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Short-term heart-rate variability in healthy small and medium-sized dogs over a five-minute measuring period

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Abstract

Introduction: Five-minute heart-rate variability (HRV) measurement is a useful tool for assessing the autonomic nervous system (ANS) balance in humans, but there are no studies on healthy dogs. The aim of the study was, therefore, to provide the reference ranges in small and medium-sized breeds for short-term HRV time and frequency domain (TFD) analyses. **Material and Methods:** A total of 79 healthy dogs were included in the study between 2015 and 2019. Grouping by age with the breakpoint at six years and subgrouping by reproductive status and sex was imposed. All the dogs were included after physical and cardiological examinations and blood analyses. The TFD of HRV were analysed from a five-minute-long digital ECG recording after removal of non-sinus complexes. **Results:** There were no statistically significant differences in any TFD parameters between age, reproductive status or sex groups. A mild increase in all time domain parameters and the high-frequency (HF) band was observed in older dogs, and the low frequency (LF):HF ratio decreased in these dogs. In males, the time domain parameters and HF band increased slightly. **Conclusion:** The normal ranges for HRV derived from short-term ECG recording in the usual clinical environment now have proposed reference ranges. Our findings suggest that accommodation time, age, sex, or reproductive status do not influence the results derived from these recordings, indicating that this method is reliable for assessing the ANS function in small and medium-sized dog breeds.

Keywords: dog, heart-rate variability, frequency domain, time domain.

Introduction

Heart-rate variability (HRV) represents the oscillations in interval between successive normal heart beats as a result of autonomic nervous system (ANS) activity (5). This is a practicable and non-invasive method for measuring ANS balance and has been proven to be simple and effective (37). The ANS plays an important role in the regulation of physiological and patho-physiological processes. Several studies have assessed its modulation through HRV analyses in dogs with different pathologies such as mitral valve degeneration, sick sinus syndrome, epilepsy, upper airway obstruction, or diabetes (4, 15, 20–22, 24, 36). HRV may be evaluated through the time and frequency domains (TFD). The time domain includes the standard deviation of the normal-to-normal intervals (hereinafter SDNN) over the entire recording period in ms, representing the sympathetic and parasympathetic activity, without distinguishing between pronounced sympathetic activity and vagal tone withdrawal; it also includes the difference between consecutive R-wave-to-R-wave (R–R) intervals, which includes the percentage of successive R–R intervals >50 ms (hereinafter pNN50); and it includes the root-mean-square of successive R–R interval differences (hereinafter rMSSD) in ms representing parasympathetic activity (5). The frequency domain contains the low frequency (LF) band (0.04–0.15 Hz), reflecting the sympathetic tone in contrast to the vagal tone effect on cardiac function; it also has the high frequency (HF) band (0.15–0.4 Hz), which reflects the vagal tone variability associated with the respiratory cycle and with changes in blood pressure; and this domain also takes in the LF/HF ratio, representing the absolute and relative changes between the sympathetic and parasympathetic tones (37). In human and some animal studies, HRV is analysed from a 24-h Holter

recording to assess the autonomic nervous system balance in an accustomed environment and to capture the changes through the entire circadian rhythm (2, 21). However, recent studies reported that short-term and ultra-short-term recordings may also give useful heart-rate variability data (17, 26). Human studies have evaluated HRV parameters from five-minute recordings in both normal and diseased patients (6, 14, 16, 27, 28). To the authors' knowledge, there are no published norms to bracket short-term (5 min) recording HRV analyses in normal small and medium-sized dog breeds. The aim of the study was, therefore, to provide the reference ranges in dogs for short-term (5 min) HRV analyses derived from electrocardiography (ECG) recording, expressed through the time and frequency domains.

Material and Methods

This retrospective study included healthy client-owned dogs referred to the Cardiology Service of the Veterinary Teaching Hospital between January 2015 and July 2019 for complete cardiological examination for different reasons such as pre-anaesthesia, routine, or cardiological and reproduction-related checkups. Following the example of a previous study reporting normal ranges of HRV in dogs for one-hour ECG recordings (3), the dogs in the present study were divided into two age groups, one of animals ≤ 6 years and the other of those > 6 years. The study included 79 dogs aged between 1 and 15 years with body weights between 2 and 21 kg, among which 39 (49.4%) were males and 40 (50.6%) were females. The intact animals numbered 47 and 32 dogs were neutered. The breeds were bichon frise (n = 23), mixed breed and Cavalier King Charles (n = 9), poodle (n = 7), Yorkshire terrier and Shih-Tzu (n = 6), beagle (n = 4), dachshund (n = 3), pug, pinscher and West Highland terrier (n = 2), and American Staffordshire, French bulldog, small schnauzer, field spaniel, fox terrier and Chihuahua (n = 1). All dogs included in the study were subjected to a complete cardiological examination consisting of history, physical examination, at least five-minute six-lead electrocardiography (PolySpectrum 8E/8V, Neurosoft, Ivanovo, Russia), blood pressure measurement (Vet HDO oscillometric device, S+B medVET, Babenhausen, Germany), thoracic radiography in at least one lateral view, and echocardiography (Logiq V5 equipped with a 4–7 MHz phased-array, GE Medical Systems, Wuxi, China), as previously described (32, 33). Echocardiography was comprehensive. It included the measurement of the left ventricular internal diameters end diastole and end systole (LVIDd and LVIDs) with the values indexed to body weight (LVIDd-n and LVIDs-n) (8). Echocardiography also took in the measurement of the left atrial dimension on the short axis with this scaled to aortic diameter (La/Ao). In addition, it encompassed evaluation of the valves and quantification of the trans-mitral, pulmonary and aortic flows with spectral Doppler. Only dogs indicated as being healthy based on the physical and cardiological examinations and with normal cell blood count and liver and renal enzyme concentrations were included in the study. Dogs with acquired or congenital cardiac pathologies and dogs with signs of any systemic pathology based on physical examination, as well as dogs receiving any pharmacotherapy for any medical condition were excluded from the study.

The electrocardiography recording was performed in the same manner for all patients, with the only variation being that it was taken in the morning in 46 cases and in the afternoon in the remainder. Dogs were accommodated for 10 to 15 min in a quiet room along with their owner. After the accommodation time, all dogs had a normal respiratory rate, which should be and was below 30 breaths per minute. Ten dogs were randomly selected and subjected to three consecutive electrocardiographic recordings at T15 after 15 min of accommodation, T30 at the 30 min point from the end of the first recording, and T60 at 60 elapsed minutes from the end of the first recording, in order to ensure the stability of the HRV parameters between different accommodation periods. The selected dogs were kept in the accommodation room for the entire period until the last recording was finished.

After the accommodation time, the patients were gently restrained in right lateral recumbence with the owner present and alligator electrodes were attached to the forelimbs and hindlimbs as previously recommended (34). All electrocardiographic tracings were recorded for at least five minutes and stored in dedicated digital software (Neurosoft v. 4.8.131). The electrocardiographic tracings were then manually edited in lead II by a single operator at a speed of 200 mm/sec and amplitude of 40 mm/mV. All non-sinus complexes or 2nd degree AV blocks were excluded from the HRV analyses along with their two flanking (previous and subsequent) sinus complexes. The R–R intervals were then transformed using an ASCII text file and uploaded in dedicated software (Kubios HRV v.2.1, Kubios, Kuopio, Finland) for further HRV analyses. The time of each ECG was recorded by the software and was assigned as morning (before 1 pm) or afternoon (after 1 pm).

Heart-rate variability was assessed using the time and frequency domain analyses using a sampling rate of 256 s with a 50% window overlap (30). Heart-rate variability was analysed using the time and frequency domains. Time domain was assessed through variability of R–R intervals, which included SDNN (ms), pNN50 (%) and rMSSD (ms). The frequency domain was calculated through the fast Fourier transform (FFT) and included total power (TP), very low frequency (VLF), LF, and HF band (HF) power expressed as units of spectral power (ms^2), and the LF/HF ratio. Further analyses were performed on the

group of dogs in aggregate and dogs divided by age, sex, and reproductive status.

Statistical analysis was performed using SPSS v.17 software (IBM, Armonk, NY, USA). Data was tested for normality using the Shapiro–Wilk test. Normally distributed data were expressed as mean ± SD, and non-normally distributed data were expressed as a median and an inter-quartile (IQR) interval. A one-way ANOVA test was used to assess the differences between groups for normally distributed data, and the Kruskal–Wallis test was used to analyse the non-normally distributed data for comparison between groups. Analyses of relative proportions between groups were performed using the Pearson chi-squared test and Fisher's exact test, and correlations between variables were assessed with Pearson's test. A $P < 0.05$ was considered statistically significant for all tests.

Results

There was no statistically significant difference in any of the HRV measurements among different accommodation times in the clinical environment ($p > 0.05$). Also, there was no statistical difference in HRV parameters between morning and afternoon recordings ($p > 0.05$). Clinical characteristics and HRV values for the entire study group as well as the 95% confidence interval are represented in Table 1.

There were no statistical differences between different age groups in dogs regarding the time and frequency domain analyses. However, we observed a mild increase in all time domain parameters and a lower heart rate in dogs older than six years of age. Frequency domain analyses showed an increase in both TP and the HF band expressed as ms^2 in dogs in this age group. The LF/HF ratio dropped gently in the older dogs. The clinical characteristics and TFD values of heart-rate variability in dogs grouped according to age are shown in Table 2.

Heart-rate variability comparative analyses were performed between the sexes. There was no statistical difference in body weight, age, or any of the HRV measurements between the two groups. In males, the SDNN, rMSSD, and the pNN50 were higher to a small extent, while the mean heart rate was lower compared to females. Also, both the LF and HF band values expressed as units of spectral power (ms^2) were higher in the male group. There were no statistically significant differences in heart-rate variability results between intact and neutered dogs ($p > 0.05$). The clinical characteristics and values of heart-rate variability analyses in groups distributed by sex are shown in Table 3.

There were no significant correlations between any of the heart-rate variability measurements and age or body weight when the entire group was analysed, or when the dogs were divided according to age in dogs under and above six years. Also, when dogs were divided by sex, no significant correlations between HRV parameters and body weight were observed.

Table 1. Clinical characteristics and values of time and frequency domains for the total study group and 95% confidence interval expressed as mean ± SD for normally distributed data and median (IQR) for non-normally distributed data

HRV	Total (n = 79)	95% CI
Age	7 ± 3.7	6–7.73
BW (kg)	8.42 ± 4.45	7.4–9.4
Sex (M/F)	39/40	-
Mean RR	477 (404–576)	471–519
Mean HR	124 (103–146)	120–132
SDNN	61.9 (38.5–87.8)	60–77.1
rMSSD	56.6 (33.2–121)	69.9–102.5
pNN50	27.6 (9.44–60.38)	29.2–41.7
TP (ms^2)	1,898 (947–3,641)	2,037–3,851
VLF (ms^2)	164 (70.2–517.7)	274–618
LF (ms^2)	652 (457–1,116)	714–1,113
HF (ms^2)	635 (284–1,952)	932–2,385
LF/HF	0.99 (0.48–1.95)	1.07–1.68

BW – body weight; mean RR – mean value of the R–R intervals; mean HR – mean value of the heart rate; SDNN – standard deviation of all R–R intervals in 5-min recording; rMSSD – root-mean-square of successive R–R interval difference; pNN50 (%) – the percentage of successive R– R intervals >50 ms; TP – total power; VLF – very-low-frequency component; HF – high-frequency component; LF – low-frequency component; LF/HF – high to low frequency ratio

Table 2. Clinical characteristics and values of time and frequency domains in dogs grouped by age as under and over six years of age, expressed as mean ± SD for normally distributed data and median (IQR) for non-normally distributed data

HRV	Dogs ≤ 6 years (n = 41)	Dogs > 6 years (n = 37)
Age (years)	4 ± 1.55	10.1 ± 2.64
BW (kg)	8.06 ± 4.9	8.82 ± 3.87
Sex (M/F)	22/19	17/21
Mean RR	475 (394–568)	488 (416–602)
Mean HR	130 (108–154)	122 (101–142)
SDNN	60.3 (38.4–84.9)	65.9 (37.7–87.8)
rMSSD	54.2 (32.2–120)	67 (31.6–124.1)
pNN50	26.7 (8.9–62.6)	29.6 (9.3–61.2)
TP (ms^2)	1,885 (971–3,752)	2,029 (917–3,350)
VLF (ms^2)	186 (94.5–627)	155 (52.5–284.5)
LF (ms^2)	653 (523–1,111)	586 (420–1,137)
HF (ms^2)	513 (256–1,858)	865 (338.5–2,034.5)
LF/HF	1.1 (0.62–2)	0.96 (0.4–1.7)

Legend as in Table 1

Table 3. Clinical characteristics and values of time and frequency domains for different groups distributed by sex, expressed as mean ± SD for normally distributed data and median (IQR) for non-normally distributed data

HRV	Males (n = 39)	Females (n = 40)
Age (years)	7.06 ± 3.9	6.89 ± 4.1
BW (kg)	8.9 ± 4.8	7.9 ± 3.6
Mean RR	513 (413–599)	449 (389–527)
Mean HR	117 (103–139)	132 (108–151)
SDNN	65.9 (38.1–103.5)	58 (38.7–82.1)
RMSSD	73 (29.9–142.8)	52.6 (34.4–116.1)
pNN50	36.2 (8–69.5)	25.2 (10.5–55.8)
TP (ms^2)	2,430 (966–4,441)	1,647 (940–2,483)
VLF (ms^2)	164 (73–526)	165 (62–437)
LF (ms^2)	657 (486–1,108)	609 (420–1,142)
HF (ms^2)	865 (271–2,980)	575 (306–1,235)
LF/HF	0.96 (0.3–1.7)	1.1 (0.6–2)

Legend as in Table 1

Discussion

This retrospective study proposes the reference ranges of heart-rate variability for both time and frequency domains obtained from five-minute electrocardiographic recordings in the clinical environment in small and medium-sized dogs. Several studies assessing HRV in dogs with different pathologies have used small groups of healthy dogs for comparative analysis of heart-rate variability data over 5 min, 60 min, and 24 h (4, 20, 21, 23, 38). One study assessed the effect of classical music on selected measurements of HRV in healthy dogs in selected five-minute intervals from one-hour recordings (13). To date, only one study proposed reference ranges for the time and frequency domains from 60-min recordings in healthy dogs (3). However, the Task Force of The European Society of Cardiology and the North American Society of Pacing and Electrophysiology recommended that the frequency domain should be preferred over the time domain when short-time HRV analyses are performed without excluding any of the time domain parameters (17). A more recent study published accepted norms for short-term heart-rate variability analyses, showing that most of the parameters can be analysed suitably from a five-minute recording (26). Moreover, one study compared the results of HRV from 24-h and five-minute recordings in workers (19). The study concluded that HRV components of a five-minute recording appear to

remain stable through the longer period, although none of the correlations were strong. In the present study we sought to evaluate and provide reference ranges for a shorter recording time, for clinical use where Holter monitoring might not be widely available. Holter monitoring requires more time for installation, and the method's analysis is time-consuming. Also, another difficulty is presented by the large amount of artefacts on the recording which interfere with the HRV analysis. Studies analysing HRV over a 24-h recording period are recommended because it has been demonstrated that results are influenced by the circadian rhythm, with higher values of LF in the daytime and higher values of HF at night (17). Our goal was to analyse HRV during the daytime while the dog is awake, in the clinical environment over a clinically practicable and relevant recording period. This study demonstrated that during the daytime, the moment of recording does not influence the HRV results.

Our study did not show any influence on any of the HRV parameters of different accommodation periods before ECG recording. Martlé *et al.* (18) removed the first ten-minute ECG trace from each recording to avoid stress and manipulation interference in a study assessing the HRV during vagus nerve stimulation in dogs. Our results support the previous proposed accommodation time, suggesting that a period of 10 to 15 minutes is sufficient for the patient to adapt to the clinical environment.

In the present study, we did not find significant differences between different age groups nor between the sexes or different reproductive statuses. One study assessing the reference ranges for HRV over 60 min recordings found an age-related decrease in SDNN, standard deviation of the averaged R–R intervals for all 5-min segments (SDANN), and the HF band (3). In contrast, our results showed an increase in time-domain parameters such as SDNN, rMSSD, and pNN50 in dogs past their sixth year, which did not reach the threshold of statistical significance; however, the 95% confidence interval of SDNN for the entire study group was similar in both studies. The SDNN measurement represents the sympathetic and parasympathetic activity, without distinguishing between a pronounced sympathetic activity or vagal tone withdrawal. The difference in the values obtained in our study compared to the previous reported reference ranges may be due to the difference in recording length (3, 37). Regarding the frequency domain, in the present study, the high frequency band expressed as units of spectral power rose with age. A study assessing the HRV of healthy individuals with different ages did not find any age-related HRV changes (29). The conclusion was, therefore, that the age-associated decline in the cardiac vagal tone suggested in several studies (3, 11) may reflect a general decline in wellbeing rather than age specifically (29). Our results assessed from short-term HRV in a clinical environment in healthy dogs suggest that age does not induce significant changes in the autonomic nervous system balance.

Another study assessing the effect of sildenafil therapy for the evaluation of the time domain in dogs with different stages of mitral valve disease showed similar results for the time domain parameters such as SDNN and pNN50 in the healthy dog group (23). One study performed on dogs with idiopathic epilepsy also revealed similar results to those from our study regarding both time and frequency domains for the control group (20). Results from the present study revealed a mild increase in heart rate in females compared to males. Also, we found higher values of SDNN, rMSSD, and pNN50 in the male group, without the differences reaching the threshold of statistical significance. One study assessing the HRV from 24-h recordings in dogs before and after supplementation with omega 3 found a mild increase in both SDNN and SDANN in males; however, rMSSD was noted at higher values in females (31). Similarly to our results, Christensen *et al.* (7) found higher values of time domain parameters such as SDNN, rMSSD, and pNN50 in healthy men than in women. Also, another study assessing the HRV from a five-minute recording in 13,214 humans found a decrease in heart rate and an increase in SDNN and LF/HF in men compared to women (10). These measurements might have been influenced by the heart rate during the recording period and also may suggest sex-dependent autonomic nervous system activity; however, some of these mechanisms are not yet completely understood (35). It has been shown that an uptick in heart rate caused by reducing or abolishing the parasympathetic regulation provoked a large fall in HRV parameters (1). Furthermore, the high frequency, which represents the vagal tone variability associated with the respiratory cycle and with changes in blood pressure, was moderately higher in the male group. Several studies assessing the HRV–sex dependence in humans obtained similar results to those of the present study (9, 12, 35). Our findings suggest that the parasympathetic function, which is closely correlated with pNN50 and rMSSD, is at lower levels in females than in males (35), a mechanism supported by the higher values of the HF band in the male group. However, none of these differences in results was sufficient for statistical significance. In our study, the time domain parameters obtained from five-minute ECG recordings were, in general, lower than the same parameters from 24-h data collection published in other studies (21, 31). Regarding the frequency domain parameters, our study showed lower values in LF, HF, and TP compared to published results of 24-h recordings (21). These differences may be the result of long-term adaptations of the autonomic nervous system, because a substantial part of the long-term HRV is contributed by the day–night differences (5). Regarding these findings, reference ranges on specific recording time are absolutely necessary.

The limitation of this study is the small number of dogs included. It is well known that HRV may be influenced by many factors, including environmental stimuli and stress during the presence in the clinic. Also, heart-rate variability may be influenced by breathing. In our study, we did not simultaneously record the breathing rhythm, yet all dogs had a respiratory rate below 30 breaths per minute after the accommodation period, which is considered normal (25). Shaffer *et al.* (26) stated that short-term HRV measurements are appropriate when breathing is at normal rates. The authors consider that these results accurately express the physiological state of the ANS during a dog's time in the clinical environment.

Animal patients will not behave perfectly cooperatively throughout their whole time in the clinical environment, therefore it is needful that the recording period for HRV analysis is short. Our data suggest that heart-rate variability derived from short-term (five-minute) electrocardiographic recording in the usual clinical environment is a reliable method for assessing the autonomic nervous system function in small and medium-sized dog breeds. Moreover, the time of day of ECG recording, accommodation period, age, sex, or reproductive status do not influence the heart-rate variability results. Further studies on larger populations are warranted to determine the implications of other factors on the time or frequency domain parameters.

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