 2^n; (c) the level of accuracy achieved in describing the fluctuations of time series depends considerably on the number of points used in the FFT (see Fig. 6). According to published recommendations, a minimum of 512 points should be used for FFT analyses of IBI data [1] (e.g. let us suppose a mean HR of 80 bpm results in an IBI series of 400 values in 5-minutes. This time series has to be lengthened to 512 values by interpolation prior to analysis.). The number of points used for FFT influences the highest frequency (Nyquist frequency) as well as the amount of power in the different frequency bands (Fig. 5).

Fig. 5

Because FFT applies "folding" of the original time series for calculating the power of the various harmonics, the highest oscillation corresponds to ½ of the number of points used for FFT, e.g. the highest frequency in a data set which contains 512 points (representing a time-series of 300 s) is the 256-harmonic with a wavelength of 1.17 s (= 300 s/ 256) that has a

frequency of 0.85 Hz. The spectrum calculated in this way is made up of 256 discrete spectral-lines. The absolute power of the different bands is limited by the number of spectral-lines within the given ranges for the different bands. Using 1024 points (instead of 512) when analysing the same 300 s time series, would result in 512 spectral-lines which means a higher number of spectral-lines and absolute power in the different bands (Fig. 6).

Fig. 6

In spite of all these considerations, one of the great benefits of FFT is the ability to assign the power in different bands to different underlying physiological functions. It is widely accepted that the power in the high frequency (HF) band (0.15 – 0.4 Hz in humans) represents vagal activity [46, 134-136]. The low frequency (LF) band (0.04 – 0.15 Hz) and the very low frequency (VLF) band (≤ 0.04 Hz) are associated with sympathetic [55] or sympathetic plus vagal activity, and their physiological meaning has been much debated [137-139]. Therefore, when calculating the LF/HF ratio as a measure of sympathovagal balance, one has to appreciate that this measure may also be influenced by other physiological functions like thermoregulation or myogenic activity of vessels. The location of vagal power in the HF band of the spectrum is influenced by the respiratory rate of the species [140]. It is, therefore, important to consider respiration rate when locating the HF power for assessment of HRV.

To account for inter-individual differences, LF and HF power may also be expressed in normalised units where the absolute value of each power component is expressed as a proportion of either total power (e.g. LF/total power) or total power minus the VLF component (e.g., LF/(total power-VLF power)) [141, 142].

Recommendations for the FFT analysis of HRV

To increase the inter-study comparability of FFT analysis of HRV, the following recommendations should be taken into consideration:

- Use data-sets of approximately 5-min in length
- Use at least 512 points from the resampled equidistant time series derived from the original IBI data to calculate the power spectrum
- Use species appropriate frequency bands widths such as the HF ranges following:

horse:	0.13 to 0.26 Hz	(corresponds to a respiratory rate of 8 – 16 /min)
foal:	0.25 to 0.33 Hz	(corresponds to a respiratory rate of 15 – 20 /min)
cattle:	0.20 to 0.58 Hz	(corresponds to a respiratory rate of 12 – 35 /min)
calf:	0.50 to 0.83 Hz	(corresponds to a respiratory rate of 30 – 50 /min)
swine (100 kg)	0.13 to 0.41 Hz	(corresponds to a respiratory rate of 8 – 25 /min)
piglet:	0.33 to 0.83 Hz	(corresponds to a respiratory rate of 20 – 50 /min)
goat/sheep:	0.20 to 0.40 Hz	(corresponds to a respiratory rate of 12 – 24 /min)
lamb:	0.33 to 0.58 Hz	(corresponds to a respiratory rate of 20 – 35 /min)
rabbit:	0.67 to 1.00 Hz	(corresponds to a respiratory rate of 40 – 60 /min)
chicken:	0.33 to 0.67 Hz	(corresponds to a respiratory rate of 20 – 40 /min)
duck:	0.83 to 1.17 Hz	(corresponds to a respiratory rate of 50 – 70 /min)

Data should be expressed in normalised units as a percentage or proportion of total power (LF/total power x 100 or HF/total power x 100).

Non-linear analysis of HRV by Recurrence Quantification Analysis

Parameters derived from various non-linear time-series analyses have been found to be sensitive indicators of changes in sympathovagal balance under both healthy and pathological conditions [143-146]. HRV can be influenced by a number of different feedback or feed-forward mechanisms and coupling of two or more oscillators can produce non-linear chaotic behaviour as non-linearity is a hallmark of complex dynamic systems [147, 148]. Such non-linear oscillations have been shown to be an integral part of HRV and a number of authors advocate that these processes are related to deterministic or non-deterministic chaos [149-

153]. Nevertheless, others studies have failed to find evidence of low-dimensional chaos in IBI data [154-156]. These contradictory findings could be partly explained by the fact that IBI data contains a periodic component originating from the basal frequency of the sinus node [157]. Furthermore, some non-linear time-series analyses require that data meet some *a priori* restrictive mathematical assumptions. One method to test whether time-series IBI data really contains non-linear dynamics or just linear relationships in the time and frequency domain is surrogate data analysis [158].

Recurrence Quantification Analysis (RQA) may be applied to IBI data to detect hidden rhythms and non-linear deterministic structures of HRV in higher dimensional space [159, 160]. This mathematical approach has already been applied successfully for estimation of non-linear processes in various physiological time series data [148, 157, 161, 162]. Since RQA is independent of limiting constraints such as data set size, stationarity, and assumptions regarding the statistical distributions of data, it seems ideally suited for investigating physiological systems characterised by non-homeostatic transients and state changes, such as cardiac activity. Validation of RQA of true non-linear components in HRV time series has been performed using surrogate data analysis [163, 164].

Recurrence plots were introduced by Eckman, Kamphorst and Ruelle [165] as a purely graphical tool to uncover non-linear properties in time series. Since then, six parameters have been introduced that may be used to quantitatively assess recurrence plots[1] [159, 160].To perform RQA, the time series is first embedded in a suitable n-dimensional Euclidean space (e.g. n=10, leaving room for up to ten operators to act on HRV) at unitary time lags. The outcome is a specific embedding matrix with a single N-dimensional vector for each point of the time series. From the embedding matrix, a distance matrix is computed determining the

[1] http://homepages.luc.edu/~cwebber/

Euclidean distances between all pairs of vectors (Fig. 7). The distance between each pair of vectors is defined as a recurrence point whenever it is below a predefined cut off value.

Fig.7

All recurrence points are plotted as black points at corresponding X,Y coordinates in the recurrence plot (Fig. 8). In other words, the recurrence plot visualises the distance matrix of all vectors of the N-dimensional embedded HRV-tachogram. According to Giuliani et al. [157] the recurrence plot represents the autocorrelation of a given signal at all possible time scales. Whereas the plot itself already gives an impressive picture of the regularity/irregularity of the tachogram, the parameters defined by Trulla, Webber and Zbilut [160, 163, 166] enable quantitative estimation of the recurrence structure of embedded time series.

Using RQA, a number of quantitative parameters can be derived from the recurrence plots that are useful in assessing HRV (Table 2). The PERCENTAGE OF RECURRENCE (% REC) quantifies the percentage of the plot occupied by recurrence points. It corresponds to the proportion of pair-wise vector distances below a predetermined radius or, equivalently, to repetition of single vectors in the multidimensional space. The PERCENTAGE OF DETERMINISM (DET) is the percentage of recurrence points forming upward diagonal lines in the plot i.e. recurrence points in consecutive sequences, where a line is defined as a sequence that is longer than a preset threshold length. Both %REC and %DET are both parameters of the regularity of HRV in multidimensional space that cannot be proven in the original time series. However, single point recurrence can be observed by chance whenever the system explores two nearby points of its state space. On the other hand, recurrence points that appear in a row, forming diagonal lines, are an important signature of deterministic structuring.

The ENTROPY (ENT) is computed as the Shannon entropy of the deterministic line segment length distributed in a histogram. It corresponds to the richness of deterministic structuring of the series. The MAXLINE (L_{MAX}) is the length of the longest line of recurrence points in a

continuous row within the plot. It is inversely related to the largest positive Lyapunov exponent. The Lyapunov exponent is a quantitative measure of the sensitive dependence of a time series on the initial conditions. A positive largest Lyapunov exponent indicates chaos [165]. A small L_{MAX} corresponds to a high Lyapunov exponent, meaning a large amount of "chaos" and vice versa. Finally, TREND describes how stationary the system is during the period of measurement. Systems showing a drift may have positive or negative TREND values, whereas systems without drift have values close to zero. For a more detailed description of the mathematical background of RQA, several detailed methodological papers have been previously published [157, 159, 160, 166]. However, as these authors emphasis, implementation of RQA is far simpler than its actual interpretation.

Table 2

Beside the calculation of “non-linear indexes” of the time series, of particular importance is the physiological meaning of these parameters. Studies in rats have shown that administration of atropine significantly increases %REC, %DET and L_{MAX} [161]. However, administration of atenolol (β1-adrenergic antagonist, sympathetic inhibitor) only increases %REC, whereas prazosin (α1- adrenergic antagonist) does not affect non-linear indexes of HRV data. In contrast, α1-sympathetic blockade increased the non-linear parameters of systolic BP [161, 167]. In human diabetic subjects with autonomic dysfunction, no relationship has been found between linear frequency-domain HRV parameters and the results from the Ewing test (which is a standard test to diagnose diabetic autonomic dysfunction), whereas the non-linear index L_{MAX} was strongly correlated to the Ewing score [164]. Theses studies concluded that the non-linear indexes of HRV were more reliable markers of sympathetic and parasympathetic activation compared to parameters generated from time- and frequency-domain analysis.

Research in ruminents indicates that combinations of linear and non-linear parametersof HRV are most promising estimates of the influence of sub-clinical or clinical stress. Results in

calves, cattle and dwarf goats indicated a loss of complexity in HRV and a more deterministic control in response to extrinsic, physiological, or pathological loads (Fig. 8) [28, 35, 36]. Further positive correlations have also been demonstrated among measures of short-term variability in the time and frequency domains (RMSSD and HF) and many non-linear parameters [35, 161, 167].

4. HRV in applied animal research: methodology and interpretation of HRV in pigs, cattle, horses, sheep, goats and poultry

4.1. Heart rate variability in pigs

A review of the literature on HRV in pigs identifies two main themes of research activity, albeit with a certain degrees of overlap: 1) use in biomedical models of human disease, and; 2) use as an indicator of stress in applied studies allied to animal well-being. Each set of literature is small in number but demonstrates an increasing interest in the area of HRV within the last 10 years.

Issues researched

In terms of biomedical research, much focus has been on Yucatan [168, 169] or Göttingen [31, 170] miniature swine, but other studies have also used commercial type pigs [171, 172]. There has been some basic research carried out into data acquisition system design for minipigs [173] and studies into circadian patterns of HRV [170] and the effects of pair housing on HRV parameters [31]. Miniature pigs have also been used as subjects for research into cardiovascular autonomic neuropathy [168] and the effects of testosterone modulation on HRV [169]. HRV of commercial piglets has been studied to elucidate asymmetric innervation

of the myocardium [171] and during research using piglets as a model for Sudden Infant Death syndrome [172].

In terms of studies allied to animal well-being, again there has been some more basic research into methodology of analysis and data acquisition system design [99], the effects of gestation on HRV parameters [97] and circadian rhythmicity in HRV. [174]. Applied studies have looked at the effects of social stress [27, 32, 67], restraint stress [32] and the effects of grooming [67] on HRV.

Methodologies used

The major aspects of methodology relate to the physical data acquisition equipment used and issues surrounding the editing and analysis of data once they have been collected. Firstly, for data acquisition, there is the choice of either implantable transmitters or externally-mounted non-invasive transmitters. The commonly-used implantable telemetric devices are manufactured by Data Sciences International (St. Paul, Minnesota, USA), and both the TA10CTA-D70 [27] and the TL11M2-D70-PCT [168] have been used successfully, transmitting to RLA receivers (DSI, St. Paul, USA) (Table 3). The major advantage of the telemetric system is that potentially, pigs can be housed with pen-mates as the recording equipment is internalized and thus protected from damage by conspecifics. Furthermore with appropriate electrode placement there is a substantial reduction in the signal to noise ratio that is at times an inhibitory factor with non-invasive monitors. However, the transmission range is currently limited to around 1 metre and thus, if the pig is housed in a larger pen, multiple receivers are needed to acquire the signal. Also, the transmitters transmit on a single frequency so only one animal can be recorded per pen at any one time.

With externally-mounted equipment, the most commonly used have been ambulatory monitors, modified for use in pigs [e.g., 31, 99, 169, 170], or various types of Polar HR monitors, including the SportTester [32], the Vantage NV [67, 99] and the R-R Recorder [99]. Other equipment used has included a telemetric ECG system [99] and static ECG systems [171, 172]. The later Polar products have the advantage that they transmit on multiple coded frequencies, thus allowing several pen-mates to be recorded simultaneously once each is locked into a different transmitter frequency. Also, if the receiver is attached to the transmitter belt, the size of pen does not present a problem as the receiver is never out of range of the transmitter. However, the external nature of the equipment means that it can become the focus of investigatory attention from pen-mates, resulting in signal disruption from physical movement of the electrode belt either directly by the rooting or chewing activity of the investigating pig, or indirectly by the physical exertion of the monitored pig trying to avoid unwanted attention.

Analysis and Indices

A summary of the most frequently reported analysis and indices is reported in Table 3. Preliminary analysis typically begins with the identification and correction of spurious beats that are a common occurrence in recording from unrestrained pigs. For the most part, data has been analysed and express in the time (e.g. mean HR, mean IBI, Q-T interval, SDNN, Variance (σ^2), pNN50, RMSSD) and frequency domain (total power, LF, HF, LF:HF ratio, SNSI (LF/HF) or PNSI (HF/Total) [e.g., 27, 67, 99] as well as geometrically (e.g. Lorenz or Poincaré plots [e.g., 171]) to determine overall variability and the amount of variability and power relating to sympathetic and vagal activities. Fast fourier transforms are the common most method applied to analyse data in the frequency domain [31, 168, 170]. Frequency bands are expressed in either cycles per beat or hertz (Hz) with the VLF frequency typically reported as 0 to 0.01 Hz, the LF frequency from 0.01 to 0.07 Hz and the HF frequency

extending from 0.01 to 1 Hz. Individual frequency bands are sometimes normalised by expressing the individual bands as a function of the total power. Moreover, the LF to HF ratio, also referred to as the SNS indicator (SNSI) is determined to reflect activity due to sympathetic activity whereas the PNS indicator (PNSI, HF/total power) is used to enumerate vagal activity (see Table 3).

Recommendations for Future Studies

Considerably work is still necessary to elucidate the regulatory mechanisms contributing to HRV in pigs. For the most part, in applied studies assumptions have been made about the location and contributing factors (e.g., respiratory sinus arrhythmia, thermoregulation, etc) of individual frequency bands based on the human literature. Simple modelling work using ANS activity inhibitors such as atropine and propranolol is still outstanding and recommended to standardise methodology and analysis prior to embarking on further controlled stress and welfare studies. More longitudinal studies looking at the effects of age and disease on HRV are also of interest together with the effects of genetics, environment, and subjective states such as fear and anxiety, pain, and general welfare status. The existing data, however, is sufficient to indicate that HRV is indeed a promising indicator of pig welfare with the ideal being that in the future appropriate research will continue in this area so that we may be able to identify specificity in magnitude and/or direction of ANS activity for different subjective and welfare states.

Table 3

4.2. Heart rate variability in cattle

A review of the current literature identifies only a small number of studies addressing HRV in cattle. This is in contrast to a relatively huge body of research reporting on the measurement

of HR alone. HR is often used as an index for stress or emotional reactivity in cattle but it provides little information on the underlying physiological mechanisms that govern its modification [136, 175, 176]. As in other species, analysis of HRV is a tool used to non-invasively determine changes in PNS and SNS activity [177].

Methodological aspects

Different types of Holter recorders or fixed systems [24, 25, 101, 136] as well as portable HR monitors (mostly from Polar Electro Öy, Finland) have been used to investigate HRV in cattle [28, 35, 176, 178]. In some cases, electrode sites were shaved prior to attaching electrodes [28] and in others not [35]. In either case, it is strongly recommended that ample electrode gel is applied to optimise electrode-skin contact. With HR monitors, electrodes and transmitters were usually secured in place by attaching them to a horse girth or similar [28, 35, 178]. A sufficiently long acclimatisation period (min.1 h) is recommended to allow the animals enough time to become accustomed to wearing the equipment even when visible reactions after fixing the belt generally only occur for about 5 to 10 minutes after fitting [28]. The general advantages, disadvantages or problems concerning accuracy of measurements and correction of artefacts and ectopic beats discussed earlier in this review are also applicable to HRV analysis in cattle.

Factors affecting HRV in cattle

As in other species, the relationship between HRV and underlying sympathovagal balance in cattle was confirmed using pharmacological blockade of the autonomic nervous system [136, 175, 177]. HRV gives insight into the regulatory processes of the cardiovascular system and various parameters describing HRV in cattle have been used to detect irregularities in the operational sequences of sympathovagal balance. Several authors have used HRV to detect alterations in the brainstem caused by bovine spongiform encephalopathy (BSE). In addition to bradycardia, these studies report an increase in vagal tone, marked by a drastic increase in

HF spectral power [25]. LF power, on the other hand, shifted between phases of high power to phases of lower power. This effect of switching between low and high spectral frequencies seemed to be quite characteristic for BSE and was not comparable to changes in HRV described after brainstem stroke [24]. Furthermore, elevated HF power was present 9 months before the animals developed any clinical signs of BSE itself [24]. Bradycardia and increased HF power, due to an increase in vagal tone has also described in fasting steers. The connection between these two effects (bradycardia and emptying of the rumen) was explained by a reduction of ruminal tensor receptor input into the medullary gastric centre that influences the nearby cardiovascular centre [177]. These observations highlight the fact that because of the multivalent input into cardiac activity, irregularities and changes in activity levels can be caused by a multitude of intrinsic and extrinsic environmental factors. From this perspective, it is not surprising that it seems possible to detect emotional or physical stress by analysing HRV [178]. It has been reported that, as in other species, cattle also exhibit anticipatory changes in HR and cardiac activity when they are about to acquire a cognitive task [176]. Other research has demonstrated that in calves, short-term variability (RMSSD), as well as long-term variability (SDNN), of HRV decreased significantly with increasing levels of stress load (from high ambient temperature combined with insect harassment to clinical signs of diarrhoea) [28]. The decrease in RMSSD in response to external stressors and pathological loads is indicative of a considerable reduction in vagal tone during stress. In contrast, SDNN, and strongly correlated parameters SDANN and $HRV_{index,}$ are more complex parameters reflecting, at least, both vagal as well as sympathetic influences on cardiac activity. These parameters are better indicators of overall sympathovagal balance than for distinguishing between the regulatory activities of the two branches of the ANS [179]. Reduced cardiac vagal tone in response to external stress has not been reflected in SDNN, SDANN or HRV_{index} parameters, possibly due to the slight delay that occurs in sympathetic regulation of cardiac activity [28]. However, a pathological challenge, like diarrhoea, causes a

considerable decrease in both vagal and sympathetic tone, as shown by simultaneous decreases in RMSSD, SDNN, SDANN and HRV_{index}. Results in the frequency domain exhibit similar patterns to time domain parameters. Changes in the HF component to varying levels of stress were similar to those found in RMSSD. A significant decrease occurred following external stress and an even stronger decline happened in response to pathological load. Simultaneously changes in the LF component were not observed, but the absence of changes in the LF component could have been a result of masking effects or interchanges between all the loops mentioned above. In summary, results in both the time domain and frequency domain indicated a reduction of vagal tone as stress level increases. No significant difference in either time or frequency domain parameters of HRV have been found to exist between lactating and non-lactating cows [28]. All cows showed similar values for all parameters within the two groups, so it seems that lactation and late pregnancy are comparable pathological loads for the animals. Cardiac activity in mammals is an integrated signal which is influenced not only by the two branches of the ANS, but also by other physiological control circuits and external influences [180]. HRV is influenced by several feedback or feed forward mechanisms [159]. In recent years, some studies have shown that IBI time series contain non-linear components in the sense of deterministic or non-deterministic chaos that is described in detail earlier in this review [121, 150, 152, 153,]. In calves, all non-linear parameters of HRV have been demonstrated to rise significantly in response to external stress, and even more to pathological load. The most obvious increment was observed for L_{MAX}, which increased significantly in all three thermal, insect and pathological stress groups [28]. In lactating cattle, %DET was the only non-linear parameter which increased significantly from non-lactating to lactating cows [28]. The results in both calves and cattle studies indicate a loss of complexity in cardiac activity and a more deterministic control of HRV in reaction to external, physiological or pathological loads. In other words, when an organism's biological systems need to focus on specific challenges, a loss in the general freedom of cardiovascular dynamics

results. Furthermore, both calves and cattle do show differences in their non-linear cardiac dynamics depending on type of stress load experienced. Both moderate physiological stress in lactating cows compared to non-lactating animals and moderate external stress in calves, cause an increase of %DET that is indicative of an increase of recurrence sequences in the time series. This is interpreted as a more quantitative growth of deterministic processes in HRV. In contrast to that, %DET did not further expand in response to pathological load in calves. However, higher values of L_{MAX}, indicate that under such circumstances, HRV persists under stringent control for much longer periods [161, 181]. It is suggested that %DET indicates quantitative changes in the level of stress load, while higher values of L_{MAX}, are signs of a qualitative different stress level.

These results were very recently confirmed by other authors who evaluated the influences of a conventional milking system versus an automatic one on non-liner dynamics of HRV [35]. Beside differences in breed, body weight and time of day, this study also reports an increase in %DET, L_{MAX}, and LF/HF ratio and a reduction in RMSSD and HF_{norm} in animals which were confronted with an automatic milking system, suggestive of higher levels of stress.

Specific conclusions and recommendations

Measuring HRV in cattle can be used to measure stress from physical, pathological and emotional origins. In addition to the general methodological recommendations given in the review the following points should be considered when measuring HRV in cattle to evaluate stress and welfare:

- Electrodes should be positioned on the left side of the chest with one electrode placed close to the sternum and the other over the right scapula
- Shaving the skin is useful but not necessary
- Ample electrode gel should be used

Table 4

4.3. Heart rate variability in horses

A search of the literature reveals 19 studies published in peer reviewed scientific journals where HRV has been evaluated in horses with different techniques and objectives. The following section discusses methodological aspects of measuring HRV in horses during a range of different conditions. The descriptions of the different general recording techniques described earlier in this review also encompass the different approaches to record IBIs in horses. This section, therefore, focuses on the major constraints of the different techniques, reports basal HRV values found in horses, and also reports on the effects of different clinical, behavioural and physiological conditions on sympathovagal regulation of cardiac activity.

By far the majority of equine HRV studies have used Holter type recordings [37, 39, 58, 100, 92, 182-191]. A smaller number have used the Polar Vantage [40, 192] or the Polar R-R [30, 33, 193]. Practical difficulties are often encountered when trying to document reliable measures of HRV in field like conditions. Published techniques have some limitations associated with them that should be considered when designing any study. Holter systems provide precise and long-term recordings but they are expensive and could be damaged when used on farms where several horses can interact during experiments. Heart rate monitors are more affordable telemetric systems that have the benefit of not requiring invasive surgery associated with them. However, they also have inherent limitations associated with them, namely that they automatically detect the R-peak of the ECG but not the ECG itself. In horses, the steepness of the t-wave can be very pronounced and systems only detecting R peaks, by looking for sharp increase in voltage, can often register false values. These artefacts

are easily identified because two false IBI values will be separated only by some milliseconds as the system triggers first on the t-wave then very soon afterwards on the R-wave. In some cases, the problem can be avoided by changing the site of the electrodes to reduce the perceptible size of t-waves. In some recording systems, the presence of artefacts caused by movements of electrodes on the skin or by muscle contractions can be detected and corrected automatically by software algorithms. Using such automatic correction tools has to be considered with due care and attention. Arrhythmias at rest are physiologically normal in the horse because of their very high basal vagal tone. In this case, normal successive IBIs may have large differences that are hardly differentiable from artefacts without reference to the original ECG. It is hard to perform an appropriate automatic identification and correction of errors that can considerably affect the interpretation of HRV.

Factors affecting HRV in horses

Previously published research has documented good stability in inter-individual levels of HRV across age [40] and high degrees of repeatability when recordings are analysed over subsequent days [30, 184]. Horses also exhibit an increase in HF spectral power at night, indicating that time of the day is an important factor that should be controlled for in equine HRV studies [38, 184]. Some studies also report gender-related differences in ANS regulation of cardiac activity, with females appearing to have higher vagal tone which is consistent with the gender differences reported in humans [182, 194]. In contrast, an unrelated study using twenty horses failed to observe any gender based differences [30]. Basal values of HRV in horses appear to contain large inter-individual variations (Table 5). The exact origin of this variation is unknown but is likely due in part to a multitude of factors including genotype, behaviour, temperament, and nutritional status.

Clinical conditions, temperament and training have been the most commonly investigated factors affecting HRV in horses. A significant change in HRV has been demonstrated in several diseases such as grass sickness [190], laminitis [193], and atrial fibrillation [188]. The effect of pain, in horses suffering from laminitis has also been investigated [193]. In this particular work, treatment with non-steroidal anti-inflammatory drugs results in changes in LF and HF power, along side simultaneous changes in adrenalin and weight shifting behaviours, leading the authors to conclude that HRV may be used to reliably assess pain in horses.

Several horse studies have shown an effect of physical effort and training on cardiac activity and sympathovagal balance [37-40, 92, 185]. In general, resting HR in horses is significantly decreased by training but one study has failed to find any training related changes in the vagal tone when HRV was recorded at rest [187]. Other work found that in challenging conditions (behaviour tests), untrained horses showed more pronounced, although not significant, increases in HR alongside associated decreases in HRV parameters [40]. Exercise on aqua-treadmill is associated with significantly higher sympathetic tone and decreased vagal tone [92], although immersion in warm spring water, without any physical effort, results in an enhancement in vagal activity, that is purportedly linked to a mental and physical state of relaxation [185]. Many studies found negative correlations between the intensity of exercise and the overall HRV due to a progressive rise in sympathetic tone [37, 39, 92].

HRV appears to be a promising indicator of temperament and coping strategies in horses also. Several studies report a relationship between behavioural reactivity and HRV in horses undergoing behavioural testing [33, 40, 184, 192]. Exposure to a novel object, as well as handling, induces a physiological state characterised by an increase in mean HR and a decrease in SDNN and RMSSD indicating that there is a reduction in PNS influence during testing [40]. Clement and Barrey [182] and Thayer et al. [37] described reduced HRV for

more reactive horses, young subjects and hot-blooded breeds. Eager et al. [184] found positive correlations between the scores of six horses to a water spray test, handler scorings on a visual analogue scale and HRV with more fearful horses showing increased total and LF power. Another study by Visser et al. [40] report a relationship between HRV parameters and riders' rating scores with respect to ten temperamental traits. Additionally, HRV analysis has also been used to assess stress and susceptibility to stress in horses. In humans, is has been reported that vagal tone is a reliable indicator of individual stress vulnerability and the magnitude of a stress response [83]. In horses, baseline resting levels of LF, HF and their ratio has been found to differ between habitual crib-biting and normal control horses [33]. Other research reports on a relationship between indices of HRV (increased mean HR, LF and HF/LF and decreased HF) and stress related behaviour exhibited as a result of enforced backward movement in horses [30].

Specific conclusions and recommendations

HRV analysis in horses appears to be a sensitive measure of both physical and emotional stress responses. Beside the general methodological recommendations given earlier in this review, the following points are recommended when measuring and analysing HRV in horses:

- Physical soundness with special attention to the cardiovascular system and atrio-ventricular blocks.
- Using a system that records ECG is preferably to one storing only IBI data due the relatively pronounced t-waves in horses.
- Shaving the electrode sites is unnecessary with certain systems but electrode gel should be applied in all instances to enhance signal transmission.

Table 5

4.4. HRV in sheep and goat

Several laboratories have undertaken extensive cardiac and HRV studies in sheep because the sheep heart is similar to that of the human in many ways, including dimensions of the chambers, coronary anatomy, and magnitude of haemodynamic variables such as BP, HR, and cardiac output [195]. Moreover, autonomic innervations of the heart in sheep are also similar to that of the human [196].

These similarities explain why a large number of studies on foetal cardiovascular regulation have been performed in the ovine foetus. Several studies [reviewed in 197] support the existence of autonomic control of circulatory function early in the development of the foetus. In the immature foetus, basal sympathetic tone is important in the maintenance of foetal arterial pressure [198, 199] and is reflected by the LF variability in the HRV power spectrum [200, 201]. Monitoring the variability in the LF range has been used in estimating the level of foetal sympathetic activation during high-risk pregnancies, foetal distress after haemorrhage [202] and hypoxia [203]. The PNS is reported to have little influence on basal foetal cardiovascular function in the immature foetus, with its influence on resting HR increases progressively during post-natal maturation [198, 204]. Although neurohumoral control is important in neonatal period, the sympathetic system appears to be the major regulator of vascular function up to 8 weeks of life [198, 205]. By 3 months age, vagal regulation dominates and the best indicies of this dominance are RMSSD and HF power [175].

Studies involving HRV and behaviour in sheep and goats are sparse. Desire, Veissier et al [41] investigated the ability of lambs to react to suddenness and novelty of an event, according to appraisal theories. They found that lambs responded to a sudden event by a startle response coupled with a transient increase in HR that did not appear to be vagally

mediated as there were no associative modifications in RMSSD levels. They responded to a novel event by orientating towards the novelty coupled with a transient increase in RMSSD.

Langbein, Nürnberg et al. [36] studied HRV and visual discrimination learning in Nigerian dwarf goats using Polar S810 monitors. To minimize the influence of physical activity, and to study the long-term effects of visual operant conditioning learning on HRV only IBI data corresponding to resting behaviour (lying, calm and undisturbed) were incorporated into the analyses in this particular research. Whereas HR increased throughout the course of a first learning task, this relationship was the opposite in two proximate tasks, indicating different effects of different learning challenge on HR that may have been related to how familiar the goats were with the function of the learning device. Moreover, this work also found significant relationships between the time taken to perform particular tasks and several HRV indices representing vagal tone. Overall, results from this research suggest that learning related changes in HR were predominantly caused by a withdrawal of vagal tone. To investigate non-linear processes in cardiac regulation, this study used RQA. Increased deterministic shares of HRV throughout task 1 and 2 indicated that the goats did not really relax until the end of task 3.

Recommendations for HRV studies in sheep and goat

Several types of equipment designed for monitoring cardiac activity in humans are also suitable for use in sheep and goats. Data loggers are useful for short-term measurements but generally do not allow for specific event marking. Radio-telemetric equipment (e.g. Life Scope System, Nihon Kohden, Japan), that transmit to data acquisition system are more useful for long-term studies and multiple events marking. Some of them permit the acquisition of behaviour recording and are equipped with HRV analysis software packages (e.g. QuickTime capture Module for Chart software, PowerLab System, ADInstruments, UK). When

measuring HRV in free ranging animals, the belt with the electrodes needs to be protected to avoid movement of the electrodes and loss of signal. The electrodes should be positioned on shaved skin on the left side of the chest corresponding to the cardiac electrical axis. To achieve this one electrode is generally placed close to the sternum and the other over the right scapula.

ECG signals should ideally be recorded at a sampling rate of 1000 Hz. Time- and frequency-domain analysis should be conducted following the procedures recommended earlier in this review. Frequency domain analysis is critical to determine the contributions of both branches of the ANS. Stationary data should be used and this is easiest to obtain in sheep and goats when they are lying undisturbed.

Since experimental conditions, such as temperature or animal metabolism, may vary greatly, the respiratory frequency of the animals should be simultaneously recorded to allow for accurate determination of the upper limit of the spectrum defined by Nyquist frequency. In warm environment, HR in sheep can reach 200 bpm and their respiratory frequency 72 bpm (1.2 Hz). In this example, if the resampling frequency of the IBI data is 3 Hz, the Nyquist frequency is 1.5 Hz and the following frequency-domain ranges are advised: total power in the range 0-1.5 Hz, LF in the range 0.04-0.15 Hz, and HF in the range 0.15-1.5 Hz, including the respiratory peak at 1.2 Hz. The limits of LF wavebands include the oscillation of the baroreflex region (0.1 Hz) as shown by pharmacological blockades studies in sheep [205].

4.5. Heart rate variability in poultry

The analysis of HRV has been used in very few studies in birds. Two main reasons can be identified: firstly, HRV analysis requires high quality data that can be difficult to obtain in

birds, particularly when using non-invasive equipment; secondly, a lack of fundamental research evaluating the physiological meaning of HRV indices in avian species inhibits the development of further research in this area.

Several studies have been carried out on HR fluctuation, rather than variability itself, in chicks, emus and quail, both before and after hatching, and most of these studies have focused on the development of the cardiac rhythms [206-211]. These experiments were not designed to evaluate welfare problems but the methods are interesting and are a useful source of HRV information in normal and non-normal chicks both during and after hatching. Cardiac activity can be measured pre-hatching by inserting specially designed electrodes through a hole in the egg in three locations. Recording in hatched chicks can be achieved using flexible Ag/AgCl gel electrodes that are attached to the skin at the lateral thoracic wall under both wings and at the ventral abdomen. The electrode wires are then fixed on the back so that the bird could move freely within a small cage. The authors [207-211] did not use the usual indices to analyse HRV and their analysis led to the identification of three types of HR fluctuations according to their frequency (high: type I HRV; low: type II HRV) and to irregularities (type III HRV). Ultradian and circadian rhythms in HR have also been reported in embryos and hatchlings, respectively, and the distinctive patterns of HR fluctuations in embryos and hatched chicks are assumed to be partly related to ANS activity.

Another innovative study on the chick embryo used time- and frequency-domain indices and demonstrated that cardiovascular function in the chick embryo was modulated by the ANS as early as day 19 of incubation and that both SNS and vagal activities have reached a ’mature’ level by this stage [212].

HRV has also been used to better understand the relationship between coping style and feather pecking [29]. Time domain analysis of HRV identified different autonomic responses in chicks from high- and low-feather pecking lines of laying hens during a stressful challenge 29. This response was assumed to be related to the different coping style of the birds as reflected in higher vagal activity in the low-feather pecking line that was perhaps related to more passive coping strategies.

HRV analysis has been used to obtain physiological information about broilers at risk of sudden death syndrome which can lead to the death of 2 to 4 % of male broiler chickens in a flock [213]. In this research, birds were equipped with telemetric transmitters when they were 15-days old. The transmitters were implanted subcutaneously at the base of the neck with one electrode placed over the right shoulder and the other one over the left groin area. The freely moving chicks could then be monitored in their home cage. Unfortunately, although only SDANN was used to evaluate HRV, the study clearly demonstrated that telemetric devices are powerful tools for accurately measuring cardiac activity and HRV at rest.

Biotelemetric devices have also been used for HRV analysis in quail [214]. The purpose of this research was to understand how the ANS responded to emotional stress in strains differentially selected for fear. ANS regulation of the quail heart has also been assessed using HRV analysis and pharmacological blockades [215]. Genetic lines of quail selected for either long or short duration of tonic immobility were compared to their controls. The transmitters were fixed to the back of the quail using a harness type setup. The positive electrode was fixed to the muscular fibres of the quail's back at the wing base and the negative one was fixed to the right *Pectoralis major* muscle. HRV analysis, in time and frequency domains, showed that the two lines did not differ in their intrinsic heart rate, i.e. heart rate during total ANS blockade. However, parameters of HRV did differ between the two strains. Vagal

activity was the highest in the line with short-tonic immobility duration while sympathetic activity was the highest in the quail from the long-tonic immobility duration line. It seems therefore that responses to tonic immobility in quail appear to be related to underlying sympathovagal control of the heart.

In conclusion, the use of HRV analysis is increasing in birds and appears to be a useful tool to study stress and welfare, especially when telemetric equipment is used. In general, equipment that has been developed for use in laboratory rodents is also appropriate for use in poultry. Future research should involve establishing the exact physiological meaning of the various HRV parameters in avian species and determining what relationship exists between these parameters and stress and welfare.

5. General conclusions and recommendations for future research

During the last decade, HRV has been successfully used as a measure of autonomic regulation of cardiac activity in farm and companion animals : (i) to assess stress and well-being under various housing and management conditions on the farm or under laboratory conditions; (ii) to study basic cardiovascular regulation in various test situations, including animal based model studies that enhance our understanding of human diseases; (iii) to evaluate pathological conditions, behavioural disorders, management and housing problems, training and fitness level (mainly horses); (iv) to characterise and understand individual traits such as temperament and coping strategies. In most studies, non-invasive ambulatory Holter monitors or telemetric HR monitors have been used for data sampling. Data are commonly analysed and expressed in the time and frequency domain as well as geometrically, although some recent studies in domestic animals and humans indicate that the non-linear indices of HRV are also reliable markers of sympathetic and vagal activation. Chronic changes (such as housing

conditions) in HRV parameters should only be measured during stationary condition with minimal, or unvarying, motor activity. In order to analyse the complex oscillatory of HRV, analyses from at least 5-min of consecutive IBI data are recommended. Age, sex and time of the day should be standardised and mentioned. Animals need to be well accustomed to the recording device before starting data collection. A within-individual change in HRV, recorded before and after a treatment is applied, is more meaningful than between groups comparisons. Recordings of IBIs should contain less than 5% of artefacts before editing and the subsequent manual editing of the data should be done to a very high standard.

This TASK FORCE has identified the following areas that warrant further study in order to improve methodology and to enhance our understanding of HRV and underlying sympathovagal balance in relation to stress and welfare of farm animals:

1. Study ranges of variation for HRV in different animal species and populations in order to estimate animal numbers needed for studies on a group level
2. Study individual traits (coping styles, temperament) in relation to HRV and physiological correlates, and use these traits as possible criteria for selection purposes
3. Measure diurnal variation and effects of season, age and metabolic state on HRV
4. Assess HRV in relation to chronic diseases and pain
5. Develop techniques for remote controlled telemetric devices for monitoring HRV as a tool for precision livestock farming
6. Improve ease of analysis by means of automatic elimination of artefacts from tachograms

7. Study regulatory mechanisms contributing to HRV by means of pharmacological inhibition or stimulation of the ANS activity

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Appendix A

Abbreviations and main definitions

ANS: **Autonomic N**ervous **S**ystem. Portion of the nervous system that controls visceral functions of the body. It is traditionally partitioned into the sympathetic (= SNS) and parasympathetic (PNS) branches in reference to the neurotransmitters released at the nerve terminals (noradrenaline for the SNS, acetylcholine for the PNS) and to the region in which the nerves have their origin (the thoracic and lumbar segments of the spinal cord for the SNS, the brainstem via the cranial nerves or the sacral segments of the spinal cord for the PNS). The vagus (= vagal nerve = 10^{th} cranial nerve) is a major component of the ANS.

AV: **A**trio**v**entricular. The AV node has autonomous heart beat stimulation properties. It is under the control of the sinus node and both sympathetic and parasympathetic (vagal) nerves.

BP: **B**lood **p**ressure.

Bpm: **B**eats **p**er **m**inute. Number of heart beats in one minute.

CLV: **C**ycle **l**ength **v**ariability. Standard deviation of IBIs calculated over 24 h.

ECG: **E**lectro**c**ardio**g**ram.

ENT: **Ent**ropy. One of the quantitative parameters derived from a non-linear mathematical analysis of HRV (see RQA). See exact definition in part III.

FFT: **F**ast **F**ourrier **t**ransformation.

HF: **H**igh **f**requency. The component of HRV determined by spectral analysis whose usual range of variation in human is between 0.15 and 0.4 Hz. In other species, it could differ depending on the respiratory frequency. It depends mainly on vagal (parasympathetic) influences.

HR: **H**eart **r**ate. Frequency of heart beats usually expressed as number of beats per min.

HRV: **H**eart **r**ate **v**ariability. Usually determined by analysing the time series of normal inter-beat intervals determined by ECG or arterial pressure tracings. Various measures of heart rate variability have been proposed.

IBI: **I**nter-**b**eat **i**nterval. Time interval between two consecutive heart beats in ms.

LF: **L**ow **f**requency. Component of HRV determined by spectral analysis whose usual range of variation in humans is between 0.04 and 0.15 Hz. It integrates both vagal (parasympathetic) and sympathetic influences.

L_{MAX}: **Maxl**ine. One of the quantitative parameters derived from a non-linear mathematical analysis of HRV (see RQA). See exact definition in part III.

Lorenz plot. See Poincaré plot.

NN interval: **N**ormal-to-**N**ormal interval = IBI.

NN50: **N**ormal-to-**n**ormal intervals greater than **50** ms. Number of differences between two successive IBIs greater than 50 ms.

pNN50: **P**ercentage of **n**ormal-to-**n**ormal intervals greater than **50** ms. Percentage of differences between two successive IBIs greater than 50 ms.

%DET: percentage of **det**ermination. One of the quantitative parameters derived from a non-linear mathematical analysis of HRV (see RQA). See exact definition in part III.

%REC: percentage of **rec**urrence. One of the quantitative parameters derived from a non-linear mathematical analysis of HRV (see RQA). See exact definition in part III.

Poincaré plot = Lorenz plot. Scatter plot where each dot represents an IBI plotted against the previous one.

PNS: **P**arasympathetic **N**ervous **S**ystem. One of the two main branches of the ANS (see above).

PSD: **P**ower **s**pectral **d**ensity. PSD analysis describes the variation of an IBI data series as a set of sine and cosine constituents. One method to calculate PSD is based on FFT.

RMSSD: Square **r**oot of the mean of the **s**um of the **s**quares of **d**ifferences between consecutive IBIs. It is the standard deviation of differences between successive IBIs.

RQA: **R**ecurrence **q**uantification **a**nalysis. A non-linear mathematical analysis of HRV.

RR: **R** wave to **R** wave. R waves are identified by electrocardiogram. RR interval = IBI =NN interval.

SD1: Standard **d**eviation 1. It represents the short-term component of HRV derived from a quantitative analysis of Poincaré plot. See exact definition in part III.

SD2: **S**tantard **d**eviation 2. It represents the long-term component of HRV derived from a quantitative analysis of Poincaré plot. See exact definition in part III.

SDANN: **S**tandard **d**eviation of the **a**verage **n**ormal-to-**n**ormal intervals. It is the standard deviation of the IBIs averages calculated on 5-min segments during the 24-h cycle.

SDNN: **S**tandard **d**eviation of **n**ormal-to-**n**ormal intervals. It is the standard deviation of all IBI measured.

$SDNN_{index}$: mean of **s**tandard **d**eviation of **n**ormal-to-**n**ormal intervals. It is the mean of the IBI standard deviations calculated on 5-min segments.

SN: **S**inus **n**ode = nodus sinu-atrialis. The heart's pacemaker that generates an intrinsic heart rate. It is under the control of both parasympathetic and sympathetic nerves.

SNS: **S**ympathetic **N**ervous **S**ystem. One of the two main branches of the ANS (see above).

Tachogram: a graphical record representing the variation of IBIs as a function of the interval number.

TINN: **T**riangular **i**nterpolation of **NN** interval histogram. One of the quantitative parameters derived from a geometrical analysis of HRV.

Trend: One of the quantitative parameters derived from a non-linear mathematical analysis of HRV (see RQA). See exact definition in part III.

VLF: **V**ery **l**ow **f**requency. It is the component of HRV determined by spectral analysis whose usual range of variation in humans is between 0.0033 and 0.04 Hz. Its physiological significance is not fully clear.

Table 1

Time-domain measures of HRV (adapted from (Task Force, 1996 396 /id))

Variable	Units	Description
Statistical measures		
SDNN	ms	Standard deviation of all IBIs of the dataset.
SDANN	ms	Standard deviation of the mean of IBIs in all 5-min segments of the entire dataset.
$SDNN_{index}$	ms	Mean of the standard deviations of all IBIs for all 5-min segments of the entire dataset (24h).
RMSSD	ms	The square root of the mean of the sum of the squares of differences between successive IBIs.
NN50 count		Number of pairs of successive IBIs differing by more than 50 ms.
pNN50	%	NN50 count divided by the total number of all IBI's.
Geometric measures		
HRV_{index}		Total number of all IBIs divided by the height of the histogram of all IBIs measured on a discrete scale with bins of 7.8125 ms (1/128 s).
$TINN_{index}$	ms	Baseline width of the minimum square difference triangular interpolation of the highest peak of the histogram of all IBIs.
Poincaré (Lorenz) plot		XY-diagram of each IBI of the dataset plotted as a function of the previous IBI.

Table 2

Quantitative parameters derived from the recurrence plot by applying the RQA.

PERCENTAGE OF RECURRENCE 127(%REC)	Percentage of recurrence points in the plot; single vector repetition in n-dimensional space.
PERCENTAGE OF DETERMINISM (%DET)	Percentage of recurrence points forming upward diagonal lines i.e. recurrence points in consecutive sequences.
ENTROPY (ENT)	Shannon information entropy of the line length distribution
MAXLINE (L_{MAX})	The longest diagonal line segment of consecutive recurrence points in the plot.
TREND	Drifting of the recurrence points away from the central diagonal line of identity.

Table 3

Summary of selected research on HRV research in pigs

Publication	**Study Objective**	**Equipment Used**	**Time Domain Indices Reported**	**Frequency Domain Indices Reported**
Mesangeau et al., 2000 [168]	Diabetes research	Data Science TL11M2-D70-DCT	Mean HR, SDRR	Total Power
Olmstead et al., 2005 [169]	Effects of testosterone on HRV	Holter	Mean HR, SDRR, SDANN, RMSSD	LF, 0.01 - 0.07 Hz HF, 0.07 - 1.0 Hz
Kuwahara et al., 1999 Kuwuhara et al., 2004 [170] [31]	Circadian rhythms Pair housing	Holter	Mean RR, SDRR, CVRR	LF, 0.01 - 0.07 Hz HF, 0.07 - 1.0 Hz Total Power Normalised LF & HF
Voss et al., 2004 [172]	Effects of endotoxins on HRV	Hook Electrodes & Grass Recorder	SDRR, SDΔRR	LF, 0.02 - 0.15 Hz HF, 0.15 - 2.0 Hz
Marchant-Forde et al., 2004 [99]	Validation of equipment and identification & correction of artefacts	Polar RR, Telemetric ECG	Mean HR, RR Max, RR Min, SD, σ^2, RMSSD	VLF, 0.003 - 0.01 Hz LF, 0.01 - 0.07 Hz HF, 0.01 - 0.5 Hz Total Power SNSI & PNSI
Marchant-Forde, Marchant-Forde, 2004 [97]	Effects of gestation on HRV	Polar NV	Mean HR, RR Max, RR Min, SD, σ^2, RMSSD	LF, 0.01 - 0.07 Hz HF, 0.01 - 0.5 Hz Total Power SNSI & PNSI
de Jong et al. 2000 [27]	Social stress	Data Science TA10CTA-D70	Mean RR, SDNN, SDRR, RMSSD	
Geverink et al. 2002 [32]	Restraint stress	Polar Sport Tester Electrodes & FM Transmitters	Mean HR, SD, SD1, SD2, SD2/SD1 ratio	

Table 4

Baseline values of HRV at lying down (mean ± SD) in

18 calves [28]

Variable	Units	mean ± SD
IBI	ms	593,47 ± 131,9
SDNN	ms	27,1 ± 8.3
RMSSD	ms	15.2 ± 8.8
LF/HF		6.1 ± 6.2
LF	normalized units	35.3 ± 12.1
HF	normalized units	9.9 ± 6.2
%REC		3.3 ± 2.2
%DET		84.0 ± 6.9
ENT		3.5 ± 0.4
L_{MAX}		50,0 ± 24.3

15 cows, breed Brown Swiss [35]

Variable	Units	mean ± SD
IBI	ms	819.11±114.6
SDNN	ms	35.6 ± 10.8
RMSSD	ms	16,2 ± 8.2
LF/HF		4.7 ± 3.2
LF	normalized units	25.9 ±5.7
HF	normalized units	11.9 ± 8.6
%REC		3.4 ±2.4
%DET		76.1 ±11.5
ENT		3.0 ±0.62
L_{MAX}		49.7 ±40.0

Table 5

Baseline values of HRV (mean ± SD) in:

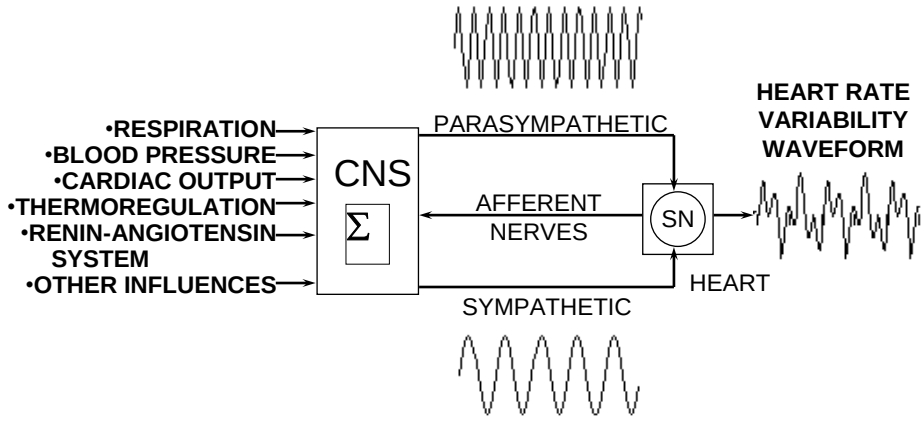
18 adult Warmblood horses [30]

Variable	Units	mean ± SD
IBI	ms	1818±152
SDNN	ms	111±50.55
LF/HF		1.70±1.69
LF	normalized units	53.3±19.5
HF	normalized units	46.8±19.5

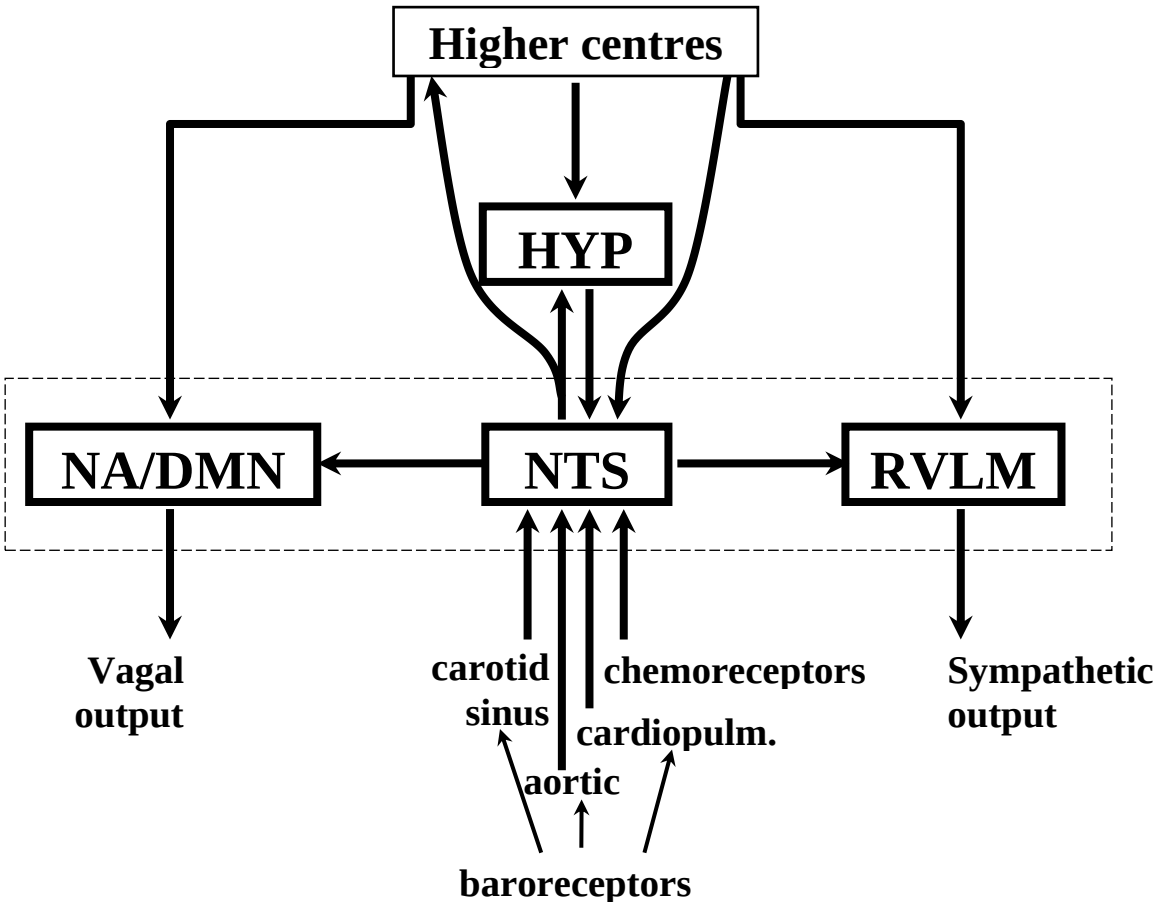
6 Thoroughbred horses (8-20 years) [100]

Variable	Units	mean ± SD
IBI	ms	1532±130
SDNN	ms	313±169
RMSSD		300±297
PNN50		37±14
Total power	ms^2	149.5±167.8
HF	ms^2	114.1±153.9
LF	ms^2	35.4±18.1

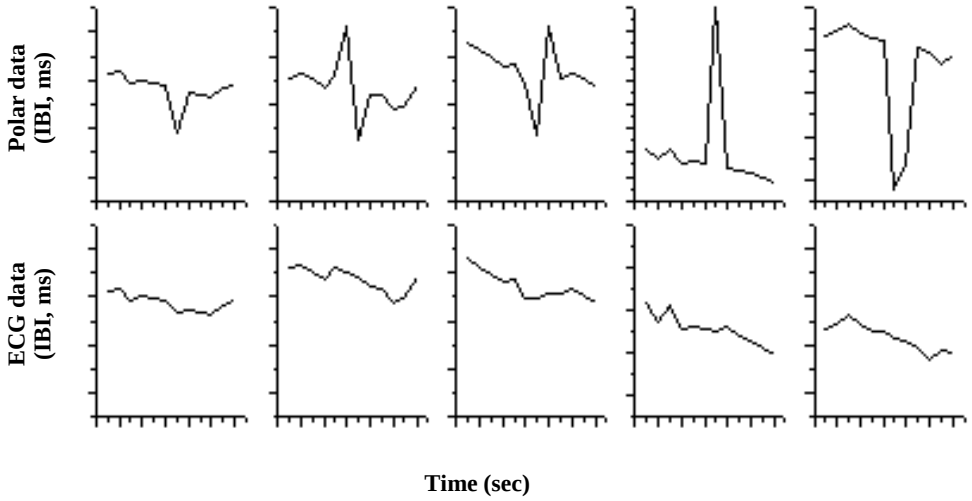
Autonomic Control of the Heart



(From McCraty & Watkins, 1996)


Higher centres
HYP
NA/DMN
NTS
RVLM
Vagal output
carotid sinus
chemoreceptors
cardiopulm.
aortic
baroreceptors
Sympathetic output

1



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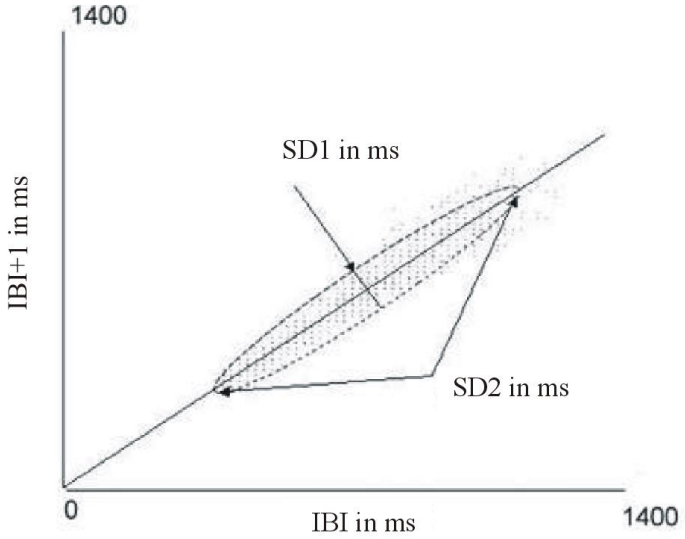
2



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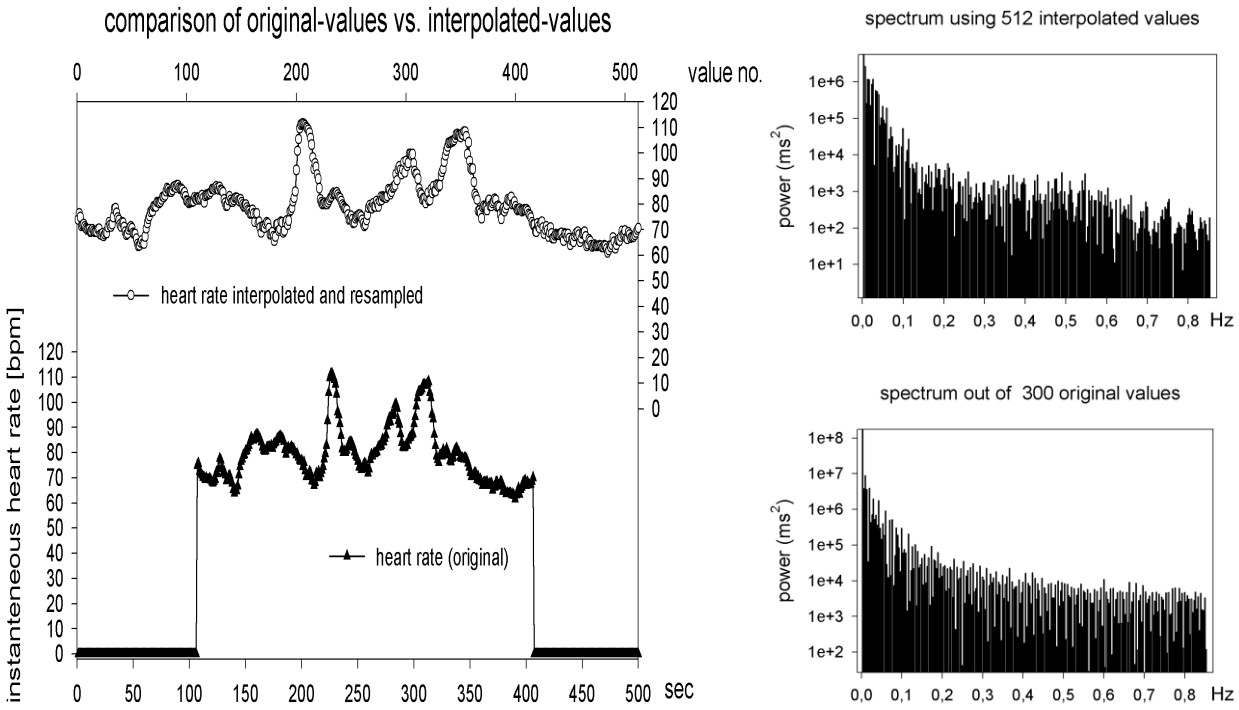
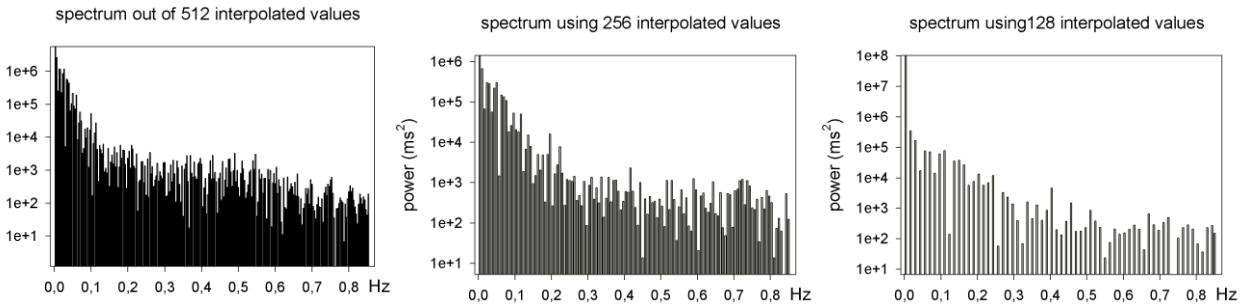
2

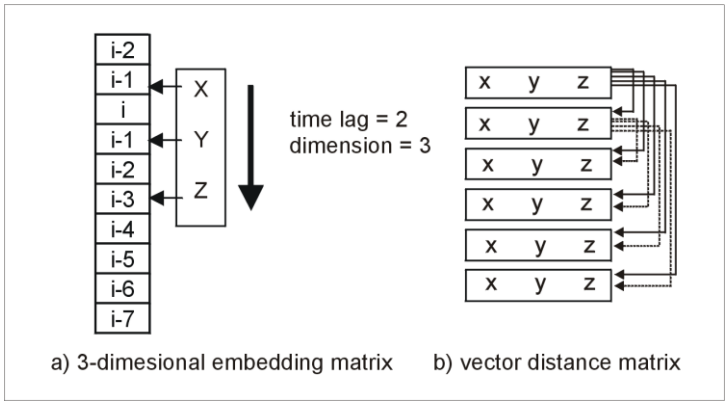
1


comparison of original-values vs. interpolated-values
value no.
heart rate interpolated and resampled
heart rate (original)
instanteneous heart rate [bpm]
sec
spectrum using 512 interpolated values
power (ms²)
Hz
spectrum out of 300 original values
power (ms²)
Hz

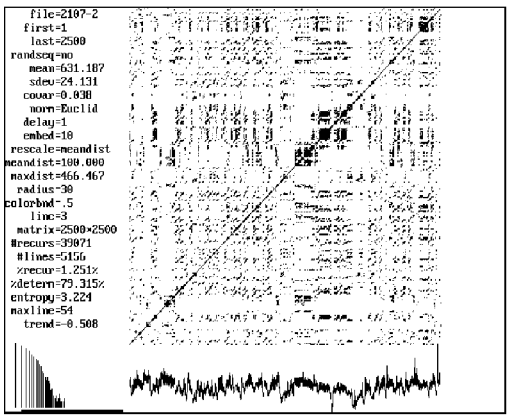
1


spectrum out of 512 interpolated values
spectrum using 256 interpolated values
spectrum using128 interpolated values
power (ms^2)
1e+1
1e+2
1e+3
1e+4
1e+5
1e+6
1e+7
1e+8
0,0 0,1 0,2 0,3 0,4 0,5 0,6 0,7 0,8 Hz

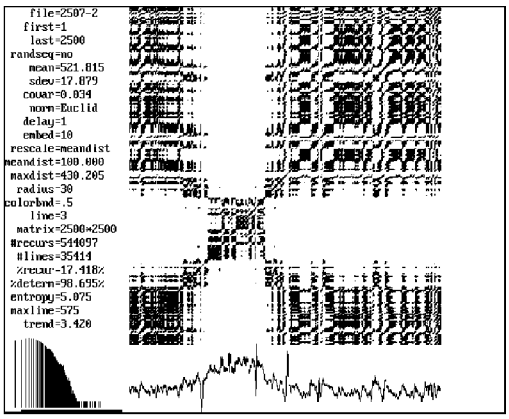
1


i-2
i-1
i
i-1
i-2
i-3
i-4
i-5
i-6
i-7
X
Y
Z
time lag = 2
dimension = 3
x y z
x y z
x y z
x y z
x y z
x y z
a) 3-dimesional embedding matrix
b) vector distance matrix

1



a. calf, 7 days old, healthy
% Rec: 2; % Det: 79; L_ _: 54



a. calf, 11 days old, diarrhea
% Rec: 17; % Det: 98; L_ _: 575

Legends for Fig.s

Fig. 1. Simplified model for the formation of HRV and the structure of the cardiovascular control (modified from [75])

Fig. 2. Structure of the cardiovascular control; modified from [64]

Centre (dotted rectangle) in the medulla. DMN=vagal Dorsal Motor Nucleus, HYP=Hypothalamus, NA=Nucleus Ambiguus, NTS=Nucleus Tractus Solitarii, RVLM=Rostral Ventrolateral Medulla

Fig. 3. Five different error types in IBI data series recorded by the Polar R-R Recorder in pigs as identified by comparison of the Polar tachogram with simultaneously recorded conventional ECG derived IBIs (modified from [99]).

Fig. 4. Quantitative analysis of Poincaré plot. SD1 is the SD of instantaneous IBI variability measured from axis 1. SD2 is the SD of long-term continuous IBI variability measured from axis 2 (modified from [132]).

Fig. 5. Differences in FFT-spectra depending on the method of correction of data length.

Fig. 6. Information content of spectra depends on the number of spectral lines.

Fig. 7. a) Computing an embedding matrix from original time series in a 3-dimensional Euklidian space with time lag = 1. b) Calculating.

Fig. 8. Recurrence plot of a tachogram (2500 IBI´s) of (a) a 7 day old healthy calf and (b) the same calf four days later when suffering from diarrhoea. Changes in the various RQA-parameters are given.