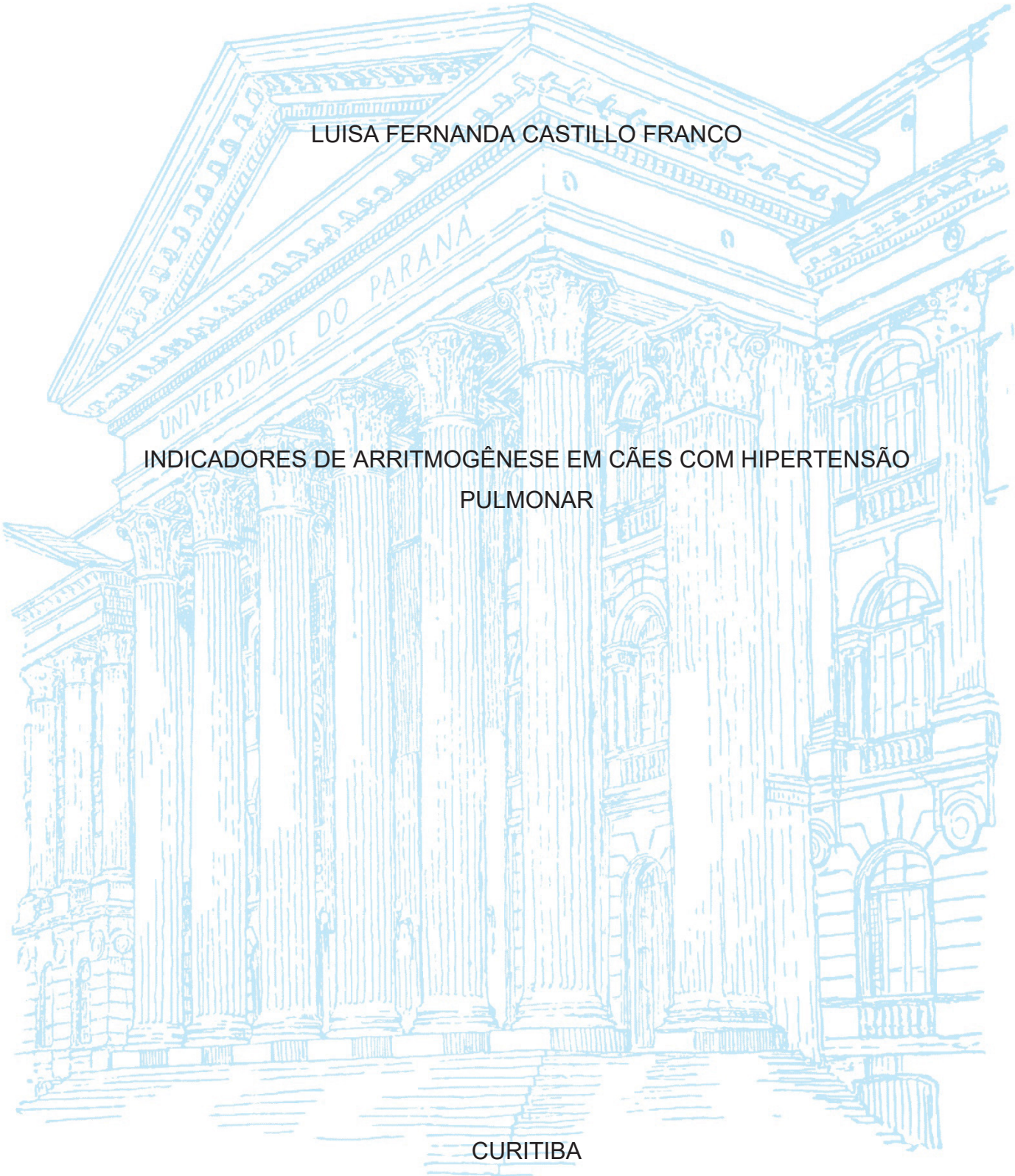
UNIVERSIDADE FEDERAL DO PARANÁ

LUISA FERNANDA CASTILLO FRANCO



INDICADORES DE ARRITMOGÊNESE EM CÃES COM HIPERTENSÃO PULMONAR

CURITIBA

2023

LUISA FERNANDA CASTILLO FRANCO

INDICADORES DE ARRITMOGÊNESE EM CÃES COM HIPERTENSÃO PULMONAR

INDICATORS OF ARRHYTHMOGENESIS IN DOGS WITH PULMONARY HYPERTENSION

Dissertação apresentada ao Programa de Pós-Graduação em Ciências Veterinárias, do Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Ciências Veterinárias.

Orientador: Prof. Dr. Marlos Gonçalves Sousa

CURITIBA

2023

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO (CIP)
UNIVERSIDADE FEDERAL DO PARANÁ
SISTEMA DE BIBLIOTECAS – BIBLIOTECA DE CIÊNCIAS AGRÁRIAS

Franco, Luisa Fernanda Castillo
Indicadores de arritmogênese em cães com hipertensão pulmonar / Luisa Fernanda Castillo Franco. – Curitiba, 2023. 1 recurso online: PDF.

Dissertação (Mestrado) – Universidade Federal do Paraná, Setor de Ciências Agrárias, Programa de Pós-Graduação em Ciências Veterinárias.
Orientador: Prof. Dr. Marlos Gonçalves Sousa

1. Hipertensão pulmonar. 2. Cães. 3. Arritmia. I. Sousa, Marlos Gonçalves. II. Universidade Federal do Paraná. Programa Pós-Graduação em Ciências Veterinárias. III. Título.

Bibliotecária: Telma Terezinha Stresser de Assis CRB-9/944

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS AGRÁRIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO CIÊNCIAS VETERINÁRIAS - 40001016023P3

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação CIÊNCIAS VETERINÁRIAS da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **LUISA FERNANDA CASTILLO FRANCO** intitulada: **INDICADORES DE ARRITMOGÊNESE EM CÃES COM HIPERTENSÃO PULMONAR**, sob orientação do Prof. Dr. MARLOS GONÇALVES SOUSA, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestra está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 27 de Fevereiro de 2023.

Assinatura Eletrônica
02/03/2023 13:59:52.0 MARLOS GONÇALVES SOUSA
Presidente da Banca Examinadora

Assinatura Eletrônica
03/03/2023 17:32:43.0
PATRÍCIA CHAMAS
Avaliador Externo (UNIVERSIDADE PAULISTA)

Assinatura Eletrônica
02/03/2023 18:03:22.0
SIMONE TOSTES DE OLIVEIRA STEDILE
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

Aos meus amados pais, Andrea e Hardany;
ao meu amado Samuel; a Ronaldo, Karina e
Donna, a razão da minha felicidade.

RESUMO

Introdução/ Objetivos: A hipertensão pulmonar (HP) é uma síndrome multifatorial que leva à sobrecarga cardíaca e distensão muscular gerando remodelamento ventricular direito associado à morte súbita cardíaca (MSC) causada por arritmias ventriculares. O eletrocardiograma (ECG) é uma ferramenta importante, mas simples, para o diagnóstico de arritmias, permitindo a detecção precoce de MSC devido a remodelamento ventricular causado por HP por meio da mensuração dos intervalos QT e T-peak T-end (TpTe) que representam a atividade elétrica ventricular total e cuja alteração pode sugerir predisposição a morte súbita. Este estudo visa determinar o papel dos indicadores de arritmogênese na HP canina. Métodos: Avaliação cardiológica retrospectiva de 53 cães com HP (n=23 diagnosticados com HP-pós, e n=30 diagnosticados com HP-pré) e 21 cães saudáveis (controle) foram recrutados neste estudo. Registros de ECG foram usados para determinar ritmo, frequência cardíaca, duração do QRS, dispersão do QRS, intervalo QT corrigido para frequência cardíaca (QTc); Intervalo de dispersão QT, variância do intervalo QT (QTv), intervalo TpTe e razão TpTe/QT. Informações de prognóstico e sobrevida também foram registradas para o grupo de HP. Resultados: A dispersão do QRS foi significativamente diferente (p 0,035) em cães com HP em comparação com os animais saudáveis e quando um valor de corte de 16,5 foi aplicado, houve um tempo médio de sobrevivência de 278 dias em cães com QRSdisp >16,5. QT, QTc, TpTe falharam em demonstrar risco arritmogênico em cães com HP. Conclusão: Cães com HP-pós apresentaram maior risco arritmogênico em comparação com animais com HP-pré e animais controle. A dispersão do QRS pode ser usada como indicador de arritmogênese em cães com HP.

Palavras-chave: Canino; Indicadores eletrocardiográficos; condução e repolarização ventricular; prognóstico.

ABSTRACT

Abstract/Objectives: Pulmonary hypertension (PH) is a multifactorial syndrome that leads to cardiac muscle overload and distension. This generates right ventricular remodeling associated with sudden death (SCD) caused by ventricular arrhythmias. Electrocardiogram (ECG) is an important but simple tool for diagnosing arrhythmias allowing the early detection of SCD in ventricular remodeling due to PH through the measurement of the QT and T-peak T-end (TpTe) intervals which represent total ventricular electric activity and whose alteration may suggest a predisposition to sudden death. This study aims to determine the role of arrhythmogenesis indicators in canine PH. Methods: Retrospective cardiologic evaluation of 53 dogs with PH (n=23 diagnosed with post-PH, and n=30 diagnosed with pre-PH), and 21 healthy (Control) dogs were used in this study. ECG records were used to determine, rhythm, heart rate, QRS duration, QRS dispersion, QT interval then corrected for heart rate (QTc); QT dispersion interval, QT interval variance (QTv), TpTe interval, and TpTe/QT. Prognostic and survival information were also recorded for the PH group. Results: QRS dispersion was significatively different (p 0.035) in dogs with PH compared with the healthy animals and when a cut-off value of 16.5 was applied there was a median survival time of 278 days in dogs with QRSdisp >16.5. QT, QTc, TpTe failed to demonstrate arrhythmogenic risk in dogs with PH. Conclusions: Dogs with post-PH showed a higher arrhythmogenic risk compared to animals with pre-PH and control animals. QRS dispersion may be used as an indicator of arrhythmogenesis in dogs with PH.

Keywords: Canine; Electrocardiographic indicators; ventricular conduction and repolarization; prognosis.

LIST OF ILLUSTRATIONS

LIST OF TABLES

LIST OF ABBREVIATIONS

1

ECG	Electrocardiogram
HR	Heart rate
LTI	long-term instability
MMVD	myxomatous mitral valve disease
PH	Pulmonary hypertension
Post-PH	Post-capillary pulmonary hypertension
Pre-PH	Pre-capillary pulmonary hypertension
QTa	average QT
QTc	QT corrected for heart rate
QTv	QT variance
STI	short-term instability
TI	total instability
SCD	sudden cardiac death
TpTe	T-peak T-end
TAPSE	Tricuspid Annular Plane Systolic Excursion
TRPV	Tricuspid regurgitation peak velocity

SUMMARY

1 INTRODUCTION

Pulmonary Hypertension (PH) is a multifactorial syndrome. In veterinary medicine mainly affects dogs, and has been associated with sudden cardiac death (SCD) in various species [1,2]. It is characterized by an abnormal increase in pulmonary vasculature pressure (>25mmHg), which leads to muscle overload and distension, generating right ventricular remodeling and failure [3]. The PH is classified into pre-capillary (pre-PH) and post-capillary (post-PH). Dogs with left heart failure in which there is an increase in left atrial (LA) pressure more commonly have post-PH due to passive back transmission of increased left-ventricular filling pressure to the pulmonary capillaries [4], this pressure increases the volume load of the right ventricle (RV) thereby indirectly increasing the RV systolic pressure [5].

Pre-PH is defined as the presence of increased pulmonary arterial pressure and pulmonary vascular resistance associated with each other, together with the absence of an increase in left atrial pressure [6,7]. Currently, PH is classified as low, intermediate, or high PH probability. Patients with PH are considered to have echocardiographic signs of alterations in the ventricles, pulmonary artery, right atrium, caudal vena cava, and tricuspid regurgitation peak velocity (TRPV) according to the ACVIM consensus of PH [6]. RV enlargement caused by increased pressure or volume overload, which are common findings in patients with PH, can be evidenced electrocardiographically. Ventricular hypertrophy secondary to volume and pressure overload, caused by subaortic or pulmonary valve stenosis, in addition to arterial systemic or PH cases, can trigger rhythm disturbances and sudden death from arrhythmias [8]. These arrhythmias are triggered by ischemia, increased sympathetic tone, and myocardial fibrosis, caused by ventricular hypertrophy [9]. Thus, PH can lead to RV overload generating RV instability and lethal arrythmias [2].

The Electrocardiogram (ECG) is a simple but important tool for the diagnosis of rhythm disturbance secondary to heart disease and the establishment of prognosis and prediction of sudden death through the measurement of the QT and T-peak T-end (TpTe) intervals [10,11]. The QT interval represents the period of total ventricular repolarization and is affected by repolarization and depolarization disturbances, which predispose to the emergence of ventricular arrhythmias [12,13]. On the other hand, TpTe and TpTe/QT ratio have been used as clinical risk

indicators for ventricular arrhythmias and sudden death in patients with myxomatous mitral valve disease (MMVD) [11]. QRS interval represents ventricular depolarization and changes in this parameter may appear with ventricular enlargement or intraventricular conduction disorders [14].

In this study, we aimed to evaluate the QT, QRS dispersion (QRSdisp) and duration (QRSdur), and TpTe intervals in dogs with PH. Our objectives were three-fold: (1) To evaluate different indicators of arrhythmogenesis in dogs with PH. (2) To determine the sensitivity and specificity of QT interval, QRSdisp, QRSdur, TpTe, and TpTe/QT ratio measurements as indicators of increased arrhythmogenesis in dogs with PH. (3) To assess whether dogs present instability associated with the progression of PH. We hypothesized that repolarization markers have a high sensibility to predict arrhythmias forthcoming and prognosis in dogs with PH.

2 MATERIALS AND METHODS

This retrospective, cross-sectional study was conducted at a veterinary teaching faculty. The study was approved by the Institutional Animal Care and Use Committee (protocol 006/2022) and complied with the National Institutes of Health Guide for the Use and Care of Laboratory Animals

2.1 ANIMALS

This retrospective cross-sectional study recruited electrocardiographic and echocardiographic records of dogs admitted to the cardiology sections between January of 2018 to May of 2022 at a veterinary teaching hospital. The inclusion criteria were patients with a probability of PH according to the American College of Veterinary Internal Medicine guidelines (ACVIM 2020) [6], regardless of breed, age, sex, or body weight. PH dogs were classified according to the origin of the disease in pre-PH and post-PH and according to the probability of PH in low, intermediate, or high. Patients with a history of antiarrhythmic treatment were excluded. Data from clinically healthy dogs admitted for elective procedures were selected for the control group.

2.2 ECHOCARDIOGRAPHIC ANALYSIS

The echocardiographic evaluation was performed by experienced veterinary cardiologists using an echocardiographic machine [a]. The echocardiographic

parameters known to change with disease progression were recorded, such as the ratio of the left atrial to aortic root diameters (LA/Ao), left ventricular internal diameter at end-diastole (LVIDd), TRPV, pulmonary valve flow velocities (PV) and tricuspid annular plane systolic excursion (TAPSE). Data obtained from echocardiographic examinations were used to properly classify the probability of PH and rule out cardiac alterations in the control group.

2.3 ELECTROCARDIOGRAPHIC ANALYSIS

For the electrocardiographic assessments, three to five-minute of ten or twelve leads tracking records of electrical activity were acquired for each patient at a scan speed of 50 mm/s, using a computerized ECG recorder [b]. Bipolar limb electrodes were placed as follows: DI: upper left extremity (+) - upper right extremity (–), DII: lower left extremity (+) - upper right extremity (–), DIII: lower left extremity (+) - left edge top left edge (–); unipolar peripheric leads aVR: right upper limb (+), aVL: left upper limb (+), aVF: left lower limb (+) [9].

[a] Affinity 50, Philips.

[b] Recorder ECG model ECGP V6 TEB Brazilian electronic technology Ltda. - São Paulo, SP, Brazil.

The precordial leads were placed following one of the two known systems, the modified Lannek, and the modified Wilson precordial systems. For the Lannek system, RV2, positioned in the fifth intercostal space at the level of the sternochondral junction; V2, in the sixth left intercostal space at the level of the sternochondral junction; V4, in the sixth left intercostal space at the level of the costocontral junction; and V10, located in the dorsal region of the spinous process of the seventh thoracic vertebra [15].

For the modified Wilson precordial system V1, was placed in the first right intercostal space, at the level of the sternochondral junction; V2, in the sixth left intercostal space at the level of the sternochondral junction; V3, in the sixth left intercostal space and equidistant from V2 and V4; V4, in the sixth left intercostal space at the costochondral junction; V5, in the sixth left intercostal space above V4, keeping the same distance between V5 and V4 as between V4 and V3; finally, V6 was located in the sixth left intercostal space above V5 keeping the same distance between V6 and V5 as between V5 and V4 [9].

The electrocardiographic parameters obtained for each dog, were R-R intervals; heart rate (HR); rhythm; QRSdisp defined as the maximum difference between the QRS obtained in each lead; QRSdisp; QT interval; QT interval dispersion (QTdisp); variance of QT interval (QTv); and the TpTe interval in each one of the front leads (I, II, III, aVR, aVL, aVF) and precordial Lannek system (RV2, V2, V4, V10) and modified Wilson system (V1, V2, V3, V4, V5, V6) leads. For QRS and TpTe parameters, was considered an average of 3 consecutive beats. Subsequently, QT was corrected for heart rate (QTc) for each dog as proposed by Fridericia [16]. The ratio of TpTe and QT (TpTe/QT) and QTc (Tpte/QTc) were included in the study.

In the case of biphasic morphology, the interval between the nadir of the first component was used as a peak, followed by the end of the T wave (Fig. 1) [17]. Fifty consecutive QT intervals of sinus origin were measured and corrected for heart rate using the formula “corrected QT” for heart rate (QTc), proposed by Fridericia [16].

When arrhythmias were detected, they were registered but not included in the formulas. After this, both corrected and uncorrected QT intervals were used to calculate the average QT (QTa) and QT variance (QTv). Also, was calculated the total instability (TI), the short-term instability (STI), and the long-term instability (LTI) of the QT intervals from the ECG tracings of each dog enrolled using the technique proposed by Van der Linde et al. (2005) [18] to quantitatively assess QT instability per a Poincaré plot. (Fig. 2). The calculation of these indices was based on the following equations:

$$\mathrm{TI_n} = \sqrt{((\mathrm{cg(x)} - \mathrm{QT_n})^2 + ((\mathrm{cg(y)} - \mathrm{QT_{n+1}}))^2}$$

$$\mathrm{TI} = \mathrm{M}\ (\mathrm{TI_n})$$

$$\mathrm{LTI_n} = [\mathrm{Rcg(x)} - ((\cos\theta \times\ \mathrm{QT_{n+1}}) - (\sin\theta \times\ \mathrm{QT_n}))]$$

$$\mathrm{LTI} = \mathrm{M}\ (\mathrm{LTI_n})$$

$$\mathrm{STI_n} = [\mathrm{Rcg(y)} - ((\mathrm{sen}\,\theta \times \mathrm{QT_{n+1}}) - (\cos\theta \times \mathrm{QT_n}))]$$

$$\mathrm{STI} = \mathrm{M}\,(\mathrm{STI_n})$$

The median of the distances of the 50 data points to the center of gravity in the data cluster is represented by M, cg represents the center of gravity, Rag represents the rotated centers, and $\theta = 45$. These parameters were previously obtained from the following equations used by Brüler et al. (2018) [10].

$$\mathrm{cg(x)} = \sum_{\mathrm{i=m}}^{\mathrm{m+49}} (\mathrm{QTi})\,/50$$

$$\mathrm{cg(y)} = \sum_{\mathrm{i=m+1}}^{\mathrm{m+50}} (\mathrm{QTi})\,/50$$

$$\mathrm{Rcg(x)} = (\cos\theta \times \mathrm{cg(x)}) - (\mathrm{sen}\,\theta \times \mathrm{cg(y)})$$

$$\mathrm{Rcg(y)} = (\mathrm{sen}\,\theta \times \mathrm{cg(x)}) + (\cos\theta \times \mathrm{cg(y)})$$

In this study, we obtained the QT instability from consecutive measures in the same ECG, and this reflects ventricular repolarization instability. This methodology allows to determine and quantify the instability by three different dynamic ventricular repolarization markers. In synthesis, the morphological properties of the plot are used to determine the indices, based on the distribution and their distances from the center of gravity. The distance to the y coordinate (width) of the plot reflects the STI, the distance to the x coordinate (length) reflects the LTI, and a width- and length-dependent parameter reflects the TI [10].

2.4 PROGNOSIS AND SURVIVAL ANALYSIS

Clients were contacted by phone and invited to answer questions about the patient's condition; if they were still alive, the date of telephone contact was recorded; if animals had died, the date and probable cause of death was noted. When clients were lost to contact or information was unavailable for both survival and development of clinical signs, the date on which the animal was last seen alive was registered.

3 STATISTICAL ANALYSIS

The Statistical software GraphPad Prism, version 9.0 [c] was used for the analysis of the data. Dogs were divided by the origin of PH in Pre and Post capillary and in PH probability which depended on echocardiographic characteristics. All data underwent the Shapiro-Wilk normality test to classify into parametric or non-parametric data. The Chi-square test was calculated to evaluate the association between groups and nominal categorical variables, such as the sex, rhythm, polarity of the T wave, and presence of arrhythmias. TpTe, TpTe/QT, and data obtained from uncorrected and heart rate corrected QT intervals were compared between the different groups using Kruskal-Wallis or Mann-Whitney test depending on the normality test results.

The Student T–test (two-tailed, independent) was used to find the significance when it was compared two groups, in PH with the control group; pre-PH and post-PH; and referring to precordial systems in Lannek and modified Wilson groups. Kaplan-Meier curves were used to assess all-cause mortality and the first recurrence of clinical signs of PH (for all dogs). Spearman's test was used to assess whether correlations existed between QT indices and some of the echocardiographic parameters previously mentioned [19].

The Receiver Operating Characteristic (ROC) curves were used to determine the sensibility and specificity of the parameters to differentiate dogs with and without pulmonary hypertension. Finally, to access intra-observer variability of the measurements of repolarization parameters, the same investigator (L.F.C.F.) randomly selected 20.2% of the ECG tracings to repeat the measurements. In addition, to assess interobserver agreement, the same method was chosen for reevaluation by another observer without knowledge of the previous results. Intra-class correlation coefficient estimates were generated using the Real Statistics

Resource Pack [d] software and were calculated based on a mean-rating (k = 2), absolute agreement, two-way mixed-effects model. Intra-class correlation coefficient values were classified as indicative of poor reliability (< 0.5), moderate reliability (0.5 - 0.75), good reliability (0.75- 0.9), and excellent reliability (> 0.9) [20]. All analyses were performed using statistical software with default settings. Statistical significance was defined as $p < 0.05$.

[c] GraphPad Prism version 8.0.2 for Windows, GraphPad Software, San Diego, California, USA, www.graphpad.com.

[d] Real Statistics Resource Pack software (Release 7.6). Copyright (2013 – 2021) Charles Zaiontz. www.real-statistics.com.

4 RESULTS

Based on the establish criteria, 53 dogs with PH and 21 healthy dogs were included and were allocated to the respective control (n=21), pre-PH (n=30), and post-PH (n=23) groups. Several breeds were included, but mixed breeds (n=23) were overrepresented. The other ones were, Shih-Tzu (n=8), Poodle (n=7), Cocker spaniel (n=4), Beagle, Maltese, Pinscher and English bulldog (n=3 animals for each breed), Pitbull, Schnauzer, Labrador and Border collie (n=2 animals for each breed), Bullterrier, Dogue de Bordeaux, Pekingese, Yorkshire, Airedale Terrier, Pomeranian, Dalmatian, Pug, Bloodhound, Belgian shepherd, Malinois Shepherd, Dutch Shepherd (n=1 animal for each dog).

The patients aged from one to 17 years and the weight ranged from 2.2 to 37 kg being significatively different between groups (P<0.0001). The average heart rate oscillated between 57 to 235 bpm with a significative difference of (P= 0.0006). From the analyzed sample, 60% of the patients of pre-PH and post-PH groups and 38% of the control group were female. A significant difference in average heart rates was documented between pre-PH, post-PH, and control groups ($P < 0.001$), showing a higher heart rate in post-PH patients with an average of 154 bpm compared with 122 and 125 bpm in pre-PH and control groups, respectively (Fig. 3).

Cardiac rhythm was also assessed in the study patients, with sinus arrhythmia being predominant in the control group (n=13/21) and in the Pre-PH group (n=15/30), in contrast to sinus tachycardia in the post-PH group (n=9/23), followed by sinus

192 rhythm (n=9/23), with only three individuals presenting arrhythmias among patients
193 with PH. The arrhythmias registered were supraventricular tachycardia (n=1),
194 accelerated idioventricular rhythm (n=1), right bundle branch block (n=1), left
195 anterior fascicular block (n=1), and sinus arrest (n=1).

196 As for the polarity of the T wave, it was negative 57% (n=17/30) of the pre-PH and
197 74% (n=17/23) of the post-PH groups; 43% (n=13/30) of the pre-PH and 22%
198 (n=5/23) of the post-PH groups had positive T wave and, 4% (n=1) had a biphasic
199 T wave in the post-PH group, however, in the control group, the negative polarity of
200 the T wave was also predominant, represented by 52% (n=11/21) of the dogs,
201 followed by a positive T wave in 29% (n=6/21), and a biphasic T wave in
202 19%(n=4/21). Most ventricular depolarization and repolarization variables
203 measured in frontal and precordial leads of both, the Lannek and modified Wilson
204 systems were higher in patients with post-PH, but no significant difference was
205 found, besides there was no significant difference between the methods used.

206 The QRSdisp was demonstrated to be the best arrhythmogenic indicator with a
207 significant difference (p 0.035) (Fig. 4) in dogs with PH compared with the healthy
208 animals, when a cut-off value of 16.5 was applied there was a median survival time
209 of 278 days in dogs with QRSdisp >16.5, much lesser than the control group which
210 had a median survival time of 1687 days (p 0.001) (Fig. 5). There was a significative
211 difference in TpTe/QT in lead II between post-PH and pre-PH with a P = 0.0371,
212 but not between the control and PH groups.

213 Other repolarization markers such as QT, QTc, and TpTe did not show statistically
214 significant difference between groups and failed in demonstrating arrhythmogenic
215 risk in dogs with PH. There was no statistical difference in ventricular repolarization
216 parameters between the different methods of precordial systems (Lannek and
217 Wilson). The majority of leads showed higher ventricular repolarization values in
218 post-PH dogs except for TpTe in V2, which showed a higher value in pre-PH dogs.

219 When only the visual characteristics of the Poincaré plot are taken into
220 consideration, it is also clear that QT instability increases in patients with pre- and
221 post-PH, as shown in (Fig. 2). QT instability was compared in different groups: all
222 patients with PH, pre-PH and post-PH patients, and finally with PH probabilities
223 (low, intermediate, and high). Of note, these measurements in dogs with pre-PH

were lower than measurements in dogs with post-PH. Referring to PH probability dogs with a high probability of PH had an increase in TI, STI, and LTI.

QRS duration showed higher values but no statistical difference between PH and the control group. The survival information was obtained from owners of 53 animals with PH who were contacted by telephone, of which 7 (13%) owners did not report the date when their dog died, 11(20%) owners were not possible to contact and 29 (54%) reported when the dog died. Only 6 (11%) dogs were still alive at the date of telephone contact. Sixteen of these dogs had developed clinical cardiac signs and only 9 died from congestive heart failure. The low proportion of patients (<50%) that reached this endpoint impairs the construction of a Kaplan-Meier curve (data not shown). Median survival all-cause mortality was 526 days. Higher survival probability was for dogs with low PH probability with an average of 1420 days compared with 317 and 427 days in intermediate and high probability respectively. Dogs with high PH probability were 3 times more likely to die earlier, than patients with low PH probability as showed in Fig. 6.

The QT intervals corrected for heart rate were used in the correlation tests. For the interpretation of the Spearman correlation magnitude, the following classification was adopted: correlation coefficients < 0.3 (poor), 0.3 - 0.5 (fair), 0.6 - 0.8 (moderately strong), and > 0.8 (very strong) [19]. Negatively poor but significant correlations were found to exist between QTv and TRPV (R= 0.324- P = 0.020); TI and TRPV (R = 0.295- P =0.035), STI and TRPV (R = 0.340- P = 0.014), Poor correlation was found between TpTe in lead II and weight (R= 0.006 - P = 0.95), QTa and weight (R= 0.266 - P = 0.053).

To investigate the sensitivity and specificity of the variables, ROC curves were constructed. The area under the curve (AUC) for each of the parameters with the best results was 0.70 (TpTe/QT in lead II), 0.6 (QT), 0.7 (TpTe in lead II), and 0.7 (QRSdisp). The parameters with AUC >0.6, with their respective cut-off values, sensitivity, specificity, likelihood, and p value, are provided in Table 2.

When evaluating the agreement of indicators of ventricular repolarization between different observers using Intra-class correlation coefficient, all ECG indices measured in this study had variable score coefficients, thus we found the best

repeatability and reliability with a score of good (0.7 to 0.9) or excellent (> 0.9) in QRS disp and QT intervals for all leads in inter and intraobserver analysis.

5 DISCUSSION

It is known that PH increases the probability for arrhythmias to occur by several methods such as the modulation of autonomic activity, delayed cardiac repolarization, and right ventricular myocardial ischemia [21]. In this investigation, we studied the applicability of different ventricular repolarization markers such as QT, QRS, and TpTe intervals in dogs with PH.

A significant difference in average heart rates was observed between pre-PH, post-PH, and control groups ($P < 0.001$), showing a higher heart rate in post-PH patients with an average of 153 bpm; this is due to probably activation of autonomic activity. Post-PH is more common in dogs with congestive left heart failure with an increased LA pressure, leading to congestion of the pulmonary veins, increasing pulmonary pressure, and reducing cardiac output (CO) [5]. A reduction in CO induces changes in autonomic activity which increases the sympathetic system [21], speculations on the origin of neurohumoral dysfunction in PH are based on the current state of knowledge about the neurohumoral imbalance in left heart failure [22].

In this study, regardless of the group analyzed, there was a variety in the polarity of the T wave, and the majority was negative, followed by positive and biphasic polarity. This characteristic was presented in the same proportion in each group, different from a study made with 129 and another with 15 healthy dogs where the positive polarity predominated followed by the negative and biphasic [11, 23], agreeing with the biphasic minority in all studies including this one. Vila et al (2021) [11] also observed a higher prevalence of negative T waves in patients with MMVD stages B2 and C. In humans, may be normal to have a negative T wave until 12 years of age and then it becomes positive at 16 years old [24]. However, these studies may not be comparable because different breeds and ages were used in each one. It would be interesting to study the polarity of the T wave in an homogeneous group and perform laboratory analysis, as electrolyte, hemogasometry, and hormone analysis.

On the other hand, QRSdisp is defined as the difference between the maximum and minimum QRS duration measured in the standard twelve lead ECG and it has been

poorly studied in dogs. In people it has been suggested that an increased QRSdisp is a marker of inhomogeneous ventricular depolarization and could be associated with a negative prognosis [25]. It was founded that an increase in QRSdisp in humans after 48 hours of acute myocardial infarction could predict arrhythmias [26]. Turrini et al (2021) [27] demonstrated that augmented QRSdisp was the strongest independent predictor of SCD in human patients with arrhythmogenic right ventricular cardiomyopathy [27]. All these findings demonstrate the importance of the statistically significant difference of this variable (P= 0.0359) in dogs with PH compared with the control group and the shortest median survival time of dogs with QRSdisp >16.5 observed in the present study.

A study performed in people with hypertrophic cardiomyopathy who developed ventricular tachycardia and sudden cardiac death observed a TpTe/QT larger in people affected, compared with QTc [28]. Furthermore, a previous study in dogs with MMVD showed that TpTe/QT is a good marker to predict ventricular arrhythmias and all-cause mortality [11]. In chronic obstructive pulmonary disease in people TpTe and TpTe/QT are higher than in the control group [29]. Although TpTe/QT in lead II should not be used as an isolated parameter for diagnosis of arrhythmogenic risk, it can be useful as a complementary tool as it was in this investigation because it was proved that dogs with a TpTe/QT in lead II > 0.2026 could have minor survival probabilities. The TpTe/QT in lead II in our study had a sensitivity of 80.0 % and specificity of 56.2% in identifying dogs with pulmonary hypertension. This result has potential applicability in a clinical setting because most electrocardiographic measurements in veterinary medicine are performed in lead II.

In children with PH and congenital heart diseases, the QTdur was a good predictor for the occurrence of various arrhythmias [30], opposite to the results we found, in which there were no differences between the groups regarding this parameter. In this study, QT and QTc (all leads), failed to differentiate PH dogs and healthy dogs and showed no reasonable sensibility and specificity results for predicting augmented ventricular arrhythmogenesis. This is in agreement with the findings in dogs with MMVD, where no increase was found in the QT interval with the progression of the disease and the appearance of arrhythmias [11], and in Boxers with arrhythmogenic right ventricular cardiomyopathy (ARVC) in which there was

no correlation with QT and disease severity, so the evaluation of this indices did not appear to be useful in the identification of familial ventricular arrhythmias [31].

In people, there is a positive correlation between TpTe and mean pulmonary artery pressure calculated by right catheterization [32]. This study showed a statistical difference between TpTe values in lead III in dogs with and without PH. A poor correlation was founded between TpTe in lead II and weight, differently from a study in people that found a correlation between these two parameters [33]. This could be explained by the significant difference between the weights from the different groups, with larger breed dogs in the control group that could alter repolarization conditions.

In this study, no difference was found between the two methods of pre-cordial leads used to record the electrocardiogram, even though in humans it has been established that the use of a 12-lead ECG is necessary to identify certain types of arrhythmias and variations in QRS duration [24,25]. However, it should be noted that only the durations of the repolarization indices were evaluated and future studies will be convenient to determine if there is a variation in the amplitude or duration of other parameters between both methods.

The Poincaré plot is a theory of equilibrium points used to give visual summarized information of the dynamic processes of a variable and could be used to describe QT instability [34]. In this study we found higher values of QT instability (TI, STI and LTI) in post-PH group and high PH probability, with no statistical difference compared with pre-PH, control and, low and intermediate PH probability groups agreeing with an investigation in dogs with MMVD in stage C, in which the authors showed longer STI and identified dogs with more probability of arrhythmias occurrence [10].

The results of this study showed that dogs with high PH probability are 3 times more likely to die earlier than patients with low PH probability with a median survival time of 427 days. This median survival time was longer than that in dogs with pos-PH (368 days) [35] and similar to 456 days in PH dogs secondary to MMVD stage B2 and C [4]. Factors such as actual medications for current diseases and owner care may have had influence, making survival times not comparable between different studies.

Excellent repeatability and reliability were founded in inter and intra-observer in QRSdisp interval in this study. Although good inter and intraobserver and excellent repeatability and reliability have been found in TpTe interval measurement in cats with hypertrophic cardiomyopathy [36], this was not seen in this study, maybe due to the variable polarity of the T wave observed in the dogs included, this could limit the correct identification of the peak and the end of the T wave.

The reason why this study did not find risk indicators of arrhythmogenesis in pre-PH remains unclear, but we hypothesize that it may have an association with the fact that in the precapillary group, there were few dogs with a high probability of PH, thus there could be discrete remodeling in the RV that was insufficient to lead to increased or altered repolarization markers. The results found in the post-PH group could be due to the influence of left heart failure caused by MMVD in the majority of the dogs in post-PH group. Regardless of the origin, PH syndrome does not bring a real risk for developing arrhythmias, and the reason to it still remains unclear. Further research is needed to elucidate the electrophysiological phenomena that underlie changes in various ECG parameters that reflect electrical changes in dogs with pre-PH.

6 LIMITATIONS

The study must be interpreted under some limitations. Most of the data obtained were collected retrospectively. The majority of patients did not have blood tests available (eg arterial blood gas analysis that could reveal changes in T wave due to electrolyte and/or hormone disturbances). QT intervals were measured only in lead II. There was no uniform distribution of weight within the different groups, therefore, a more homogeneous population of dogs would be evaluated in future studies. Although dogs in treatment with antiarrhythmic drugs were excluded, there was no information about some dogs that probably were receiving another treatment for heart disease, that could change the patient cardiac condition and consequently alter repolarization indicators. Finally, even following the recommendations proposed by the consensus for the diagnosis of PH, echocardiographic measurements were not performed by the same observer, which can generate variability in the measurements presented.

7 CONCLUSIONS

In conclusion, dogs with post-PH have a higher arrhythmogenic risk compared to animals with pre-PH and control animals. QRSdisp and TpTe/QT in lead II may be used for clinical and arrhythmogenic risk stratification in dogs with PH. These parameters identified animals at a higher risk for ventricular arrhythmias, predicted mortality, and poor prognosis. The measure of QRSdisp has good repeatability and intra-rater reproducibility. Future studies that investigate ventricular repolarization markers in pre-PH dogs and with a larger and more homogeneous population are warranted to improve the understanding of the role played by altered loading conditions and cardiac remodeling on electrical instability in dogs with pulmonary hypertension.

Conflicts of interest statement

The authors do not have any conflicts of interest to disclose.

Acknowledgments

This research was supported by the Coordination of Superior Level Staff Improvement (CAPES), Brazil.

REFERENCES

1. Kuriyama T. [Clinical aspects of precapillary pulmonary hypertension]. Nihon Kyobu Shikkan Gakkai Zasshi. 1992 Jan;30(1):3–11.
2. Tanaka Y, Takase B, Yao T, Ishihara M. Right ventricular electrical remodeling and arrhythmogenic substrate in rat pulmonary hypertension. Am J Respir Cell Mol Biol. 2013 Sep;49(3):426–36.
3. Chin KM, Rubin LJ. Pulmonary Arterial Hypertension. Vol. 51, Journal of the American College of Cardiology. 2008. p. 1527–38.
4. Borgarelli M, Abbott J, Braz-Ruivo L, Chiavegato D, Crosara S, Lamb K, et al. Prevalence and Prognostic Importance of Pulmonary Hypertension in Dogs with Myxomatous Mitral Valve Disease. J Vet Intern Med. 2015 Mar 1;29(2):569–74.
5. Guazzi M, Naeije R. The Present and Future Pulmonary Hypertension in Heart Failure Pathophysiology, Pathobiology, and Emerging Clinical Perspectives. Journal of the American College of Cardiology . 2017;69(13).
6. Reinero C, Visser LC, Kellihan HB, Masseau I, Rozanski E, Clercx C, et al. ACVIM consensus statement guidelines for the diagnosis, classification, treatment, and monitoring of pulmonary hypertension in dogs. J Vet Intern Med. 2020 Mar 1;34(2):549–73.
7. Kellihan HB, Stepien RL. Pulmonary hypertension in canine degenerative mitral valve disease. Vol. 14, Journal of Veterinary Cardiology. Elsevier B.V.; 2012. p. 149–64.
8. Tse G, Yan BP. Traditional and novel electrocardiographic conduction and repolarization markers of sudden cardiac death. Vol. 19, Europace. Oxford University Press; 2017. p. 712–21.
9. Santilli R, Sydney Moïse N, Pariaut R, Perego M. Electrocardiography of the dog and cat DIAGNOSIS OF ARRHYTHMIAS 2 nd edition 2. 2018.
10. Brüler BC, Jojima FS, Dittrich G, Giannico AT, Sousa MG. QT instability, an indicator of augmented arrhythmogenesis, increases with the progression of myxomatous mitral valve disease in dogs. Journal of Veterinary Cardiology. 2018 Aug 1;20(4):254–66.
11. Vila B de CP, Camacho AA, Sousa MG. T-wave peak-end interval and ratio of T-wave peak-end and QT intervals: novel arrhythmogenic and survival markers for dogs with myxomatous mitral valve disease. Journal of Veterinary Cardiology. 2021 Jun 1;35:25–41.
12. Berger RD, Kasper EK, Baughman KL, Marban E, Calkins H, Tomaselli GF. Beat-to-beat QT interval variability: Novel evidence for repolarization lability in ischemic and nonischemic dilated cardiomyopathy. Circulation. 1997;96(5):1557–65.

13. Tilley LP, Smith FWK. Electrocardiography. Manual of Canine and Feline Cardiology (Fourth Edition); 2008.

14. Santilli R; NSM. Electrocardiography of the dog and cat. 2nd ed. 2018.

15. Detweiler DK, Patterson DF. PART V. Some spontaneous cardiovascular diseases in animals t h e prevalence and types of cardiovascular disease in dogs. Annals New York Academic of Sciences. 1965;481–516.

16. Indik JH, Pearson EC, Fried K, Woosley RL. Bazett and Fridericia QT correction formulas interfere with measurement of drug-induced changes in QT interval. Heart Rhythm. 2006 Sep;3(9):1003–7.

17. Emori T, Antzelevitch C. Cellular Basis for Complex T Waves and Arrhythmic Activity Following Combined I K r and I K s Block. Journal Cardiovascular Electrophysiology. 2001;12:1369–78.

18. van der Linde H, van de Water A, Loots W, van Deuren B, Lu HR, van Ammel K, et al. A new method to calculate the beat-to-beat instability of QT duration in drug-induced long QT in anesthetized dogs. J Pharmacol Toxicol Methods. 2005 Jul;52(1):168–77.

19. Chan YH. Biostatistics 104: Correlational Analysis. Singapore Med J. 2003;44(12):614–9.

20. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. J Chiropr Med. 2016 Jun 1;15(2):155–63.

21. Rajdev A, Garan H, Biviano A. Arrhythmias in Pulmonary Arterial Hypertension. Prog Cardiovasc Dis. 2012 Sep;55(2):180–6.

22. Floras JS, Toronto F. Clinical Aspects of Sympathetic Activation and Withdrawal in Heart Failure. J Am Coll Cardiol . 1993;22(4):72A-84A.

23. Romito G, Castagna P, Pelle NG, Testa F, Sabetti MC, Cipone M. The canine T wave: a retrospective analysis on qualitative and quantitative T wave variables obtained in 129 healthy dogs and proposed reference intervals. Journal of Veterinary Cardiology. 2022 Aug 1;42:52–64.

24. Hiss RG, Averill KH, Lamb LE. Electrocardiographic findings in 67,375 asymptomatic subjects. Am J Cardiol. 1960 Jul;6(1):178–89.

25. Chávez-González E, Rodríguez Jiménez AE, Moreno-Martínez FL. Duración y dispersión del QRS para predecir arritmias ventriculares en las fases iniciales del infarto agudo de miocardio. Med Intensiva. 2017 Aug 1;41(6):347–55.

26. Chávez-González E, Rodríguez-Jiménez AE, Ferrer-Rodríguez CJ, Donoiu I. Ventricular arrhythmias are associated with increased QT interval and QRS dispersion in patients with ST-elevation myocardial infarction. Revista Portuguesa de Cardiologia. 2022;

27. Turrini P, Corrado D, Basso C, Nava A, Bauce B, Thiene G. Dispersion of ventricular depolarization-repolarization: A noninvasive marker for risk stratification in arrhythmogenic right ventricular cardiomyopathy. Circulation. 2001 Jun 26;103(25):3075–80.

28. Shimizu M, Hayashi K, Konno T, Mabuchi H. T-Peak to T-End Interval May Be a Better Predictor of High-Risk Patients with Hypertrophic Cardiomyopathy Associated with a Cardiac Troponin I Mutation Than QT Dispersion. Clinic Cardiology. 2002;25:335–9.

29. Cosgun A, Oren H, Turkkani MH. The relationship between systolic pulmonary arterial pressure and Tp-e interval, Tp-e/QT, and Tp-e/QTc ratios in patients with newly diagnosed chronic obstructive pulmonary disease. Annals of Noninvasive Electrocardiology. 2020 May 1;25(3).

30. Saleh A, Shabana A, el Amrousy D, Zoair A. Predictive value of P-wave and QT interval dispersion in children with congenital heart disease and pulmonary arterial hypertension for the occurrence of arrhythmias. J Saudi Heart Assoc. 2019 Apr 1;31(2):57–63.

31. Spier AW, Meurs KM, Muir WW, Lehmkuhl LB, Hamlin RL. Correlation of QT dispersion with indices used to evaluate the severity of familial ventricular arrhythmias in Boxers. Vol. 62, AJVR. 2001.

32. Elitok A, Emet S, Karaayvaz EB, Erdogan O, Aydogan M, Engin B, et al. The relationship between T-wave peak-to-end interval and hemodynamic parameters in patients with pulmonary arterial hypertension. Annals of Noninvasive Electrocardiology. 2020 Sep 1;25(5).

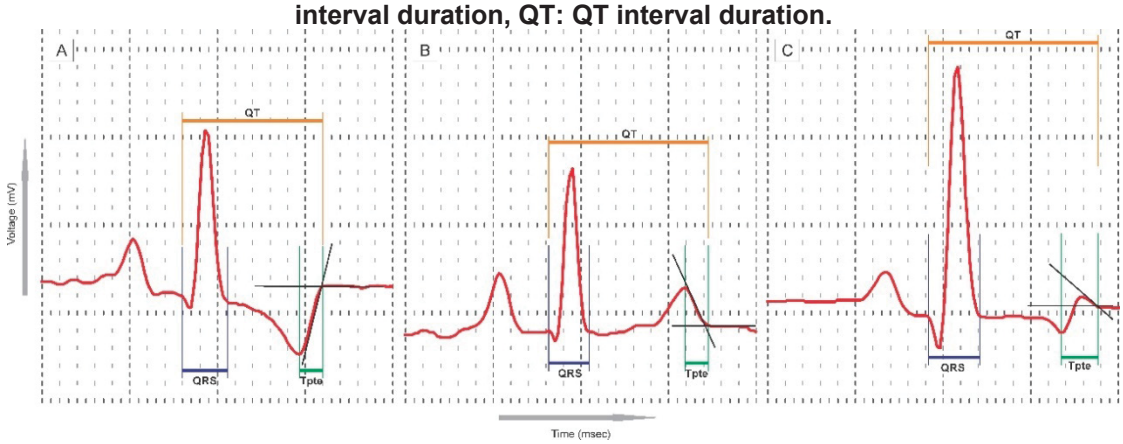
33. Heist EK, Ruskin JN. Drug-induced arrhythmia. Vol. 122, Circulation. 2010. p. 1426–35.

34. Kamen PW, Tonkin AM. Application of the Poincare plot to heart rate variability: a new measure of functional status in heart failure. Aust NZ J Med . 1995;25.

35. Udomkiattikul J, Kirdratanasak N, Siritianwanitchakul P, Worapunyaanun W, Surachetpong SD. Factors related to survival time in dogs with pulmonary hypertension secondary to degenerative mitral valve disease stage C. Int J Vet Sci Med. 2022;10(1):25–32.

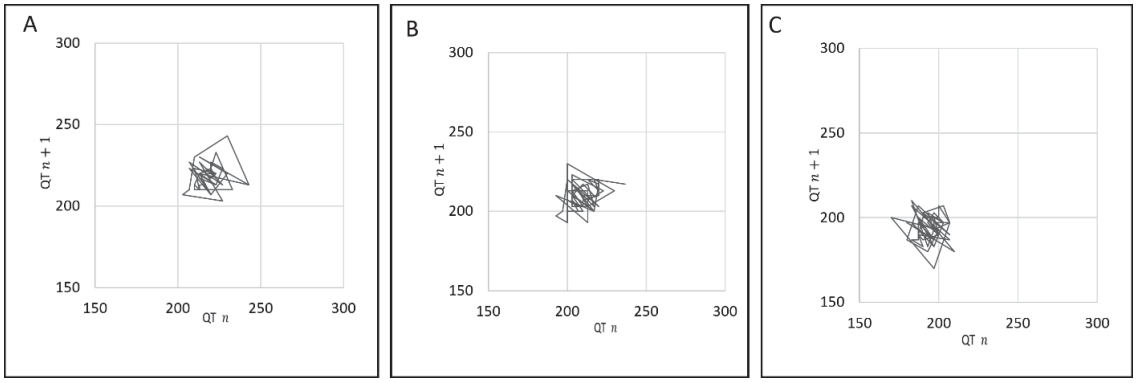
36. Bastos RF, Tuleski GLR, Franco LFC, Sousa MG. Tpeak—Tend, a novel electrocardiographic marker in cats with hypertrophic cardiomyopathy—a brief communication. Vet Res Commun. 2022.

Figure 1 – The 'tangent' method for defining the end of the T wave. (A) The peak of the T wave was defined as the time point where the T wave had the maximal amplitude, whereas the end of T wave was considered as the intersection of the tangent to the down slope of the T wave and the isoelectric line. In the case of inverted (B) or biphasic (C) T-wave morphologies, the interval between the nadir of the first component of T wave and the end of T wave was used. QRS: QRS complex duration, TpTe: T-wave peak-end interval duration, QT: QT interval duration.



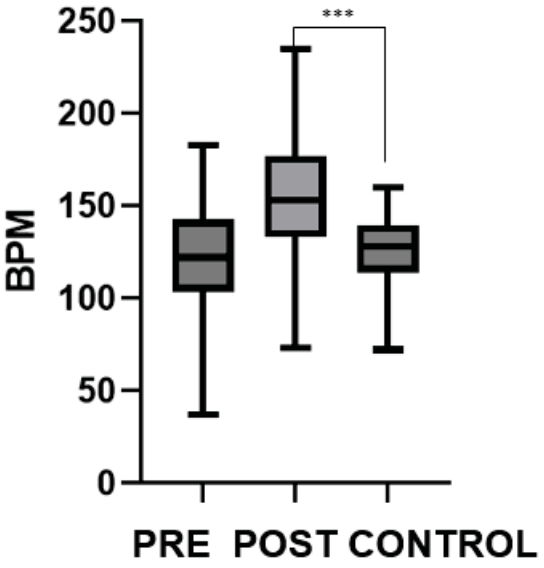
SOURCE: The author (2022)

Figure 2 – Modified Poincare plots in which a line is connecting the 49 combinations of 50 successive QT intervals to depict QT instability of three different patients, (A) Precapillary pulmonary hypertension dog with a total instability of 2.04; (B) Postcapillary pulmonary hypertension dog with a total instability of 1.02; (C) Healthy dog with a total instability of 2.12.



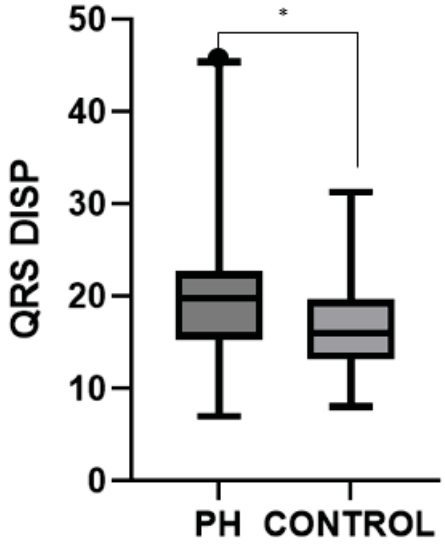
SOURCE: The author (2022)

Figure 3 – Box plot showing the difference between heart rates in beats per minute (BPM) in pre-PH, post-PH, and control groups (P < 0.001), showing a higher heart rate in post-PH patients with an average of 154 bpm compared with 122 and 125 bpm in pre-PH and control groups respectively.



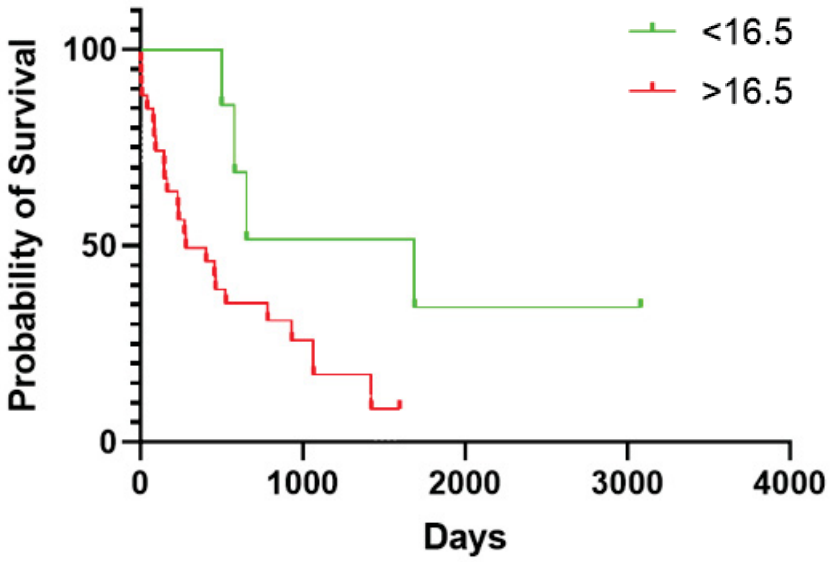
SOURCE: The author (2022)

Figure 4 – Box plot showing QRS dispersion between PH group and control group significatively different (p 0.0359) in dogs with PH.



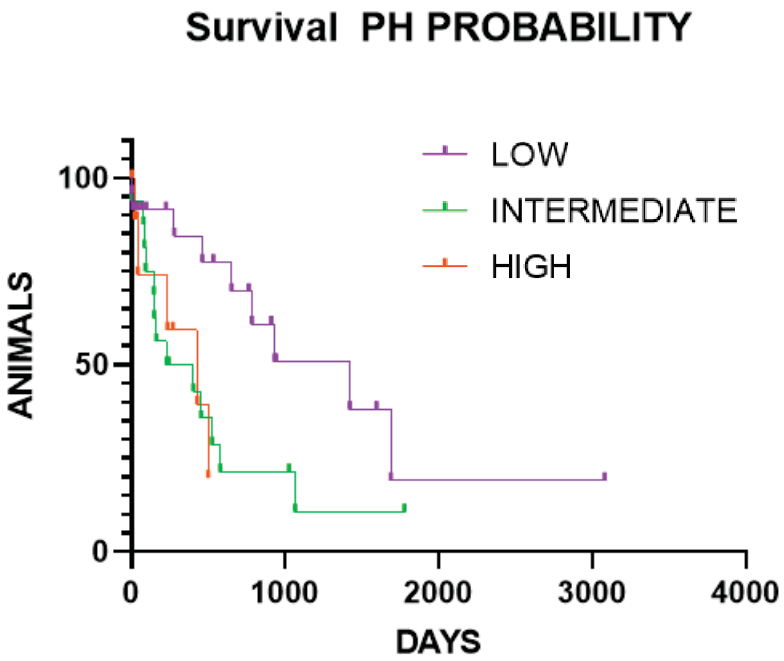
SOURCE: The author (2022)

Figure 5 – Kaplan - Meier curves were constructed to investigate survival in dogs with a cut-off of 16.5 in QRSdisp when all causes of death were included in the dataset with p 0.001.



SOURCE: The author (2022)

Figure 6 – Kaplan - Meier curves constructed to investigate survival in dogs with different pulmonary hypertension probabilities when all causes of death were included in the dataset.



SOURCE: The author (2022)

Table 1 – ECG features related to rhythm and prevalence of arrhythmias in healthy dogs and dogs with PH.

Rhythm	Control (n=21)	Pre-PH (n=30)	Post-PH (n= 23)
SA (n)	13 (61.90%)	15 (50.0%)	4 (17.39%)
SR (n)	8 (38.10%)	10 (33.33%)	9 (39.13%)
ST (n)	0	3 (10.0%)	9 (39.13%)
SB (n)	0	1 (3.0%)	0
SVT (n)	0	0	1 (4.34%)
IVR (n)	0	1 (3.0%)	0
Arrhythmia (n)	0	2 (6.66%)	1 (4.34%)

SA: Sinus arrhythmia; SR: Sinus rhythm; ST: Sinus tachycardia; SB: Sinus bradycardia; SVT: supraventricular tachycardia; IVR: idioventricular rhythm, and another type of arrhythmias

Table 2 – Area under the curve (AUC), cut-off, sensitivity, specificity, likelihood and P value of electrocardiographic and parameters with an area under the curve (AUC) >0.6 to differentiate between dogs with pulmonary hypertension.

Parameter	AUC	Cut-off	Sensitivity %	Specificity %	Likelihood	P value
QT	0.6	>198.5	76.1	43.4	1.4	0.42
TPTE in lead II	0.7	>37.3	66.6	71.4	2.3	0.06
TPTE/QT in lead II	0.7	>0.2	80.0	56.2	1.8	0.01
QRSdisp	0.7	>16.5	71.7	52.38	1.5	0.03