

## 1 Abstract

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5 regulation of cardiac activity for assessing stress and welfare in farm animals – a review.  
6 *PHYSIOL BEHAV* xx (x) 000-000, 2006.-Measurement of heart rate variability (HRV) is a  
7 non-invasive technique that can be used to investigate the functioning of the autonomic  
8 nervous system, especially the balance between sympathetic and vagal activity. It has been  
9 proven to be very useful in humans for both research and clinical studies concerned with  
10 cardiovascular diseases, diabetic autonomic dysfunction, hypertension and psychiatric and  
11 psychological disorders. In the last 10 years, HRV has been used increasingly in animal  
12 research to analyse changes in sympathovagal balance related to diseases, psychological and  
13 environmental stressors or individual characteristics such as temperament and coping  
14 strategies. This paper summarizes the state of the art of HRV research in farm animals. First,  
15 it describes how cardiac activity is regulated and the relationships between HRV,  
16 sympathovagal balance and stress and well-being. Then it outlines the types of equipment and  
17 methodological approaches that have been adapted and developed to measure inter-beats  
18 intervals (IBI) and estimate HRV in farm animals. Finally, it briefly describes experiments  
19 and conclusions derived from the measurement of HRV in pigs, cattle, horses, sheep, goats  
20 and poultry. Emphasis has been placed on deriving recommendations for future research  
21 investigating HRV, including approaches for measuring and analysing IBI data. On the  
22 whole, data from published research demonstrate that HRV is indeed a useful tool for  
23 evaluating stress and emotional states and has the potential to contribute much to our  
24 understanding and assessment of the underlying neurophysiological processes of stress  
25 responses and welfare in farm animals.

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

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5 Running head: Heart rate variability in farm animals

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## 1 **1. Introduction**

2

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

16

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

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

4

## 5 **2. Regulation, interpretation and significance of heart rate variability (HRV) in relation** 6 **to stress and well-being**

7

### 8 ***2.1. Regulation of heart beat activity***

9

10 The sinus node or sinoatrial node (SN) acts as the main pulse generator for heart beats [43,  
11 44] although other parts of the cardiac nervous system such as atrioventricular (AV) nodes  
12 are also capable of exhibiting autonomous heart beat stimulation properties. The SN, as the  
13 primary pacemaker, has the highest discharge frequency and excites other cardiac centres  
14 (such as the AV node) before they themselves initiate their own electrical impulses [43]. The  
15 SN contains two types of cells, elongated and round. The round, or pacemaker, cells are  
16 capable of spontaneous depolarisation that initiates electrical activation within the heart itself.  
17 In the absence of autonomic innervations, or under complete autonomic blockage, the  
18 discharge rate of pacemaker cells represents intrinsic heart rate (HR). In humans, the SN  
19 generates an intrinsic HR between 100 and 120 beats per minute (bpm) in the absence of any  
20 neural and hormonal influences [45].

21

22 The SN is under the control of the parasympathetic (vagal, PNS) and sympathetic nervous  
23 system (SNS) [46]. Both left and right vagus nerves stimulate the SN (the right nerve is  
24 dominant and reduces HR), the AV node (left nerve is dominant and prolongs AV conductor)  
25 as well as the atrium muscle, whereas efferent control of the ventricle muscle is unclear [43,  
26 47]. In general, activity within the vagal nerves decreases HR when the stimulatory effect of

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

7  
8 Rapid changes in HR are always caused by shifts in vagal regulation [47, 50-52]. The SN  
9 responds to vagal activity within one or two heart beats, but its overall effects are relatively  
10 short-lived. Vagal induced changes in HR typically occur within five seconds [47] whereas  
11 cardiac responses to SNS regulation occur more slowly with initial response delays of up to  
12 five seconds, followed by a progressive change and a maximum response after 20 to 30  
13 seconds [47, 53]. These differences in response times are due in part to the relatively slow  
14 exocytotic release of noradrenaline from sympathetic nerve terminal through which the SNS  
15 regulates cardiac activity. Also, unlike PNS acetylcholine mechanisms, a secondary  
16 messenger (adenylyl cyclase) is involved in SNS regulation which further slows the process.  
17 Other anatomical disparities between the autonomic branches may also contribute to the  
18 slower response rate to SNS regulation. For instance, the preganglionic cell bodies of the PNS  
19 neurons are located within the heart itself, whereas those of the sympathetic neurons are  
20 comparatively isolated in the paravertebral ganglia. Furthermore, preganglionic fibres are also  
21 myelinated contributing to faster electrical transmission of vagal regulatory signals compared  
22 to transmission rates in unmyelinated SNS fibres.

23

## 24 ***2.2. Heart rate variability (HRV)***

25

1 Healthy cardiac activity is characterized by irregular intervals between consecutive heart beats  
2 [54]. This variability is a result of rhythmic oscillation of the regulatory components of  
3 cardiac activity that function to maintain cardiovascular homeostasis within a defined range  
4 [46, 55]. They also serve to orchestrate and facilitate appropriate responses to intrinsic and  
5 extrinsic challenges [55].

6

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

13

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

20 Fig. 1

21 Fig. 2

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

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

7

### 8 ***2.3. Interpretation and significance***

9

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

16

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



1 combination of changes in activity within both branches. In reality, however, the interplay  
2 between the branches is quite complex and not yet fully appreciated. Mean HR parameters  
3 provide information on the net effects of all components inputting into cardiac activity and are  
4 of limited use for accurately assessing sympathovagal regulation [71].  
5  
6 HRV analysis, on the other hand, allow a much more accurate and detailed determination of  
7 the functional regulatory characteristics of the ANS. HRV is a particularly good indicator for  
8 the non-invasive assessment of ANS activity in response to psychophysiological stress [55].  
9 Psychological states may have an impact on sympathovagal balance in the absence of any  
10 palpable changes in heart and/or respiration rates [72]. For example, Sleight, Henderson [73]  
11 failed to demonstrate any relationship between mean HR and anxiety during pre-surgery  
12 stress but found a reduction in the relative intensity of the HF-component in the power  
13 spectrum of the HRV (representing a decrease in vagal activity) which was positively  
14 correlated with pre-surgery anxiety levels. Furthermore, McCraty, Atkinson et al. [74] found a  
15 significant decrease in HRV in relation to reduced well-being, particularly in the frequencies  
16 of the power spectrum that are sensitive to PNS modulations, whereas no effect was seen on  
17 overall mean HR. Catipovic-Veselica et al. [75] reported interactions between basic emotions,  
18 such as fear and aggression, and HRV that were not evident in HR responses. Numerous  
19 studies on social behaviour in children have employed measures of HR and HRV. Porges,  
20 Doussard-Roosevelt et al. [76] compared HR and HRV in 9-month-old children with their  
21 social behaviour at 3 years of age and found a negative relationship between HRV (vagal  
22 tone, both basal levels and magnitude of change) and behavioural problems, contact  
23 problems, aggressiveness, depression, and sleep disorders when no significant correlations  
24 with HR parameters were evident.

25

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

10

#### 11 ***2.4. Vagal tone as a stress indicator***

12

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

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

7  
8 Positive emotions may significantly increase the HF-component of a power spectrum [72, 74]  
9 whereas the opposite occurs with negative emotions. Indeed, Friedman and Thayer [86]  
10 examined HRV (time and frequency analysis) in people who suffered from panic attacks or  
11 blood phobia and found that subjects who experienced panic attacks has lower vagal tone than  
12 those with a blood phobia, whereas normal controls exhibited the highest tone. In another  
13 study, the extent to which the vagal tone of small children changes in test situations 86 has  
14 been correlated to their subsequent behaviour. Those that exhibited greater flexibility in vagal  
15 tone in response to social and attention tasks also had fewer behavioural problems at a later  
16 age [76]. It seems apparent, therefore, that vagal tone is a useful indicator for determining the  
17 physiological and psychological flexibility of an organism and for measuring their  
18 susceptibility to, and ability to respond, to stress [86].

19

## 20 ***2.5. HRV and behaviour***

21

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

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

3

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

14

### 15 **3. Methodology of measurement and analysis of HRV in farm animals**

#### 16 ***3.1. Equipment for recording cardiac activity***

17

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

1 peaks of the ECG during recording and then store IBI data in digital form. To our knowledge,  
2 there is currently only one commercial manufacturer (Polar Electro Oy, Finland) who has  
3 developed such devices that are primarily marketed for sport and research in sport medicine.  
4 There are two different models available on the market that record cardiac activity and detect  
5 IBIs at a sampling rate 1000 Hz. The storage capacity of the first model (S810i) can  
6 continuously record up to 16,000 IBIs. Postulating a mean HR of around 70 beats per minute  
7 (bpm), its maximum recording time is therefore about 4 hours. The S810i, and its predecessor  
8 the Vantage NV (stores 4,000 IBIs), have been widely applied in veterinary and behavioural  
9 research [30, 35, 36, 67, 97]. Another model, the Polar R-R Recorder, is a digital 24-h  
10 ambulatory monitor that can record IBI data over much longer periods [98, 99]. Moreover,  
11 this device can also store short (20 sec) epochs of ECG which is not possible with the other  
12 model. These devices use an electrode belt containing two coated electrodes that fits around  
13 the thorax of the wearer. Detection of the IBIs is carried out during recording and the  
14 resulting IBI data are transmitted wirelessly and stored in a data logger. These data may then  
15 be downloaded onto a PC for later analysis of HRV. There are two different types of electrode  
16 chest belts available to use with these monitors. For smaller animals like goats, sheep, pigs,  
17 and calves, a standard belt with an elastic strap can easily be adapted to fix around the thorax  
18 of the animal. For large animals, like cattle or horses, a specific transmitter with two separated  
19 electrodes should be used to optimise electrode positioning. All can transmit detected IBIs as  
20 coded signals to avoid any cross talk between different devices recording at the same time  
21 within a given area. Beside the Polar system, various non-commercial mobile systems have  
22 also been developed to record not only IBI data but also the ECG in unrestrained animals [28,  
23 100].

24

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

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

9  
10 Various approaches, from simple visual correction to more sophisticated algorithms, have  
11 been developed to correct IBI data for artefact that occur due to the misidentification of R-  
12 peaks and ectopic beats. Artefacts may occur as a result of poor electrode-skin conductance,  
13 equipment malfunction, noise from muscle action potentials and environmental  
14 electromagnetic interference [105-110]. In any case, the editing of the IBI data should be  
15 performed to a very high standard ensuring correct identification and correction of IBIs.  
16 Marchant-Forde et al. [99] recently identified five (Type 1 – 5) distinct types of errors in IBI  
17 data series recorded by the Polar R-R Recorder in pigs (see Fig 3). Type 1 errors were  
18 characterised as single point discrepancies, either positive or negative, between the Polar data  
19 and IBIs derived conventional ECG data. Type 2 errors were identified as a long IBI  
20 immediately followed by a short IBI. In contrast, with type 3 errors, where a short IBI was  
21 followed by a compensatory long IBI. Type 4 errors were characterized by a large peak  
22 representing more than one IBI, and in type 5 errors, the recorder generated two or more short  
23 IBIs in the place of a single IBI. As one can see in Fig. 3, none of these artefacts had ectopic  
24 origins, as they were not present in the IBIs derived from the conventional ECG. Type 2 – 5  
25 errors were successfully corrected using an algorithm for recovering IBIs from the  
26 information available within the anomalous IBIs themselves [108]. In type 1 errors, the

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

3 Fig. 3

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

11

### 12 ***3.2. General recommendations for recording of IBIs***

13

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

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

10

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

19

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

24

### 25 ***3.3. Methods of HRV analysis***

26



1 Early on, HRV analysis primarily focused on time and frequency domain analysis [1, 55, 68,  
2 119]. However, cardiac activity is an integrated signal that is influenced not only by the two  
3 branches of the ANS, but also by a number of other underlying physiological mechanisms and  
4 various extrinsic factors. More recent research reports that cardiac signals also contain non-  
5 linear components in the dimension of deterministic or non-deterministic chaos [120, 121].  
6 Nowadays, investigation of non-linear components of HRV has been established as a further  
7 important field of analysis of HRV [122-124].

## 8 9 *Time Domain Analysis*

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

15  
16 In the first subclass of time-domain measures, mean IBI and HR are the easiest to calculate  
17 but are also the least informative. The standard deviation of all IBIs over a 24-h period or the  
18 standard deviation of IBIs of a single 5-min period (SDNN, msec) are good predictors of  
19 overall variability present at the time of recording. As the total variance of HRV increases  
20 with the length of analysed recording, in practice, it is inappropriate to compare SDNN  
21 measures obtained from IBI data series of varying durations. If the SDNN is reported for a  
22 24-h recording it is sometimes referred to as cycle length variability (CLV). The SDANN is  
23 the standard deviation of the mean IBI of all 5-min segments in the data. It is also measured  
24 and reported in milliseconds and is highly correlated with SDNN. Finally,  $SDNN_{index}$  is the  
25 mean of the standard deviation of all 5-min segments of a 24-h recording. All these

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

3

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

14

### 15 *Geometric analyses*

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

26 Table 1

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

12

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

24 Fig. 4

25

26 *Spectral analysis of HRV by FFT – basic requirements and limits*

1 Fast Fourier Transformation (FFT) is a widespread approach used decrypt and analyse  
2 dynamical changes in signals in general. The fundamental principle of this method is based on  
3 the fact that every curve can be described by a set of harmonic waves which when added to  
4 one another make up the complete waveform. FFT can be used to ‘decompose’ a waveform  
5 into its sine and cosine constituents. There are some prerequisite factors which should be  
6 taken into account before using FFT, namely: (a) the time difference between the values of  
7 the time series has to be equidistant. In the case of cardiac signals, this means that before  
8 analysing IBI data, the tachogram has to be converted into an equidistant time-series by  
9 interpolating (preferably using cubic spline functions) and resampling the data. In other  
10 words, an instantaneous IBI data series (e.g. for every second of the measuring time) has to be  
11 constructed; (b) the FFT-function works on data sets of  $2^n$  numbers. If the data set is not  $2^n$  in  
12 length, some programs pad “0” at the beginning and end of the data range to reach the length  
13  $2^n$ . This leads to substantial alterations of the power-spectrum (see Fig. 5.) so it is strongly  
14 recommended to extend, or shorten, the data sets by interpolation to get a data set size of  $2^n$ ;  
15 (c) the level of accuracy achieved in describing the fluctuations of time series depends  
16 considerably on the number of points used in the FFT (see Fig. 6). According to published  
17 recommendations, a minimum of 512 points should be used for FFT analyses of IBI data [1]  
18 (e.g. let us suppose a mean HR of 80 bpm results in an IBI series of 400 values in 5-minutes.  
19 This time series has to be lengthened to 512 values by interpolation prior to analysis.). The  
20 number of points used for FFT influences the highest frequency (Nyquist frequency) as well  
21 as the amount of power in the different frequency bands (Fig. 5).

22 Fig. 5

23 Because FFT applies “folding” of the original time series for calculating the power of the  
24 various harmonics, the highest oscillation corresponds to  $\frac{1}{2}$  of the number of points used for  
25 FFT, e.g. the highest frequency in a data set which contains 512 points (representing a time-  
26 series of 300 s) is the 256-harmonic with a wavelength of 1.17 s (= 300 s/ 256) that has a

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

6 Fig. 6

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

18

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

23

#### 24 *Recommendations for the FFT analysis of HRV*

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

- 1       ➤ Use data-sets of approximately 5-min in length
- 2       ➤ Use at least 512 points from the resampled equidistant time series derived from the
- 3               original IBI data to calculate the power spectrum
- 4       ➤ Use species appropriate frequency bands widths such as the HF ranges following:
- |    |                |                 |   |
|----|----------------|-----------------|---|
| 5  | horse:         | 0.13 to 0.26 Hz | (corresponds to a respiratory rate of 8 – 16 /min)  |
| 6  | foal:          | 0.25 to 0.33 Hz | (corresponds to a respiratory rate of 15 – 20 /min) |
| 7  | cattle:        | 0.20 to 0.58 Hz | (corresponds to a respiratory rate of 12 – 35 /min) |
| 8  | calf:          | 0.50 to 0.83 Hz | (corresponds to a respiratory rate of 30 – 50 /min) |
| 9  | swine (100 kg) | 0.13 to 0.41 Hz | (corresponds to a respiratory rate of 8 – 25 /min)  |
| 10 | piglet:        | 0.33 to 0.83 Hz | (corresponds to a respiratory rate of 20 – 50 /min) |
| 11 | goat/sheep:    | 0.20 to 0.40 Hz | (corresponds to a respiratory rate of 12 – 24 /min) |
| 12 | lamb:          | 0.33 to 0.58 Hz | (corresponds to a respiratory rate of 20 – 35 /min) |
| 13 | rabbit:        | 0.67 to 1.00 Hz | (corresponds to a respiratory rate of 40 – 60 /min) |
| 14 | chicken:       | 0.33 to 0.67 Hz | (corresponds to a respiratory rate of 20 – 40 /min) |
| 15 | duck:          | 0.83 to 1.17 Hz | (corresponds to a respiratory rate of 50 – 70 /min) |

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

18  
 19 *Non-linear analysis of HRV by Recurrence Quantification Analysis*

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

1 153]. Nevertheless, others studies have failed to find evidence of low-dimensional chaos in  
2 IBI data [154-156]. These contradictory findings could be partly explained by the fact that IBI  
3 data contains a periodic component originating from the basal frequency of the sinus node  
4 [157]. Furthermore, some non-linear time-series analyses require that data meet some *a priori*  
5 restrictive mathematical assumptions. One method to test whether time-series IBI data really  
6 contains non-linear dynamics or just linear relationships in the time and frequency domain is  
7 surrogate data analysis [158].

8

9 Recurrence Quantification Analysis (RQA) may be applied to IBI data to detect hidden  
10 rhythms and non-linear deterministic structures of HRV in higher dimensional space [159,  
11 160]. This mathematical approach has already been applied successfully for estimation of  
12 non-linear processes in various physiological time series data [148, 157, 161, 162]. Since  
13 RQA is independent of limiting constraints such as data set size, stationarity, and assumptions  
14 regarding the statistical distributions of data, it seems ideally suited for investigating  
15 physiological systems characterised by non-homeostatic transients and state changes, such as  
16 cardiac activity. Validation of RQA of true non-linear components in HRV time series has  
17 been performed using surrogate data analysis [163, 164].

18

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

---

<sup>1</sup> <http://homepages.luc.edu/~cwebber/>

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

3 Fig.7

4 All recurrence points are plotted as black points at corresponding X,Y coordinates in the  
5 recurrence plot (Fig. 8). In other words, the recurrence plot visualises the distance matrix of  
6 all vectors of the N-dimensional embedded HRV-tachogram. According to Giuliani et al.  
7 [157] the recurrence plot represents the autocorrelation of a given signal at all possible time  
8 scales. Whereas the plot itself already gives an impressive picture of the regularity/irregularity  
9 of the tachogram, the parameters defined by Trulla, Webber and Zbilut [160, 163, 166] enable  
10 quantitative estimation of the recurrence structure of embedded time series.

11

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

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



1 continuous row within the plot. It is inversely related to the largest positive Lyapunov  
2 exponent. The Lyapunov exponent is a quantitative measure of the sensitive dependence of a  
3 time series on the initial conditions. A positive largest Lyapunov exponent indicates chaos  
4 [165]. A small  $L_{MAX}$  corresponds to a high Lyapunov exponent, meaning a large amount of  
5 "chaos" and vice versa. Finally, TREND describes how stationary the system is during the  
6 period of measurement. Systems showing a drift may have positive or negative TREND  
7 values, whereas systems without drift have values close to zero. For a more detailed  
8 description of the mathematical background of RQA, several detailed methodological papers  
9 have been previously published [157, 159, 160, 166]. However, as these authors emphasis,  
10 implementation of RQA is far simpler than its actual interpretation.

#### 11 Table 2

12 Beside the calculation of "non-linear indexes" of the time series, of particular importance is  
13 the physiological meaning of these parameters. Studies in rats have shown that administration  
14 of atropine significantly increases %REC, %DET and  $L_{MAX}$  [161]. However, administration  
15 of atenolol ( $\beta$ 1-adrenergic antagonist, sympathetic inhibitor) only increases %REC, whereas  
16 prazosin ( $\alpha$ 1- adrenergic antagonist) does not affect non-linear indexes of HRV data. In  
17 contrast,  $\alpha$ 1-sympathetic blockade increased the non-linear parameters of systolic BP [161,  
18 167]. In human diabetic subjects with autonomic dysfunction, no relationship has been found  
19 between linear frequency-domain HRV parameters and the results from the Ewing test (which  
20 is a standard test to diagnose diabetic autonomic dysfunction), whereas the non-linear index  
21  $L_{MAX}$  was strongly correlated to the Ewing score [164]. Theses studies concluded that the non-  
22 linear indexes of HRV were more reliable markers of sympathetic and parasympathetic  
23 activation compared to parameters generated from time- and frequency-domain analysis.

24

25 Research in ruminants indicates that combinations of linear and non-linear parameters of HRV  
26 are most promising estimates of the influence of sub-clinical or clinical stress. Results in

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

6

#### 7 **4. HRV in applied animal research: methodology and interpretation of HRV in pigs,** 8 **cattle, horses, sheep, goats and poultry**

9

##### 10 *4.1. Heart rate variability in pigs*

11

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

17

##### 18 *Issues researched*

19 In terms of biomedical research, much focus has been on Yucatan [168, 169] or Göttingen  
20 [31, 170] miniature swine, but other studies have also used commercial type pigs [171, 172].

21 There has been some basic research carried out into data acquisition system design for  
22 minipigs [173] and studies into circadian patterns of HRV [170] and the effects of pair  
23 housing on HRV parameters [31]. Miniature pigs have also been used as subjects for research  
24 into cardiovascular autonomic neuropathy [168] and the effects of testosterone modulation on  
25 HRV [169]. HRV of commercial piglets has been studied to elucidate asymmetric innervation

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

3  
4 In terms of studies allied to animal well-being, again there has been some more basic research  
5 into methodology of analysis and data acquisition system design [99], the effects of gestation  
6 on HRV parameters [97] and circadian rhythmicity in HRV. [174]. Applied studies have  
7 looked at the effects of social stress [27, 32, 67], restraint stress [32] and the effects of  
8 grooming [67] on HRV.

9  
10 *Methodologies used*

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

25

1 With externally-mounted equipment, the most commonly used have been ambulatory  
2 monitors, modified for use in pigs [e.g., 31, 99, 169, 170], or various types of Polar HR  
3 monitors, including the SportTester [32], the Vantage NV [67, 99] and the R-R Recorder [99].  
4 Other equipment used has included a telemetric ECG system [99] and static ECG systems  
5 [171, 172]. The later Polar products have the advantage that they transmit on multiple coded  
6 frequencies, thus allowing several pen-mates to be recorded simultaneously once each is  
7 locked into a different transmitter frequency. Also, if the receiver is attached to the transmitter  
8 belt, the size of pen does not present a problem as the receiver is never out of range of the  
9 transmitter. However, the external nature of the equipment means that it can become the focus  
10 of investigatory attention from pen-mates, resulting in signal disruption from physical  
11 movement of the electrode belt either directly by the rooting or chewing activity of the  
12 investigating pig, or indirectly by the physical exertion of the monitored pig trying to avoid  
13 unwanted attention.

14

### 15 *Analysis and Indices*

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

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

6

### 7 *Recommendations for Future Studies*

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

21 Table 3

22

### 23 **4.2. Heart rate variability in cattle**

24

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

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

5

#### 6 *Methodological aspects*

7 Different types of Holter recorders or fixed systems [24, 25, 101, 136] as well as portable HR  
8 monitors (mostly from Polar Electro Öy, Finland) have been used to investigate HRV in cattle [28,  
9 35, 176, 178]. In some cases, electrode sites were shaved prior to attaching electrodes [28] and in  
10 others not [35]. In either case, it is strongly recommended that ample electrode gel is applied to  
11 optimise electrode-skin contact. With HR monitors, electrodes and transmitters were usually  
12 secured in place by attaching them to a horse girth or similar [28, 35, 178]. A sufficiently long  
13 acclimatisation period (min.1 h) is recommended to allow the animals enough time to become  
14 accustomed to wearing the equipment even when visible reactions after fixing the belt generally  
15 only occur for about 5 to 10 minutes after fitting [28]. The general advantages, disadvantages or  
16 problems concerning accuracy of measurements and correction of artefacts and ectopic beats  
17 discussed earlier in this review are also applicable to HRV analysis in cattle.

18

#### 19 *Factors affecting HRV in cattle*

20 As in other species, the relationship between HRV and underlying sympathovagal balance in  
21 cattle was confirmed using pharmacological blockade of the autonomic nervous system [136,  
22 175, 177]. HRV gives insight into the regulatory processes of the cardiovascular system and  
23 various parameters describing HRV in cattle have been used to detect irregularities in the  
24 operational sequences of sympathovagal balance. Several authors have used HRV to detect  
25 alterations in the brainstem caused by bovine spongiform encephalopathy (BSE). In addition  
26 to bradycardia, these studies report an increase in vagal tone, marked by a drastic increase in

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

1 considerable decrease in both vagal and sympathetic tone, as shown by simultaneous  
2 decreases in RMSSD, SDNN, SDANN and  $HRV_{index}$ . Results in the frequency domain exhibit  
3 similar patterns to time domain parameters. Changes in the HF component to varying levels  
4 of stress were similar to those found in RMSSD. A significant decrease occurred following  
5 external stress and an even stronger decline happened in response to pathological load.  
6 Simultaneously changes in the LF component were not observed, but the absence of changes  
7 in the LF component could have been a result of masking effects or interchanges between all  
8 the loops mentioned above. In summary, results in both the time domain and frequency  
9 domain indicated a reduction of vagal tone as stress level increases. No significant difference  
10 in either time or frequency domain parameters of HRV have been found to exist between  
11 lactating and non-lactating cows [28]. All cows showed similar values for all parameters  
12 within the two groups, so it seems that lactation and late pregnancy are comparable  
13 pathological loads for the animals. Cardiac activity in mammals is an integrated signal which  
14 is influenced not only by the two branches of the ANS, but also by other physiological control  
15 circuits and external influences [180]. HRV is influenced by several feedback or feed forward  
16 mechanisms [159]. In recent years, some studies have shown that IBI time series contain non-  
17 linear components in the sense of deterministic or non-deterministic chaos that is described in  
18 detail earlier in this review [121, 150, 152, 153,]. In calves, all non-linear parameters of HRV  
19 have been demonstrated to rise significantly in response to external stress, and even more to  
20 pathological load. The most obvious increment was observed for  $L_{MAX}$ , which increased  
21 significantly in all three thermal, insect and pathological stress groups [28]. In lactating cattle,  
22 %DET was the only non-linear parameter which increased significantly from non-lactating to  
23 lactating cows [28]. The results in both calves and cattle studies indicate a loss of complexity  
24 in cardiac activity and a more deterministic control of HRV in reaction to external,  
25 physiological or pathological loads. In other words, when an organism's biological systems  
26 need to focus on specific challenges, a loss in the general freedom of cardiovascular dynamics



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

11

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

### 17 *Specific conclusions and recommendations*

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

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

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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

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

12

### 13 *Factors affecting HRV in horses*

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

25

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

8

9 Several horse studies have shown an effect of physical effort and training on cardiac activity  
10 and sympathovagal balance [37-40, 92, 185]. In general, resting HR in horses is significantly  
11 decreased by training but one study has failed to find any training related changes in the vagal  
12 tone when HRV was recorded at rest [187]. Other work found that in challenging conditions  
13 (behaviour tests), untrained horses showed more pronounced, although not significant,  
14 increases in HR alongside associated decreases in HRV parameters [40]. Exercise on aqua-  
15 treadmill is associated with significantly higher sympathetic tone and decreased vagal tone  
16 [92], although immersion in warm spring water, without any physical effort, results in an  
17 enhancement in vagal activity, that is purportedly linked to a mental and physical state of  
18 relaxation [185]. Many studies found negative correlations between the intensity of exercise  
19 and the overall HRV due to a progressive rise in sympathetic tone [37, 39, 92].

20

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

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

### 13 *Specific conclusions and recommendations*

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

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

23

24 Table 5

25

#### 1 *4.4. HRV in sheep and goat*

2

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

8

9 These similarities explain why a large number of studies on foetal cardiovascular regulation  
10 have been performed in the ovine foetus. Several studies [reviewed in 197] support the  
11 existence of autonomic control of circulatory function early in the development of the foetus.  
12 In the immature foetus, basal sympathetic tone is important in the maintenance of foetal  
13 arterial pressure [198, 199] and is reflected by the LF variability in the HRV power spectrum  
14 [200, 201]. Monitoring the variability in the LF range has been used in estimating the level of  
15 foetal sympathetic activation during high-risk pregnancies, foetal distress after haemorrhage  
16 [202] and hypoxia [203]. The PNS is reported to have little influence on basal foetal  
17 cardiovascular function in the immature foetus, with its influence on resting HR increases  
18 progressively during post-natal maturation [198, 204]. Although neurohumoral control is  
19 important in neonatal period, the sympathetic system appears to be the major regulator of  
20 vascular function up to 8 weeks of life [198, 205]. By 3 months age, vagal regulation  
21 dominates and the best indices of this dominance are RMSSD and HF power [175].

22

23 Studies involving HRV and behaviour in sheep and goats are sparse. Desire, Veissier et al  
24 [41] investigated the ability of lambs to react to suddenness and novelty of an event,  
25 according to appraisal theories. They found that lambs responded to a sudden event by a  
26 startle response coupled with a transient increase in HR that did not appear to be vagally

1 mediated as there were no associative modifications in RMSSD levels. They responded to a  
2 novel event by orientating towards the novelty coupled with a transient increase in RMSSD.  
3  
4 Langbein, Nürnberg et al. [36] studied HRV and visual discrimination learning in Nigerian  
5 dwarf goats using Polar S810 monitors. To minimize the influence of physical activity, and to  
6 study the long-term effects of visual operant conditioning learning on HRV only IBI data  
7 corresponding to resting behaviour (lying, calm and undisturbed) were incorporated into the  
8 analyses in this particular research. Whereas HR increased throughout the course of a first  
9 learning task, this relationship was the opposite in two proximate tasks, indicating different  
10 effects of different learning challenge on HR that may have been related to how familiar the  
11 goats were with the function of the learning device. Moreover, this work also found  
12 significant relationships between the time taken to perform particular tasks and several HRV  
13 indices representing vagal tone. Overall, results from this research suggest that learning  
14 related changes in HR were predominantly caused by a withdrawal of vagal tone. To  
15 investigate non-linear processes in cardiac regulation, this study used RQA. Increased  
16 deterministic shares of HRV throughout task 1 and 2 indicated that the goats did not really  
17 relax until the end of task 3.

18

### 19 *Recommendations for HRV studies in sheep and goat*

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

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

6  
7 ECG signals should ideally be recorded at a sampling rate of 1000 Hz. Time- and frequency-  
8 domain analysis should be conducted following the procedures recommended earlier in this  
9 review. Frequency domain analysis is critical to determine the contributions of both branches  
10 of the ANS. Stationary data should be used and this is easiest to obtain in sheep and goats  
11 when they are lying undisturbed.

12  
13 Since experimental conditions, such as temperature or animal metabolism, may vary greatly,  
14 the respiratory frequency of the animals should be simultaneously recorded to allow for  
15 accurate determination of the upper limit of the spectrum defined by Nyquist frequency. In  
16 warm environment, HR in sheep can reach 200 bpm and their respiratory frequency 72 bpm  
17 (1.2 Hz). In this example, if the resampling frequency of the IBI data is 3 Hz, the Nyquist  
18 frequency is 1.5 Hz and the following frequency-domain ranges are advised: total power in  
19 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  
20 the respiratory peak at 1.2 Hz. The limits of LF wavebands include the oscillation of the  
21 baroreflex region (0.1 Hz) as shown by pharmacological blockades studies in sheep [205].

22

#### 23 ***4.5. Heart rate variability in poultry***

24

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



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

4

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

20

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

25

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

7

8 HRV analysis has been used to obtain physiological information about broilers at risk of  
9 sudden death syndrome which can lead to the death of 2 to 4 % of male broiler chickens in a  
10 flock [213]. In this research, birds were equipped with telemetric transmitters when they were  
11 15-days old. The transmitters were implanted subcutaneously at the base of the neck with one  
12 electrode placed over the right shoulder and the other one over the left groin area. The freely  
13 moving chicks could then be monitored in their home cage. Unfortunately, although only  
14 SDANN was used to evaluate HRV, the study clearly demonstrated that telemetric devices are  
15 powerful tools for accurately measuring cardiac activity and HRV at rest.

16

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

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

5

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

12

### 13 **5. General conclusions and recommendations for future research**

14

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

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

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

12

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

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

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**Appendix A**

**Abbreviations and main definitions**

**ANS: Autonomic Nervous System.** 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<sup>th</sup> cranial nerve) is a major component of the ANS.

**AV: Atrioventricular.** 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: Blood pressure.**

**Bpm: Beats per minute.** Number of heart beats in one minute.

**CLV: Cycle length variability.** Standard deviation of IBIs calculated over 24 h.

**ECG: Electrocardiogram.**

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

**FFT: Fast Fourier transformation.**

**HF: High frequency.** 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: Heart rate.** Frequency of heart beats usually expressed as number of beats per min.

**HRV: Heart rate variability.** 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: Inter-beat interval.** Time interval between two consecutive heart beats in ms.

**LF: Low frequency.** 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.

1 **L<sub>MAX</sub>: Maxline.** One of the quantitative parameters derived from a non-linear mathematical  
2 analysis of HRV (see RQA). See exact definition in part III.

3 **Lorenz plot.** See Poincaré plot.

4 **NN interval: Normal-to-Normal interval = IBI.**

5 **NN50: Normal-to-normal intervals greater than 50 ms.** Number of differences between two  
6 successive IBIs greater than 50 ms.

7 **pNN50: Percentage of normal-to-normal intervals greater than 50 ms.** Percentage of  
8 differences between two successive IBIs greater than 50 ms.

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

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

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

15 **PNS: Parasympathetic Nervous System.** One of the two main branches of the ANS (see  
16 above).

17 **PSD: Power spectral density.** PSD analysis describes the variation of an IBI data series as a  
18 set of sine and cosine constituents. One method to calculate PSD is based on FFT.

19 **RMSSD: Square root of the mean of the sum of the squares of differences between**  
20 **consecutive IBIs.** It is the standard deviation of differences between successive IBIs.

21 **RQA: Recurrence quantification analysis.** A non-linear mathematical analysis of HRV.

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

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

26 **SD2: Standard deviation 2.** It represents the long-term component of HRV derived from a  
27 quantitative analysis of Poincaré plot. See exact definition in part III.

28 **SDANN: Standard deviation of the average normal-to-normal intervals.** It is the standard  
29 deviation of the IBIs averages calculated on 5-min segments during the 24-h cycle.

- 1 **SDNN**: Standard **d**eviation of **n**ormal-to-**n**ormal intervals. It is the standard deviation of all  
2 IBI measured.
- 3 **SDNN<sub>index</sub>**: mean of **s**tandard **d**eviation of **n**ormal-to-**n**ormal intervals. It is the mean of the  
4 IBI standard deviations calculated on 5-min segments.
- 5 **SN**: **S**inus **n**ode = nodus sinu-atrialis. The heart's pacemaker that generates an intrinsic heart  
6 rate. It is under the control of both parasympathetic and sympathetic nerves.
- 7 **SNS**: **S**ympathetic **N**ervous **S**ystem. One of the two main branches of the ANS (see above).
- 8 **Tachogram**: a graphical record representing the variation of IBIs as a function of the interval  
9 number.
- 10 **TINN**: **T**riangular **i**nterpolation of **NN** interval histogram. One of the quantitative parameters  
11 derived from a geometrical analysis of HRV.
- 12 **Trend**: One of the quantitative parameters derived from a non-linear mathematical analysis of  
13 HRV (see RQA). See exact definition in part III.
- 14 **VLf**: **V**ery **l**ow **f**requency. It is the component of HRV determined by spectral analysis  
15 whose usual range of variation in humans is between 0.0033 and 0.04 Hz. Its physiological  
16 significance is not fully clear.
- 17



1 Table 1

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

3 Variable	Units	Description
4 <b>Statistical measures</b>		
5		
6 SDNN	ms	Standard deviation of all IBIs of the dataset.
7 SDANN	ms	Standard deviation of the mean of IBIs in all 5-min segments
8		of the entire dataset.
9 SDNN <sub>index</sub>	ms	Mean of the standard deviations of all IBIs for all 5-min
10		segments of the entire dataset (24h).
11 RMSSD	ms	The square root of the mean of the sum of the squares of
12		differences between successive IBIs.
13 NN50 count		Number of pairs of successive IBIs differing by more than 50
14		ms.
15 pNN50	%	NN50 count divided by the total number of all IBI's.
16		
17 <b>Geometric measures</b>		
18		
19 HRV <sub>index</sub>		Total number of all IBIs divided by the height of the
20		histogram of all IBIs measured on a discrete scale with bins of
21		7.8125 ms (1/128 s).
22 TINN <sub>index</sub>	ms	Baseline width of the minimum square difference triangular
23		interpolation of the highest peak of the histogram of all IBIs.
24 Poincaré (Lorenz) plot		XY-diagram of each IBI of the dataset plotted as a function of
25		the previous IBI.
26		

1 Table 2

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

3	PERCENTAGE OF	Percentage of recurrence points in the plot; single vector repetition
4	RECURRENCE 127(%REC)	in n-dimensional space.
5	PERCENTAGE OF	Percentage of recurrence points forming upward diagonal lines i.e.
6	DETERMINISM (%DET)	recurrence points in consecutive sequences.
7		
8	ENTROPY (ENT)	Shannon information entropy of the line length distribution
9	MAXLINE ( $L_{MAX}$ )	The longest diagonal line segment of consecutive recurrence
10		points in the plot.
11	TREND	Drifting of the recurrence points away from the central diagonal
12		line of identity.

1 Table 3

2 Summary of selected research on HRV research in pigs

3	<b>Publication</b>	<b>Study Objective</b>	<b>Equipment Used</b>	<b>Time Domain Indices Reported</b>	<b>Frequency Domain Indices Reported</b>
5	Mesangeau et al., 2000	Diabetes research	Data Science	Mean HR, SDRR	Total Power
6	[168]		TL11M2-D70-DCT		
7	Olmstead et al., 2005	Effects of testosterone	Holter	Mean HR, SDRR,	LF, 0.01 - 0.07 Hz
8	[169]	on HRV		SDANN, RMSSD	HF, 0.07 - 1.0 Hz
9	Kuwahara et al., 1999	Circadian rhythms	Holter	Mean RR, SDRR,	LF, 0.01 - 0.07 Hz
10	Kuwuhara et al., 2004	Pair housing		CVRR	HF, 0.07 - 1.0 Hz
11	[170]				Total Power
12	[31]				Normalised LF & HF
13	Voss et al., 2004	Effects of endotoxins	Hook Electrodes &	SDRR, SDARR	LF, 0.02 - 0.15 Hz
14	[172]	on HRV	Grass Recorder		HF, 0.15 - 2.0 Hz
15	Marchant-Forde et al., 2004	Validation of equipment	Polar RR,	Mean HR, RR Max,	VLF, 0.003 - 0.01 Hz
16	[99]	and identification &	Telemetric ECG	RR Min, SD,	LF, 0.01 - 0.07 Hz
17		correction of artefacts		$\sigma^2$ , RMSSD	HF, 0.01 - 0.5 Hz
18					Total Power
19					SNSI & PNSI
20	Marchant-Forde, Marchant-Forde, 2004	Effects of gestation	Polar NV	Mean HR, RR Max,	LF, 0.01 - 0.07 Hz
21	[97]	on HRV		RR Min, SD, $\sigma^2$ ,	HF, 0.01 - 0.5 Hz
22				RMSSD	Total Power
23					SNSI & PNSI
24	de Jong et al. 2000	Social stress	Data Science	Mean RR, SDNN,	
25	[27]		TA10CTA-D70	SDRR, RMSSD	
26	Geverink et al. 2002	Restraint stress	Polar Sport Tester	Mean HR, SD, SD1,	
27	[32]		Electrodes & FM	SD2, SD2/SD1 ratio	
28			Transmitters		
29					



1 Table 4

2 Baseline values of HRV at lying down (mean  $\pm$  SD) in

3 18 calves [28]

4	Variable	Units	mean $\pm$ SD
5	IBI	ms	593,47 $\pm$ 131,9
6	SDNN	ms	27,1 $\pm$ 8.3
7	RMSSD	ms	15.2 $\pm$ 8.8
8	LF/HF		6.1 $\pm$ 6.2
9	LF	normalized units	35.3 $\pm$ 12.1
10	HF	normalized units	9.9 $\pm$ 6.2
11	%REC		3.3 $\pm$ 2.2
12	%DET		84.0 $\pm$ 6.9
13	ENT		3.5 $\pm$ 0.4
14	L <sub>MAX</sub>		50,0 $\pm$ 24.3

15

16 15 cows, breed Brown Swiss [35]

17	Variable	Units	mean $\pm$ SD
18	IBI	ms	819.11 $\pm$ 114.6
19	SDNN	ms	35.6 $\pm$ 10.8
20	RMSSD	ms	16,2 $\pm$ 8.2
21	LF/HF		4.7 $\pm$ 3.2
22	LF	normalized units	25.9 $\pm$ 5.7
23	HF	normalized units	11.9 $\pm$ 8.6
24	%REC		3.4 $\pm$ 2.4
25	%DET		76.1 $\pm$ 11.5
26	ENT		3.0 $\pm$ 0.62
27	L <sub>MAX</sub>		49.7 $\pm$ 40.0

28

1 Table 5

2 Baseline values of HRV (mean  $\pm$  SD) in:

3 18 adult Warmblood horses [30]

4 Variable	Units	mean $\pm$ SD
5 IBI	ms	1818 $\pm$ 152
6 SDNN	ms	111 $\pm$ 50.55
7 LF/HF		1.70 $\pm$ 1.69
8 LF	normalized units	53.3 $\pm$ 19.5
9 HF	normalized units	46.8 $\pm$ 19.5

10

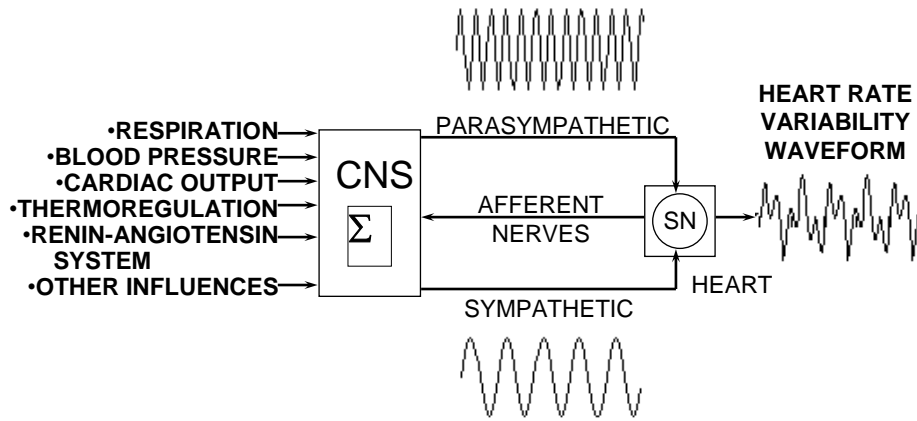
11 6 Thoroughbred horses (8-20 years) [100]

12 Variable	Units	mean $\pm$ SD
13 IBI	ms	1532 $\pm$ 130
14 SDNN	ms	313 $\pm$ 169
15 RMSSD		300 $\pm$ 297
16 PNN50		37 $\pm$ 14
17 Total power	ms <sup>2</sup>	149.5 $\pm$ 167.8
18 HF	ms <sup>2</sup>	114.1 $\pm$ 153.9
19 LF	ms <sup>2</sup>	35.4 $\pm$ 18.1

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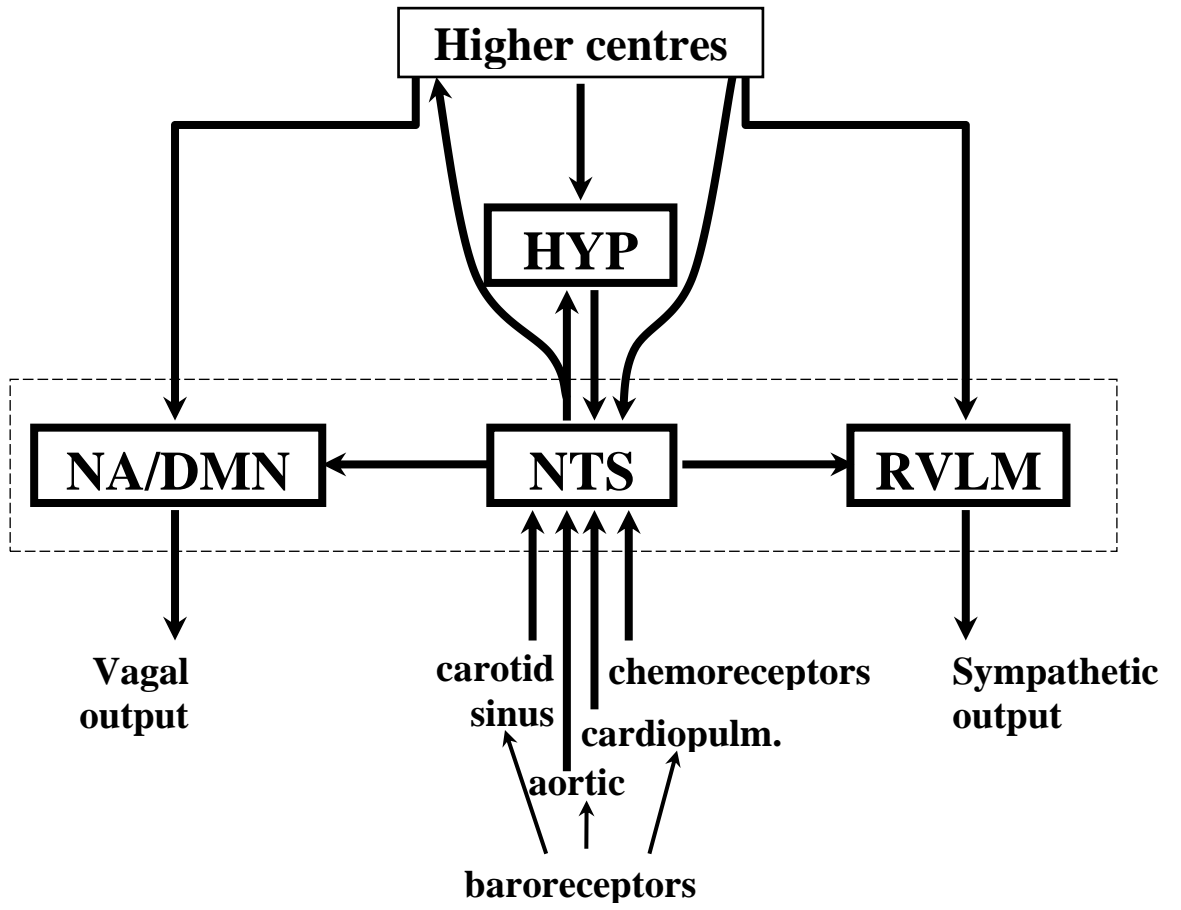
# Autonomic Control of the Heart



(From McCraty & Watkins, 1996)

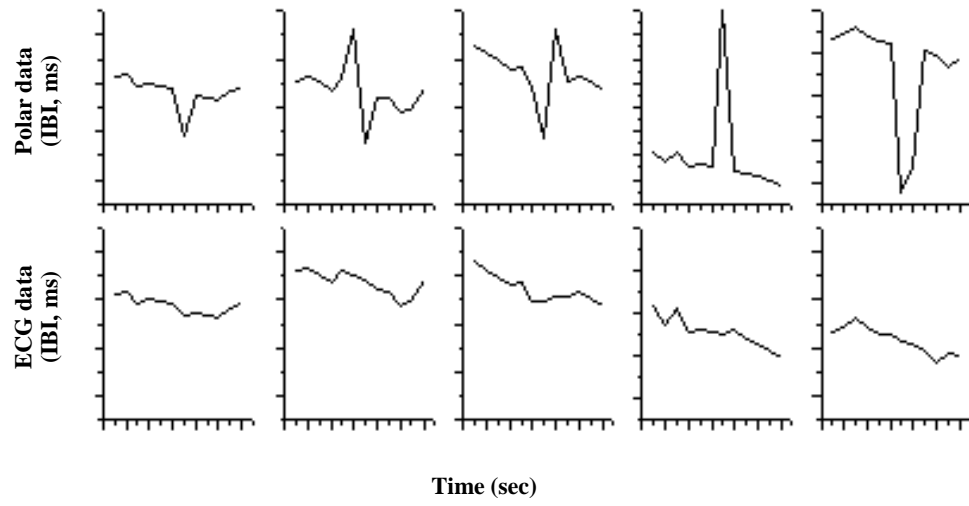
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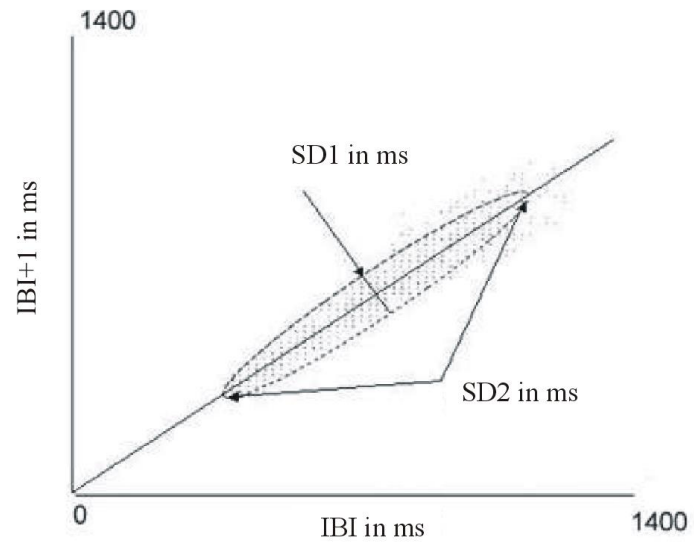


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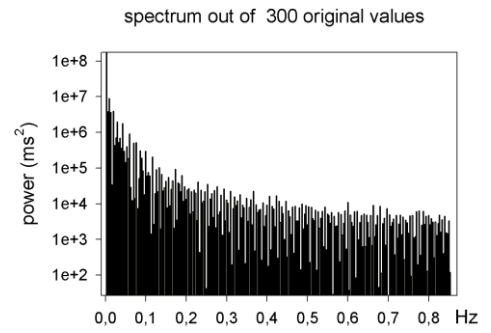
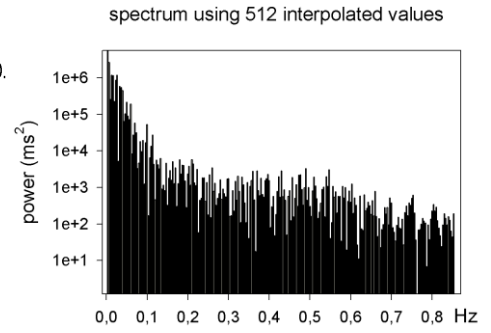
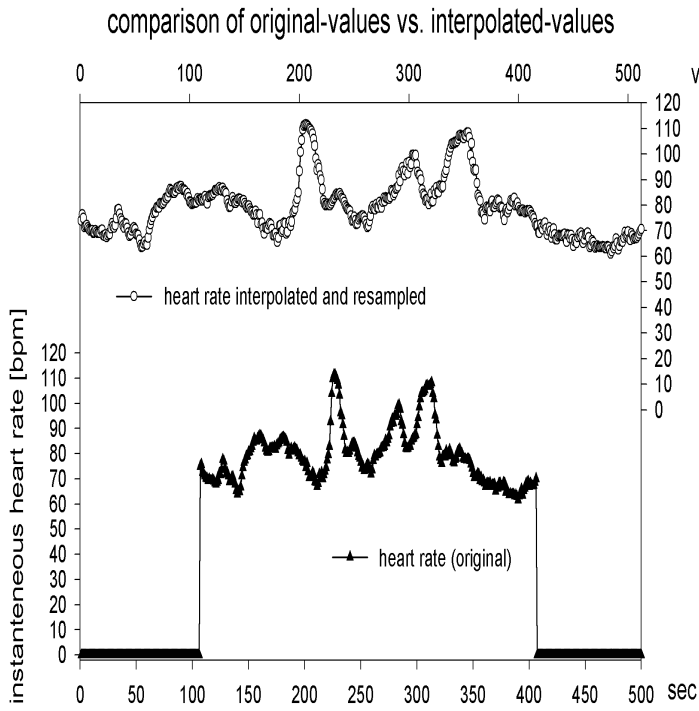


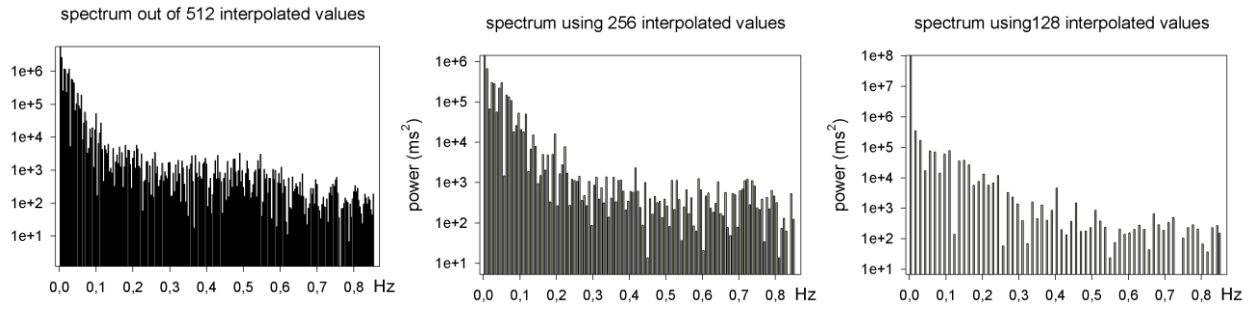
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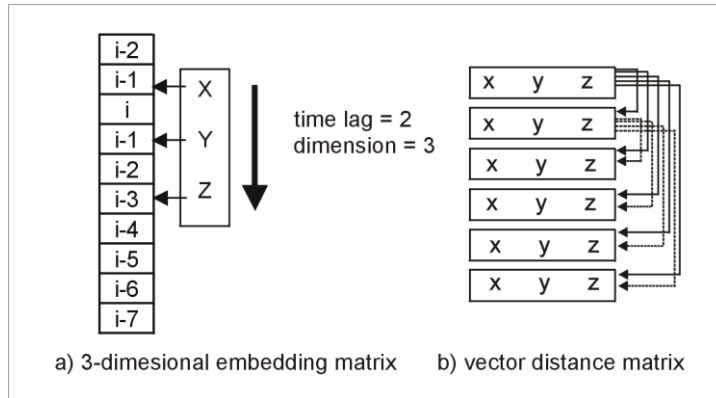


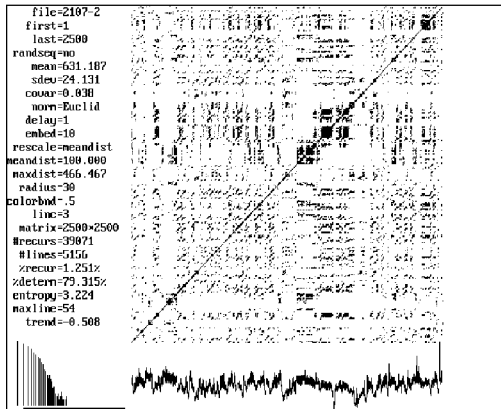
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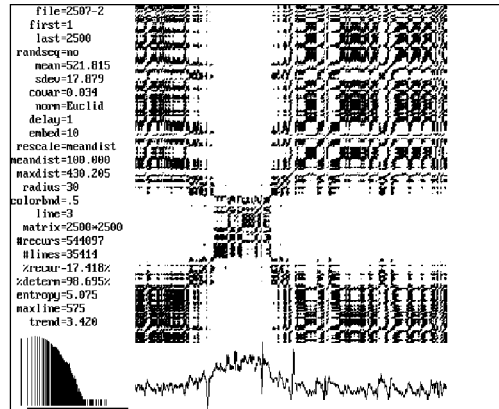








a. calf, 7 days old, healthy  
% Rec: 2; % Det: 79; L : 54



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

1 **Legends for Fig.s**

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

4

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

6 Centre (dotted rectangle) in the medulla. DMN=vagal Dorsal Motor Nucleus,

7 HYP=Hypothalamus, NA=Nucleus Ambiguus, NTS=Nucleus Tractus Solitarii,

8 RVLM=Rostral Ventrolateral Medulla

9

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

13

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

17

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

19

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

2

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

5

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