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# Ambulatory electrocardiographic evaluation of overt cardiomyopathies in dogs

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#### **Abstract**

Holter monitoring has emerged as a reliable diagnostic tool in veterinary cardiology for identifying and analysing cardiac diseases associated with varying stages of abnormal electrophysiological activity. In conjunction with echocardiography, it is currently considered as the gold standard for diagnosing dilated cardiomyopathy (DCM) in dogs. This study aimed to evaluate heart rate, electrocardiographic pauses, heart rate variability and arrhythmias associated with overt phase of DCM in dogs. A total of eight dogs diagnosed with overt DCM underwent 24-hour Holter monitoring, following established protocols. The collected Holter data were analysed statistically, and ECG waveforms were manually reviewed to assess different arrhythmias. Ventricular premature complexes were the most common arrhythmia observed (87.50 per cent), followed by atrial fibrillation which may indicate advanced stages of cardiovascular compromise. Significantly elevated minimum and average heart rates, along with reduced electrocardiographic pause beats and pause times suggested cardiac compensation and sympathetic overstimulation. Among heart rate variability (HRV) parameters, time-domain measures such as SDNN, SDANN, and pNN50, indicative of sympathetic activity and compromised cardiovascular health were notably altered. Furthermore, frequency-domain HRV parameters, including VLF and LF, which reflect baroreceptor-mediated sympathetic stimulation, were significantly reduced in dogs with overt DCM. In conclusion, Holter monitoring serves as both a vital diagnostic tool for identifying predominant arrhythmias and assessing autonomic imbalances, and as a prognostic test to determine the severity and stage of overt DCM in dogs.

Keywords: Holter monitoring, cardiomyopathy, ventricular premature complex, heart rate variability

The concept of cardiac electrophysiology began with the demonstration of changing chest potentials by Ludwig and Waller in 1887 (Goodwin, 1998). However, it was actually the discovery of electrocardiography (ECG) by the Dutch physiologist Willem Einthoven in 1902 that revolutionised the diagnosis of cardiac diseases in humans (Fye, 1994). While the clinical use of ECG advanced very rapidly in humans since the early 1900s, its application in dogs remained very modest until 1950s (Detweiler, 2010). It was in 1957, Dr. Norman J. Holter, known as the "father of ambulatory electrocardiography developed an "electro-cardiorecorder" which was later known as Holter electrocardiograph (Holter, 1961). Holter's electrocardiorecorder underwent numerous advancements over time, such that the latest Holter monitor

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had the size of a cell phone or even like a wearable patch weighing about 15 g and could record good quality traces for up to 14 days (Kennedy, 2013 and Schreiber et al., 2023). Holter monitoring is now being used in veterinary medicine for detecting intermittent arrhythmias, establishing correlation of arrhythmias with clinical signs, assessing the need and efficacy of antiarrhythmic treatment and for the screening of occult cardiomyopathy. A Holter monitoring setup constitute a Holter ECG monitor, lead wires, electrodes, recording tape, battery and a vest to secure the Holter machine and electrodes to the animal body (Petrie, 2005).

Dilated cardiomyopathy (DCM) is defined as a heart muscle disease of unknown etiology marked by the progressive dilation of ventricles with loss of contractile power of myocardium (Oyama, 2016), in the absence of coronary artery disease, hypertension, valvular heart disease and congenital heart disease sufficient to cause the observed myocardial abnormality (Alvarez and Tang, 2017). The condition had a prolonged asymptomatic phase denoted as occult DCM which might extend up to 2 to 4 years without clinical signs (Estrada and Maisenbacher, 2014). The standard 12-lead intra-hospital ECG has been shown to be neither sensitive nor specific for identifying dogs predisposed to develop DCM or those with asymptomatic disease, and may erroneously interpret cardiac pathology (Calvert and Wall, 2001). In addition, a normal resting ECG could not rule out the presence of cardiomyopathy in dogs (Oyama, 2016). Currently, the gold standard approach for diagnosing DCM in dogs relied on 24-hour Holter electrocardiographic and echocardiographic assessments in conjunction with monitoring clinical presentation and signalment (Simpson et al., 2021).

Several cardiac parameters can be monitored using Holter ECG, with heart rate variability (HRV) being one of the most significant. Heart rate variability is denoted as a quantitative marker of autonomic activity of the heart which could measure the heart rate (HR) fluctuations according to sympathetic-parasympathetic oscillations in different time intervals between individual heart beats. It was measured as the periodical changes in the duration of intervals between consecutive QRS complexes during a sinus rhythm (Harada *et al.*, 2005; Bogucki and Noszczyk-Nowak, 2015). In normal heart, HRV allowed the heart to respond to pressure changes inside, over a short or prolonged period of time. That is how the heart and blood

vessels responded to these changes in a meticulous and synchronised path, maintaining haemodynamic stability of the cardiovascular system. These adaptations to changes or HRV decreased with an increase in myocardial damage (Grutter *et al.*, 2012; Valencia *et al.*, 2015).

The research data pertaining to the analysis of ambulatory electrocardiographic data in canine DCM remains very limited. Hence, the present study was carried out to identify and characterise major arrhythmias, and to evaluate the parameters like heart rate, electrocardiographic pause and heart rate variability in dogs with overt stages of DCM.

#### Materials and methods

#### Selection of animals

Dogs, which were presented to the Teaching Veterinary Clinical Complex at Mannuthy or University Veterinary Hospital, Kokkalai with clinical signs and symptoms suggestive of overt stage of dilated cardiomyopathy like lethargy, respiratory distress, exercise intolerance, nocturnal cough, ascites and syncope were subjected to detailed trans-thoracic echocardiography to assess cardiac pathology. A total of eight dogs which were diagnosed with DCM as per the guidelines for diagnosing idiopathic DCM issued by the European Society of Veterinary Cardiology as per Dukes-McEwan et al. (2003) were selected for the study. Eight apparently healthy dogs with normal values for standard 12-lead ECG, cardiac markers like creatine kinase-MB, N terminal proBNP and echocardiography were subjected to 24-hour Holter ECG for obtaining the normal values.

## Instrumentation

A three lead digital Holter dynamic ECG analyser and recorder system (TLC 9803; Dimensions: 11 cm (Length) x 6 cm (Width) x 2.5 cm (Height) and weighing 105g without batteries) from Contec Medical Systems Co., Ltd., China was used to record and analyse ECG waveforms from dogs for a continuous period of 24-hours, using a PC software (3 Channels ECG Holter System\_TF (OS) (V5.5.2.5)) compatible with the machine. The machine was powered during the entire recording time using two, AA Alkaline batteries (Duracell Ultra, 1.5V and weighing 23g/cell).

Table 1. Chest lead attachments in Holter monitoring

SI. No.	Name of the electrode	Site on the thorax
1	V1	Right of the sternum at the 5 <sup>th</sup> intercostal space
2	V3	Left side, midway between sternum and costo-chondral junction at 6th intercostal space
3	V5	Left side, halfway between the spine and the sternum at 6th intercostal space
4	V Negative	Right side, halfway between the spine and the sternum at 5th intercostal space
5	Neutral	Dorsal side, in between the two shoulder blades (a slight lateral position towards right was also employed if frequent displacement of the electrodes happened)

#### **Procedure**

Five rectangular spots of five cm (length) x four cm (width) dimensions were prepared over the lateral thorax according to Kraus *et al.* (2002) and Anon (2021) (Table 1), using clipper and blade, sterilised with 70 per cent ethyl alcohol and left to dry off.

Disposable solid gel ECG electrodes (VIMED, Cardioart, India) were attached to the shaved spots by pressure. The corresponding, colour coded ECG lead wires were connected to the respective ECG electrode patches by pressing the lead wire tip against the patches and locked by a click mechanism. The electrodes and lead wires were secured tightly to the body of the dog by wrapping those using a specially fabricated and sized Holter vest manufactured by Heartvet, UK. The lead wires drawn forward were inserted through a hole on the vest towards the dorsal part of the body where the vest has a pocket for inserting the Holter ECG machine.

All five lead wire cables were then inserted into the respective cable ports on the machine and the machine was turned on by pressing the power key continuously for three seconds and new recordings were initiated by selecting the new record option in the machine interface and pressing the function key for affirmance. The machine was then placed in the protective pouch after noting the time and inserted in to the pocket in the vest. After finishing the placement of the machine, animal was allowed to carry out normal daily routines and activities.

Once the recording time was completed, the machine was taken out from the pouch and recording was terminated by long pressing on the down arrow button on the machine. The ECG waveforms that were recorded from the dog was analysed using the computer software after connecting the recorder to the PC using an USB cable.

The Holter data from each animal was analysed statistically for following parameters: heart rate (including minimum, maximum and average), pause (maximum pause time in msec and number of pause beats with a duration of more than one second) and heart rate variability (timedomain parameters like standard deviation of normal-to-normal (R-R) intervals (SDNN), standard deviation of averaged normal-to-normal intervals (SDANN), the percentage of successive normal-to-normal intervals >50 msec (pNN50) and root-mean-square of successive R-R interval difference (rMSSD), and frequency-domain parameters like ultra-low frequency (ULF), very-low frequency (VLF), low frequency (LF) and high frequency (HF)).

The parameters of various observations in the present study were analysed statistically using SPSS Version 24.0 and comparison between dogs with overt DCM and healthy control was carried out using independent t test. The abnormal waveforms and arrhythmias recorded

during 24-hour Holter ECG readings were manually analysed and quantified.

#### Results and discussion

#### Heart rate

The different parameters of heart rates in dogs with overt DCM and healthy controls are presented in Table 2.

Both minimum and average heart rates of dogs with overt DCM (97.25  $\pm$  10.23/min and 132.50  $\pm$  12.38/min respectively) demonstrated significant increase (p<0.01) than those values in healthy control (44.50  $\pm$  2.35/min and  $71.50 \pm 3.95$ /min respectively). Abnormally elevated heart rates in dogs with overt DCM occur possibly from multiple cardiac compensation mechanisms of a failing heart for restoring the cardiac output. These include activation of sympathetic nervous system, baroreceptor mediated reflex, renin-angiotensin-aldosterone system (RAAS) and reflex response to hypo-perfusion induced hypoxia and acidosis (Scollan and Sisson, 2017). Uechi et al. (2002), demonstrated a pathological rise in sympathetic hormonal activity responsible for the abnormally elevated heart rates in dogs with naturally acquired heart failure. Increase in the heart rate in overt DCM has been attributed to sympathetic activation aimed at restoring falling cardiac output (Stern and Meurs, 2017).

## Electrocardiographic pause

The electrocardiographic term 'pause' refers to temporary absence of cardiac electrical activity and characterised by prolonged R-R interval in ECGs representing an interruption in ventricular depolarisation (Viljoen et al., 2017). The present study defined pause beats as those beats with an R-R interval of more than one sec duration. Mean  $\pm$  S.E values for number of pause beats of more than one sec duration (beats per 24-hour) and maximum pause time (msec) for healthy and overt DCM dogs are presented in Table 3.

Overt DCM group showed a significant decrease in number of pause beats of more than one sec duration and maximum pause time (3167.25±1951.50 and 1913.75±318.85 msec respectively) when compared to that of healthy controls (20585.50 $\pm$ 3500.24 and 2964.38  $\pm$ 314.17 msec respectively). In normal dogs, it is considered normal to have sinus pauses for up to five seconds, HR less than 17 beats per min and presence of second degree AV block in a sleeping dog (Petrie, 2005). Cardiac pathologies affecting contractility typically decrease electrocardiographic pause times and R-R intervals. The strong negative correlation established between heart rate, electrocardiographic pause and the number of pause beats as per Kazmi et al. (2016) help to explain the lower values for pause beats and pause times in the overt DCM group, which exhibited elevated heart rates.

# Heart rate variability (HRV)

Heart rate variability denotes a set of parameters analysing variations in duration between consecutive heart beats to obtain information about different physiological and pathological mechanisms affecting HR (García et al., 2024). It demonstrates a shift from parasympathetic predominance in normal hearts to sympathetic overstimulation in DCM hearts, as indicated by the decreased values of HRV parameters, which are typically higher under parasympathetic predominance. Time domain parameters are calculated directly from the R-R interval times using basic statistical measures, such as the mean, standard deviation and percentage. Frequency domain parameters are derived from time domain parameters using mathematical algorithms, such as the Fast Fourier Transform (FFT), which convert the time-domain data into the frequency domain across a range of frequencies, from below 0.003 Hz in the ULF band to 0.4 Hz in the HF band (Marek et al., 1996).

Details of HRV parameters between dogs with overt DCM and healthy controls during 24-hour Holter monitoring are presented in Table 4.

Values for the time domain heart variability parameters like SDNN and SDANN (90.71  $\pm$  10.71 msec and 67.78  $\pm$  9.82 msec respectively) and frequency domain parameters like VLF and LF (1317.44 $\pm$ 419.60 msec² and 437.28 $\pm$ 105.89 msec² respectively) showed a significant decrease (p<0.01) in dogs with overt DCM when compared to that in healthy dogs. Also, the time domain parameter pNN50 (27.04  $\pm$  6.94 per cent) showed a significant decrease (p<0.05) in overt DCM than that from healthy dogs.

The values for HRV parameters showed a decreasing trend whenever myocardial damage is present. Kazmi *et al.* (2016) established a strong negative correlation between heart rate and HRV parameters in both human and animal subjects. Similarly, Billman (2013) reported a significant inverse relationship between heart rate, R-R interval variability and HRV parameters. The

reduction in HRV indices observed in dogs with overt DCM might be attributed to their elevated heart rate and impaired autonomic control over the heart.

Both SDNN and SDANN were employed to assess long-term components of HRV, such as the circadian rhythm (Sztajzel, 2004). The HRV parameter, SDANN is considered as a more refined and less error-prone version of SDNN, as it included averages over each 5-minute interval (Kleiger et al., 2005). Reduced values typically reflect a predominance of sympathetic activity. The lower values observed in the present study align with findings by Spier and Meurs (2004), who reported reduced values in dogs with cardiomyopathy. In human medicine, SDNN values between 50-100 msec are indicative of compromised cardiovascular health (Kleiger et al., 1987). While SDANN may not provide additional information beyond SDNN, it could serve as an earlier indicator of poor autonomic balance in canine DCM. The time domain parameter, pNN50 is related to the parasympathetic regulation over heart (Fernandes and Seara, 2021) and reduced values in dogs with overt DCM indicate reduced parasympathetic activity in DCM hearts.

The VLF rhythm was believed to originate from the cardiac afferent neurons, reflecting both sympathetic and parasympathetic activity and could be influenced by baroreceptor mechanisms (Shaffer and Ginsberg, 2017). In contrast, the LF parameter was more strongly associated with baroreceptor-mediated sympathetic stimulation of the heart and was considered as a reliable marker for sympathetic modulation (Piccirillo *et al.*, 2009). The observed decrease in both VLF and LF in the current study agrees with the reports of Calvert and Wall (2001) and Oliveira *et al.* (2014) and could be due to the sympathetic stimulation of the failing heart in overt DCM.

#### Number of ventricular premature complexes (VPCs)

Number of VPCs obtained in overt DCM dogs and healthy controls are presented in Table 5.

Mean  $\pm$  S.E value for number of VPCs/24-hours obtained in dogs with overt cardiomyopathy

Table 2. Comparison of heart rate parameters between overt cardiomyopathy and healthy control

Parameters	Healthy (n=8)	DCM (n=8)	t-value	p-value
Min. heart rate (/min)	44.50 ± 2.35	97.25 ± 10.23	5.0273**	< 0.001
Max. heart rate (/min)	192.88 ± 8.62	208.00 ±14.27	0.907 <sup>ns</sup>	0.190
Avg. heart rate (/min)	71.50 ± 3.95	132.50 ±12.38	4.696**	< 0.001

<sup>\*\*</sup> Significant at 0.01 level; ns: non-significant

Table 3. Comparison of parameters for electrocardiographic pause between overt cardiomyopathy and healthy control

Parameters	Healthy (n=8)	DCM (n=8)	t-value	p-value
Pause beats (> 1 sec)	20585.50±3500.24	3167.25±1951.50	4.346 <sup>**</sup>	< 0.001
Max. pause time (msec)	2964.38 ± 314.17	1913.75±318.85	2.347 <sup>*</sup>	0.017

<sup>\*\*</sup> Significant at 0.01 level; \* Significant at 0.05 level

Table 4. Comparison of heart rate variability parameters between overt cardiomyopathy and healthy control

Parameters	Healthy (n=8)	DCM (n=8)	t-value	p-value
SDNN (msec)	162.15 ± 8.41	90.71 ± 10.71	5.247**	< 0.001
SDANN (msec)	129.05± 12.63	67.78 ± 9.82	3.829**	0.001
rMSSD (msec)	123.29 ± 19.57	77.02 ± 13.86	1.929 <sup>ns</sup>	0.037
pNN50 (%)	42.95 ± 2.68	27.04 ± 6.94	2.137 <sup>*</sup>	0.025
ULF (msec²)	2747.73 ± 516.25	1601.48 ± 767.11	1.240 <sup>ns</sup>	0.118
VLF (msec²)	5052.44±252.47	1317.44±419.60	7.627**	< 0.001
LF (msec²)	1550.84±195.98\	437.28±105.89	4.999**	< 0.001
HF (msec <sup>2</sup> )	1766.45±590.30	728.49±232.50	1.636 ns	0.062

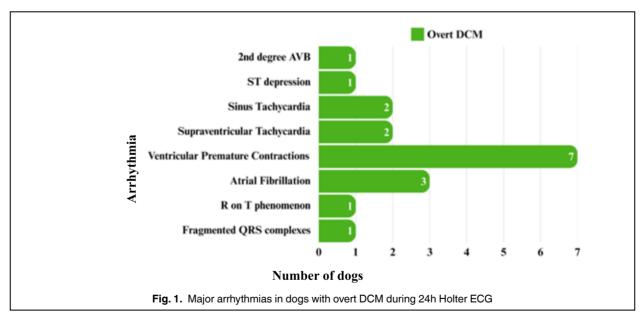
<sup>\*\*</sup> Significant at 0.01 level; \* Significant at 0.05 level; ns: non-significant

(677.88±299.29) was found to be significantly higher (p<0.05) than the number obtained in healthy controls (0.25±0.16). This finding highlights the significance of an abnormally elevated VPC count in dogs with myocardial damage or dysfunction. A threshold of 100 VPCs per 24 hours had been proposed for screening cardiomyopathy in dogs (Oyama, 2016; Wess, 2022). Meurs *et al.* (2001) concluded from their study that healthy mature dogs could have infrequent VPCs as detected by the use of 24-hour ECG and presence of numerous or sequential VPCs might be suggestive of cardiac or systemic disease.

#### Abnormalities in ECG waves

Major arrhythmias observed in dogs with overt cardiomyopathy while performing Holter monitoring are summarised and presented in Fig. 1 and Fig. 2a-2d

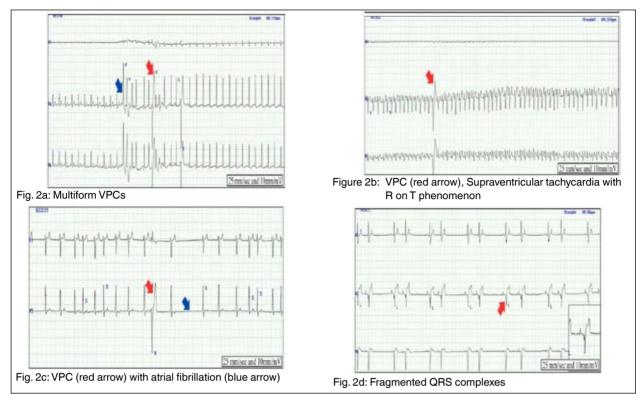
Predominant ECG abnormalities observed during 24h Holter evaluation of overt DCM group included VPCs (seven out of eight dogs; 87.50 per cent), atrial fibrillation (three dogs; 37.50 per cent), sinus tachycardia and supraventricular tachycardia (two dogs each; 25 per cent). fragmented QRS complexes, second degree AV block, ST segment depression and R on T phenomenon (one dog each; 12.50 per cent). The present study reported VPCs as the most common arrhythmia observed in overt DCM in dogs. This finding is in contrary to reports of atrial fibrillation (AF) as the most common arrhythmia in canine DCM (Dukes-McEwan et al., 2003; Wess and Torti, 2018). But most of these studies performed in resting 12 lead ECG for much shorter durations, where occurrence rate is significantly lower for infrequent arrhythmias like VPC. Colakoglu and Sahal (2015) and Teslenko et al. (2021)



**Table 5.** Comparison of number of VPCs obtained between healthy dogs and dogs with occult and overt cardiomyopathy

Parameters	Healthy (n=8)	DCM (n=8)	t-value	p-value
Number of VPCs/24h	0.25±0.16	677.88±299.29	2.264*	0.019

<sup>\*</sup> Significant at 0.05 level



observed that Holter ECGs were superior in identifying arrhythmias compared to one-minute hospital ECGs. Similarly, Meurs *et al.* (2001) reported the superior ability of ambulatory ECG to detect VPCs in dogs. Perez *et al.* (2020) observed that VPCs associated with DCM were not equally distributed over a 24-hour period. So, an intrahospital 5 min ECG might lose that critical information.

Atrial fibrillation was reported as the most common arrhythmia after VPCs associated with overt DCM in the present study. Identification of VPCs in low numbers is considered normal in some dog breeds (Wess et al., 2017), but any occurrence of AF is typically considered as pathological. In a human study, Lee et al. (2023) concluded that VPCs could precipitate the onset of new AF and it has been suggested that AF is more closely associated with the advanced stages of DCM in dogs (Arcuri et al., 2024). Presence of AF was reported to increase the risk of cardiac related death and decreased survival times in canine DCM (Guglielmini et al., 2023). According to Kittleson and Kienle (2010), wave abnormalities characteristic of DCM, such as fragmented QRS complexes and abnormalities in the T wave and ST segment were nonspecific and less significant for diagnosis.

## Conclusion

Dogs in overt phase or stage C DCM demonstrated significantly increased minimum and average heart rates, decreased electrocardiographic pause suggestive of sympathetic overstimulation and cardiac compensation. Heart rate variability parameters indicative of poor autonomic regulation with sympathetic predominance like

SDNN, SDANN and pNN50 and baroreceptor-mediated sympathetic stimulation like VLF and LF altered in overt DCM dogs. Ventricular premature complexes suggesting myocardial damage and atrial fibrillation indicating advanced stages of cardiovascular disease were the major arrhythmias noted.

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# Conflict of interest

The authors declare that they have no conflict of interest.

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