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Original article

Short-term heart rate variability (HRV) in healthy dogs

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Abstract

Heart rate variability (HRV) is a well established mortality risk factor in both healthy dogs and those with heart failure. While the standards for short-term HRV analysis have been developed in humans, only reference values for HRV parameters determined from 24-hour ECG have been proposed in dogs. The aim of this study was to develop the reference values for short-term HRV parameters in a group of 50 healthy dogs of various breeds (age 4.86 ± 2.74 years, body weight 12.2 ± 3.88 kg). The ECG was recorded continuously for at least 180 min in a dark and quiet room. All electrocardiograms were inspected automatically and manually to eliminate atrial or ventricular premature complexes. Signals were transformed into a spectrum using the fast Fourier transform. The HRV parameters were measured at fixed times from 60-min ECG segments. The following time-domain parameters (ms) were analyzed: mean NN, SDNN, SDANN, SDNN index, rMSSD and pNN50. Moreover, frequency-domain parameters (Hz) were determined, including very low frequency (VLF), low frequency (LF) and high frequency (HF) components, total power (TP) and the LF/HF ratio. The results (means ± SD) were as follows: mean NN = 677.68 ± 126.89; SDNN = 208.86 ± 77.1; SDANN = 70.75 ± 30.9; SDNN index = 190.75 ± 76.12; rMSSD = 259 ± 120.17, pNN50 = 71.84 ± 13.96; VLF = 984.96 ± 327.7; LF = 1501.24 ± 736.32; HF = 5845.45 ± 2914.20; TP = 11065.31 ± 3866.87; LF/HF = 0.28 ± 0.11.

Key words: dogs, heart, heart rate variability, cardiology

Introduction

Heart rate variability (HRV) is a physiological phenomenon defined as periodical changes in the duration of intervals between consecutive QRS complexes during a sinus rhythm. In 1847, Ludwig (1847) showed that the heart rate of healthy humans increases during inspiration and decreases during the expiration period for the first time. In 1965 Hon and Lee (1963) revealed that the loss of intrauterine heart rate variability is associated with a considerable risk of fetal death In 1978, Wolf et al. (1978) showed that a decrease in HRV parameters is associated with an increased risk of mortality in individuals with myocardial infarction. Since the implementation of computerized methods for HRV analysis, the latter gained popularity as a relatively simple and non-invasive test of cardiac autonomic function. Nowadays, HRV is

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widely used in human cardiology as a measure of risk for sudden cardiac death (Sandercock and Brodie 2006, Voss et al. 2013b). Furthermore, it proved to be a useful marker of early subclinical autonomic dysfunction associated with diabetic neuropathy (Chessa et al. 2002, Turker et al. 2013). HRV obtained during 24-hour Holter electrocardiography was also examined in dogs, both in a clinical and experimental setting and in patients with mitral valve endocardiosis, tachycardiomyopathy and diabetes (Piccirillo et al. 2009, Oliveira et al. 2012, Pirintr et al. 2012, Rasmussen et al. 2012, Chompoosan et al. 2014). Rasmussen et al. (2012) found an increase in the heart rate/min (HR) and mean HR in Cavalier King Charles Spaniels with advanced chronic mitral valve disease and a decrease in most of the measured HRV parameters (total power – TP, ultra low frequency- ULF, very low frequency – VLF, % of successive NN-intervals that differ more than 50 ms – pNN50 and the square root of the mean squared differences of successive NN-intervals – RMSSD) compared to healthy dogs and those with minimal mitral regurgitation. A decrease in the frequency-domain parameters of HRV (VLF, LF, HF, TP, LF/HF ratio) was observed in dogs with diabetes mellitus (Pirintr et al. 2012).

When an ECG recording is carried out, the varying conditions and the large number of artifacts that are present make the analysis of HRV from 24-hour electrocardiograms highly challenging. Therefore, standards for short-term HRV analysis have been developed in humans (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). However, neither such standards nor reference values for short-term HRV parameters of dogs have been proposed to date. Such reference values could be useful in dogs with heart failure, either those associated with dilated cardiomyopathy (DCM) or resulting from mitral valve degeneration (MVD), as both these conditions lead to dysautonomia (Noszczyk-Nowak 2012).

The aim of this study was to develop reference values for short-term (60-min) HRV parameters of dogs.

Materials and Methods

The study included a total of 50 healthy dogs (30 mixed-breed dogs, 9 Dachshunds, 5 Fox Terriers and 6 Beagles) aged between 2 and 11 years (mean 4.86 ± 2.74 years), with a body weight between 6 kg and 20 kg (mean 12.2 ± 3.88 kg). There were 35 females and 15 males in total. The animals were qualified for the HRV analysis after a clinical examination, an assessment of the complete blood count (using an Animal Blood Center ABC VET analyzer) and biochemical parameters of the blood (AST, ALT, urea, creatinine, Na^+, K^+, total Ca, Mg^{2+}, Cl^-) using the MaxMat Pl analyzer. None of the studied dogs showed abnormalities in any of the abovementioned laboratory parameters. Furthermore, standard transthoracic echocardiography with a determination of the ejection fraction (EF), shortening fraction (SF), left ventricular end-diastolic and end-systolic internal diameters (LVIDd and LVIDs), interventricular septal diameters at diastole and systole (IVSd and IVSs) and left ventricular end-diastolic and end-systolic posterior wall diameters (LVPWd and LVPWs), was conducted in all the dogs using an ALOKA 4000+ ultrasonograph equipped with 7.5 MHz sector probes. Furthermore, electrocardiograms of all the animals were obtained in a right-lateral recumbent position with a BTL SD08 electrocardiograph equipped with filters for power line interference and muscle noise. None of the dogs showed any clinical, electrocardiographic and echocardiographic abnormalities.

Holter electrocardiograms were obtained with an Aspel 702 recorder and analyzed with a HolCard computer module. The disposable self-adhesive electrodes were attached to shaved skin of the chest cleaned with 70% ethanol. The recorder was stabilized and protected with a bandage or specially fitted vest, so as not to limit movements of the examined dogs allowing them to stay in a comfortable position. The dogs remained in a quiet and darkened room during the 2-hour Holter ECG recording. The HRV parameters were determined from a 60-min resting electrocardiogram. The analysis of HRV was preceded by manual screening of preselected ECG segments, to identify those representing a pure sinus rhythm and lacking any artifacts. The time- and frequency-domain HRV parameters were calculated with the aid of a HolCard computer module. The mean length of the R-R intervals and the number of QRS complexes (#QRS) were determined automatically for each of the analyzed segments. Two types of time-domain HRV parameters were calculated: (a) reflecting the variability of the R-R intervals, and (b) based on the differences between the consecutive R-R intervals. Group (a) included the following parameters: SDNN (ms) – standard deviation of all R-R intervals, SDANN (ms) – standard deviation of the averaged R-R intervals for all 5-min segments, and SDNNI (ms) – mean and the standard deviations of all R-R intervals for all 5-min segments. Group (b) included: pNN50 (%) – the percentage of successive R-R intervals >50 ms, and rMSSD (ms) – the root-mean-square of successive R-R interval differences. The frequency-domain analysis was performed using the fast Fourier transform. The total spectral power (TP) of

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all R-R intervals in 5-min segments was determined, along with its high frequency (HF) (0.15-0.4 Hz), low frequency (LF) (0.04-0.15 Hz), very low frequency (VLF) (< 0.04 Hz,) components and LF/HF ratio. These components can be expressed in various units, including milliseconds (unit of amplitude), ms^2 (units of spectral power) or ms^2/Hz (units of spectral density). It is recommended to use units of spectral power. (ms^2) (Krauze T et al. 2001). HF represents parasympathetic variability of HR associated with breathing and changes in arterial blood pressure, LF reflects sympathetic effects on cardiac function, and VLF characterizes the activity of arterial chemoreceptors and baroreceptors.

The reference values for all the parameters mentioned above were determined with 95% confidence intervals (Umetani et al. 1998). Confidence intervals (Cl) were calculated as the mean ± (standard error*1,96). Additionally , the results were subjected to statistical analysis. The significance of age- and sex-related differences in the HRV parameters was verified with the Student t-test for independent variables in the case of normally-distributed parametric variables, or with the Mann-Whitney U-test for independent variables in the case of non-parametric or not normally distributed variables. The direction and power of associations between the pairs of continuous variables were determined on the basis of Spearman's R-coefficients of rank correlation. The testing was done based on the significance level $p \leq 0.05$.

The protocol of the study was approved by the 2nd Local Bioethics Committee in Wroclaw (decision No. 7/2012).

Results

The studied dogs easily accepted the place of examination (quiet, darkened room) and generally resumed in a lateral recumbent position after the initial 15-20 min of testing. The ECG recordings obtained from all the dogs were of good quality, with a low number of artifacts, and, thus, were qualified for further analysis. Clinical characteristics of the study group are presented in Table 1 and 2. Male and female dogs did not differ significantly in terms of their body weight, age, electrocardiographic and echocardiographic findings. Statistical characteristics of the HRV parameters of males and females are presented in Table 3. We did not find statistically significant sex-related differences in the analyzed parameters. The stratified results according to the animals; age are presented in Table 4. Although we did not find statistically significant differences between the parameters determined in dogs under 2 years of age, aged between 2 and 6 years, and older than 6 years, we observed a tendency for an age-related increase in SDNN and SDANN (r=-0.2) and a decrease in HF (r=-0.26). Moreover, a number of strong significant correlations were documented between the study variables: TP and SDNN (r=0.94), SDNNI and VLF (r=0.83), rMSSD and HF (r=0.78), TP and HF (r=0.94) as well as TP and VLV (r=0.75). Furthermore, we found significant inverse correlations between TP and LF/HF (r=-0.44), and between HF and LF/HF (r=-0.59).

Table 1. Clinical characteristics of the study group.

Parameter	Study Group (n=50)
Age (years)	4.86 ± 2.74
Body weight (kg)	12.20 ± 3.88
Males	15 (30%)
Ejection fraction (%)	69.21 ± 7.07
Shortening fraction (%)	37.84 ± 5.91
LA/Ao	1.27 ± 0.15
LVIDd (cm)	2.86 ± 0.36
LVIDs (cm)	1.77 ± 0.29
IVSd (cm)	0.67 ± 0.10
IVSs (cm)	0.88 ± 0.12
LVPWd (cm)	0.66 ± 0.09
LVPWs (cm)	1.00 ± 0.11
P time (ms)	36.81 ± 2.73
P amp (mV)	0.14 ± 0.03
PQ (ms)	84.88 ± 12.59
R amp (mV)	1.12 ± 0.34
QRS (ms)	53.96 ± 3.66
QT (ms)	229.18 ± 31.27
HR	102.43 ± 29.61
#QRS	5345.03 ± 1105.11
mean NN	677.68 ± 126.89
SDNN (ms)	208.86 ± 77.1
SDANN (ms)	70.75 ± 30.9
SDNNI (ms)	190.75 ± 76.12
rMSSD (ms)	259 ± 120.17
pNN50 (%)	71.84 ± 13.96
TP (ms^2)	11065.31 ± 3866.87
HF (ms^2)	5845.45 ± 2914.20
LF (ms^2)	1501.24 ± 736.32
VLF (ms^2)	984.96 ± 327.7
LF/HF	0.28 ± 0.11

LA/Ao – left atrium to aorta ratio, LVIDd – left ventricular end-diastolic internal diameter , LVIDs – left ventricular end-systolic internal diameter, IVSd – intraventricular septal diameter in diastole, LVPWd – left ventricular end-diastolic posterior wall diameter, IVSs – intraventricular septal diameter in systole, LVPWs – left ventricular end-systolic posterior wall diameter, HR – heart rate, #QRS- mean number of QRS complexes, SDNN (ms) – standard deviation of all R-R intervals, SDANN (ms) – standard deviation of the averaged R-R intervals for all 5-min segments, SDNNI (ms) – mean of the standard deviations of all R-R intervals for all 5-min segments, rMSSD (ms) – root-mean-square of successive R-R interval difference, pNN50 (%) – the percentage of successive R-R intervals >50 ms, TP – total power, HF – high frequency component, LF – low frequency component, VLF – very low frequency component

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Table 2. Blood analysis of the study group.

Parameter	Study Group (n=50)
WBC (G/l)	10.9 ± 2.1
RBC (T/l)	6,8 ± 0.5
Hb (mmol/l)	9.7 ± 0.8
Ht (l/l)	0.47 ± 0.11
Plt (G/l)	325.6 ± 107.9
ALT (U/l)	50.1 ± 12.4
AST (U/l)	56.2 ± 18.1
Urea (mg/dl)	45.9 ± 14.4
Creatinine (mg/dl)	1.05 ± 0.2
Na^+ (mmol/l)	144.8 ± 2.4
K^+ (mmol/l)	4.5 ± 0.6
total Ca (mmol/l)	2.3 ± 0.2
Mg^{2+} (mmol/l)	0.8 ± 0.1
Cl^- (mmol/l)	105.2 ± 2.6

WBC – white blood cell, RBC – red blood cell, Hb – hemoglobin, Ht – hematocrit, Plt – platelets, AST – alanine aminotransferase , ALT – aspartate aminotransferase

than 6 years. The values of the morphological and biochemical blood parameters were within the reference ranges.

Difficulties in obtaining good quality 24-hour ECG recordings stimulated research on the short-term HRV parameters of dogs. Since the time-domain parameters are tightly associated with the time point of testing, their reference values should be defined for specific intervals. This is of vital importance, since differences in the timing of ECG recording and HRV determination frequently preclude comparative analyses of the data reported by various authors. A number of studies conducted in humans confirmed the usefulness of short-term HRV. Rasmussen et al. carried out a HRV analysis in humans, rabbits, dogs and calves and analyzed these results with 11 minute ECG recordings. Differing recording times and different numbers of QRS complexes make a time analysis impossible. Since the frequency domain analysis is presented in ms^2/Hz, it cannot be compared to our results. This example highlights the need to standardize HRV analysis times, as well as the units used to measure the frequency-domain analysis. According to Voss et al. (2013b), the HRV parameters of ischemic heart failure in patients determined during the initial 30 min of testing have an almost idential classification power (81% accuracy) as those from 24-h ECG recording. Baranowski et al.

Table 3. Heart rate variability parameters in healthy dogs stratified according to their sex.

Parameter	Males (n=15)	Females (n=35)
Age (years)	4.75 ± 2.80	4.39 ± 2.33
Body weight (kg)	11.34 ± 3.29	12.13 ± 3.82
Ejection fraction (%)	66.78 ± 6.34	70.41 ± 7.27
Shortening fraction (%)	35.73 ± 4.99	38.89 ± 6.19
LA/Ao	1.29 ± 0.13	1.26 ± 0.16
LVIDd (cm)	2.84 ± 0.44	2.87 ± 0.32
P time (ms)	36.66 ± 2.45	36.88 ± 2.92
PQ (ms)	84.33 ± 13.69	85.14 ± 12.41
QRS (ms)	50.88 ± 3.29	53.5 ± 2.66
QT (ms)	230.44 ± 34.40	228.55 ± 30.61
HR	94.21 ± 13.36	92.18 ± 18.73
#QRS	5289.60 ± 888.84	5374.21 ± 1225.60
mean NN	680.50 ± 106.86	676.21 ± 139.04
SDNN (ms)	194 ± 66.65	216.68 ± 82.81
SDANN (ms)	77.9 ± 41.85	67 ± 23.86
SDNNI (ms)	170.4 ± 59.28	201.47 ± 83.10
rMSSD (ms)	241.5 ± 89.92	268.21 ± 134.76
pNN50 (%)	71.92 ± 12.16	71.80 ± 15.13
TP (ms^2)	10645.80 ± 3400.75	11286.11 ± 4162.69
HF (ms^2)	5048.00 ± 2478.93	6264.68 ± 3098.28
LF (ms^2)	1021.7 ± 341.39	1461.79 ± 772.18
VLF (ms^2)	965.63 ± 328.06	965.63 ± 328.06
LF/HF	0.33 ± 0.10	0.26 ± 0.12

Legend as in Table 1, *$p \leq 0.05$

The proposed reference values for the analyzed parameters, expressed as their means ± 95% confidence intervals, are presented in Table 5.

Discussion

The remaining morphological and biochemical blood parameters did not differ between dogs under 2 years of age, aged between 2 and 6 years, and older

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Table 4. Heart rate variability parameters in healthy dogs stratified according to their age.

Parameter	>2 years (n=10)	2-6 years (n=25)	<6 years (n=15)
Age (years)	1.77 ± 0.38	4.27 ± 0.83	7.85 ± 1.91
#QRS	5333.30 ± 1153.72	4873.37 ± 816.63	5835.75 ± 1185.98
mean R-R	666.76 ± 130.13	754.75 ± 109.38	618.37 ± 111.28
SDNN (ms)	221.92 ± 87.14#	213.37 ± 56.72#	183.12 ± 80.59#
SDANN (ms)	78.69 ± 36.94#	68.62 ± 21.67#	60.00 ± 27.64#
SDNNI (ms)	200.92 ± 86.48	196.62 ± 57.42	168.37 ± 79.10
rMSSD (ms)	275.38 ± 145.18	288.62 ± 80.56	202.75 ± 100.10
pNN50 (%)	71.35 ± 12.64	81.22 ± 7.23	63.26 ± 16.36
TP (ms^2)	12036.07 ± 4499.07	10528.12 ± 2358.46	10025.00 ± 4028.88
HF (ms^2)	6505.07 ± 3335.63#	5604.62 ± 2399.32#	5013.25 ± 2733.84#
LF (ms^2)	1652.92 ± 676.63	1069.00 ± 212.17	1687.00 ± 1021.35
VLF (ms^2)	1069.30 ± 379.47	864.87 ± 238.00	968.00 ± 309.88
LF/HF	0.29 ± 0.12	0.22 ± 0.11	0.34 ± 0.09

Legend as in Table 1, *p≤0.05, #statistical trend

Table 5. Proposed reference values for short-term (60-min) heart rate variability parameters in healthy dogs.

Parameter	Range (mean ± 95% confidence interval)
#QRS	4925-5765
mean NN	630-725
SDNN (ms)	180-238
SDANN (ms)	60-82
SDNNI (ms)	162-220
rMSSD (ms)	214-305
pNN50 (%)	66-77
TP (ms^2)	9600-12500
HF (ms^2)	4735-6950
LF (ms^2)	1220-1782
VLF (ms^2)	860-1100
LF/HF	0.24-0.33

Legend as in Table 1. Confidence intervals = mean ± (standard error*1,96).

(2001) showed that although the short-term reproducibility of heart rate variability analysis is satisfactory, heart rate reproducibility should also be considered. This is particularly important in the case of dogs whose heart rate varies considerably and is body weight-specific. Therefore, the ECG recordings for short-term HRV analyses should always be obtained under the same conditions, i.e. at rest. Although we did not find significant correlations between the HRV parameters and body weight, it should be remembered that our study included solely dogs <20 kg. The spectrum of breeds qualified for our study and/or the predefined 20-kg body weight limit were associated with the fact that dogs with such characteristics most often present with MVD, the most common cause for canine heart failure (Oliveira et al. 2012, Rasmussen et al. 2012). We observed an age-related increase in SDNN and SDANN and a decrease in HF. Although none of these relationships reached the threshold of statistical significance, this observation should be considered a clinically relevant finding as most dogs with MVD are usually older than 6 years. Human studies confirmed that time-domain HRV parameters, especially rMSSD and p50NN, decrease with age (Umetani et al. 1998, Voss et al. 2013a). The age-related decrease in both parameters followed a quadratic regression pattern, being less pronounced in older individuals. As the age-related decrease in the amplitude of the HF component was also documented in humans, the trend observed in healthy dogs seems to confirm the significant effect of aging on HRV parameters. Heart rate variability is an outcome of autonomic and humoral regulation of heart rate in response to various stimuli. Autonomic control of the heart in healthy dogs is predominantly influenced by a parasympathetic tone, which results in greater HRV than in dogs with heart failure, and vagal activity was shown to be the major contributor to the HF component in healthy dogs with a LF/HF ratio well below one (Manzo et al. 2009, Oliveira et al. 2012). As the values of the HF component vary depending on the respiratory rate, the ECG recordings for HRV analysis in dogs should always be obtained during normal rhythmic breathing. We did not document significant differences in the HRV parameters of males and females, which suggests that the same reference values for HRV parameters can be used irrespective of the dog's gender. Similar to studies in humans, we found a number of significant correlations between the values of time- and frequency-domain parameters (Bernardi et al. 2000). This likely reflects the large differences between successive beats of sinus rhythm in dogs. These, in turn, are mostly the consequence of respiratory variability, the major contributor to the HF component.

Abbreviations

HRV – heart rate variability
DCM – dilated cardiomyopathy
MVD – mitral valve degeneration

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AST – asparatate aminotranspherase
ALT – alanine aminotranspherase
EF – ejection fraction
SF – shortening fraction
LVIDd – left ventricular end-diastolic internal diameters
LVIDs – left ventricular end-systolic internal diameters
IVSd – interventricular septal diameters at diastole
IVSs – interventricular septal diameters at systole
LVPWd – left ventricular end-diastolic posterior wall diameters
LVPWs – left ventricular end-systolic posterior wall diameters
SDNN – standard deviation of all R-R intervals,
SDANN – standard deviation of the averaged R-R intervals for all 5-min segments,
SDNNI – mean of the standard deviations of all R-R intervals for all 5-min segments
pNN50 – the percentage of successive R-R intervals >50 ms
rMSSD – root-mean-square of successive R-R interval difference
TP – total spectral power
HF – high frequency (0.15-0.4 Hz, HF),
LF – low frequency (0.04-0.15 Hz, LF),
VLF – very low frequency (< 0.04 Hz, VLF)

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