

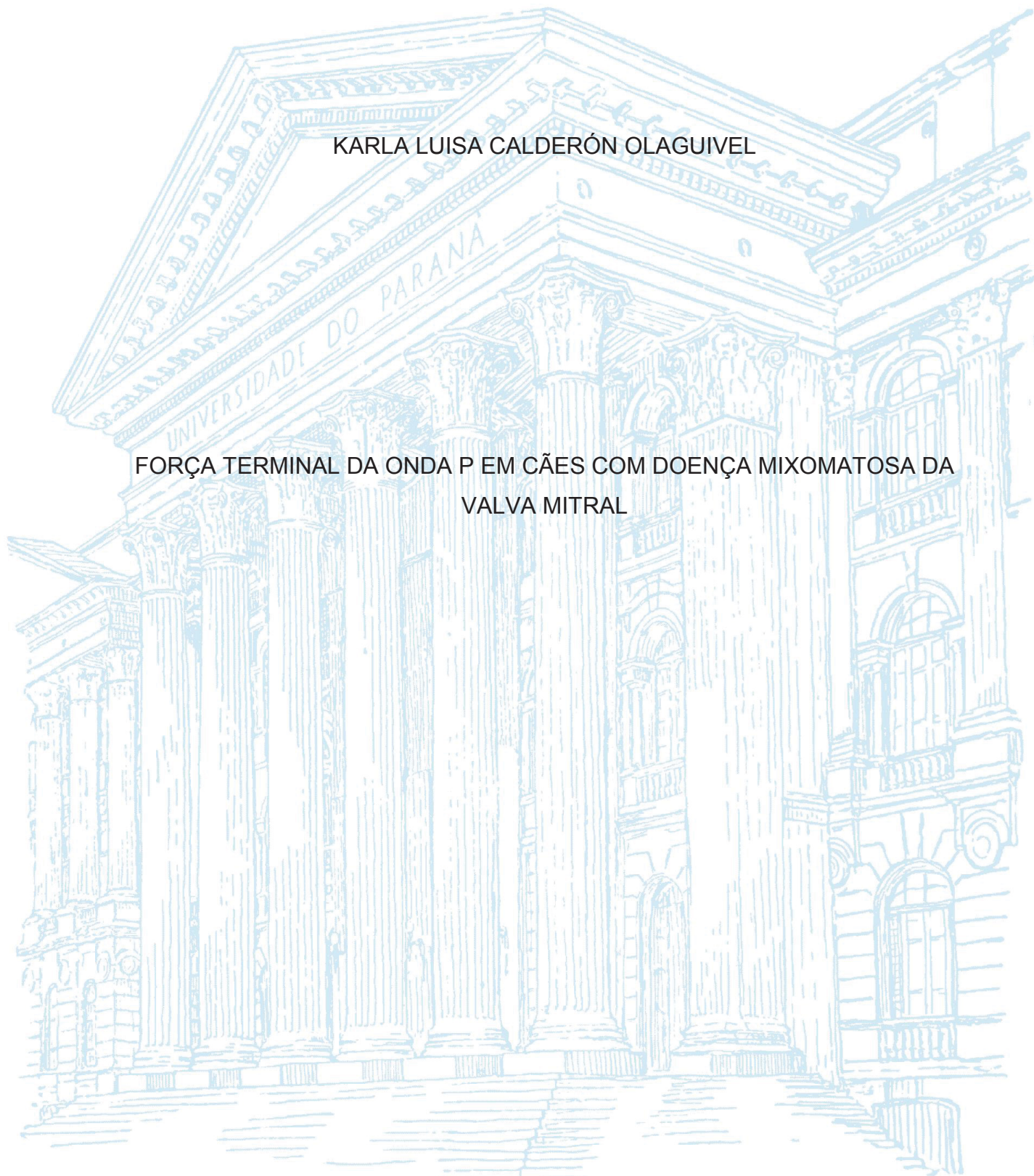
UNIVERSIDADE FEDERAL DO PARANÁ

KARLA LUISA CALDERÓN OLAGUIVEL

FORÇA TERMINAL DA ONDA P EM CÃES COM DOENÇA MIXOMATOSA DA
VALVA MITRAL

CURITIBA

2020



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FORÇA TERMINAL DA ONDA P EM CÃES COM DOENÇA MIXOMATOSA DA
VALVA MITRAL

P-WAVE TERMINAL FORCE IN DOGS WITH MYXOMATOUS MITRAL VALVE
DISEASE

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

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS VETERINÁRIAS da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de KARLA LUISA CALDERÓN OLAGUIVEL intitulada: FORÇA TERMINAL DA ONDA P EM CÃES COM DOENÇA MIXOMATOSA DA VALVA MITRAL, 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.

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“Nothing really worth having is easy to get. The hard-fought battles, the goals won with sacrifice, are the ones that matter.”

- Aisha Tayler

RESUMO

Introdução / Objetivos: Nas pessoas, a força terminal da onda P (PTF) é reconhecida como um critério eletrocardiográfico para avaliar anormalidades do átrio esquerdo. Dentro do conhecimento do autor, a PTF não foi investigada em cães. Neste estudo, procurou-se a presença de PTF em cães saudáveis e em cães com doença mixomatosa da valva mitral (MMVD). Também comparamos a PTF com outros parâmetros derivados da onda P (duração, área, produto Cornell e eixo) e procuramos correlações com substitutos ecocardiográficos de dilatação e congestão do átrio esquerdo. **Animais:** Quarenta e sete cães saudáveis e setenta e quatro cães com doença valvar mitral diagnosticada ecocardiograficamente. **Métodos:** Esta pesquisa foi delineada como um estudo observacional transversal prospectivo. Todos os cães foram submetidos a exames físicos, eletrocardiográficos e ecocardiográficos padrão antes da inclusão na investigação. As medidas eletrocardiográficas foram obtidas a partir de gravações simultâneas em três locais diferentes para a derivação precordial V_1 . A força terminal da onda P foi considerada como a deflexão após a segunda metade da onda P. Além disso, a duração e a amplitude da onda P foram obtidas para calcular os outros quatro parâmetros eletrocardiográficos. **Resultados:** A força terminal da onda P foi melhor documentada a partir de gravações obtidas no primeiro e terceiro espaços intercostais direitos. Nesses locais, a onda P era negativa e a força terminal da onda P era positiva. A força terminal da onda P e a duração da onda P correlacionaram-se com os parâmetros ecocardiográficos no primeiro e no terceiro espaços intercostais ($p < 0,05$). A força terminal da onda P, a duração da onda P, a área da onda P e o produto Cornell foram capazes de diferenciar cães com átrio esquerdo normal daqueles com aumento do átrio esquerdo ($p < 0,05$). **Conclusões:** Em cães, a força terminal da onda P é melhor registrada nos locais mais craniais para a derivação precordial V_1 . A análise da força terminal da onda P, duração da onda P, área da onda P e produto da Cornell da onda P na derivação precordial V_1 pode ser usada como uma triagem eletrocardiográfica do aumento do átrio esquerdo em cães com sopros cardíacos atribuídos à doença mixomatosa da valva mitral.

Palavras-chave: Insuficiência mitral. Derivação precordial. Índice Morris.

ABSTRACT

Introduction/Objectives: In people, P-wave terminal force (PTF) is one of the electrocardiographic criteria used to assess left atrial abnormalities. To the best of the authors' knowledge, PTF has not been investigated in dogs. In this study, we measured PTF in healthy dogs and dogs with myxomatous mitral valve disease (MMVD) and compared PTF with other P-wave derived parameters (duration, area, Cornell product and axis) and correlated it with echocardiographic surrogates of left atrial dilatation and congestion. **Animals:** Forty-seven healthy dogs and 74 dogs with echocardiographically diagnosed mitral valve disease. **Methods:** This was a prospective cross-sectional observational study. All dogs underwent physical, electrocardiographic and standard echocardiographic examinations prior to enrolment. Electrocardiographic measurements were obtained from simultaneous recordings at three different locations for precordial lead V₁. P-wave terminal force was defined as the deflection following the second half of the P-wave. P-wave duration and amplitude were also measured to calculate the other four ECG parameters. **Results:** P-wave terminal force was best documented from recordings obtained at the first and third right intercostal spaces. In those locations, the P-wave was negative and P-wave terminal force was positive. P-wave terminal force and P-wave duration correlated with echocardiographic parameters at both the first and third intercostal spaces ($p < 0.05$). P-wave terminal force, P-wave duration, P-wave area and Cornell product could be used to differentiate dogs with a normal left atrium from those with left atrial enlargement ($p < 0.05$). **Conclusions:** In dogs, P-wave terminal force is best recorded at the most cranial location for precordial lead V₁. P-wave terminal force, P-wave duration, P-wave area and P-wave Cornell product in precordial lead V₁ may be used in electrocardiographic screening for left atrial enlargement in dogs with cardiac murmurs due to myxomatous mitral valve disease.

Keywords: Mitral insufficiency. Precordial lead. Morris index.

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LISTA DE ABREVIATURAS

1CCJ	- precordial lead V ₁ in the first intercostal space at the level of the costochondral junction.
3CCJ	- precordial lead V ₁ in the third intercostal space at the level of the costochondral junction.
5ECJ	- precordial lead V ₁ in the fifth intercostal space at the level of the sternochondral junction.
E	- peak velocity of early diastolic transmitral flow
E/IVRT	- ratio of E to IVRT
IQR	- interquartile range
IVRT	- isovolumic (or isovolumetric) relaxation time
LA/Ao ratio	- left atrium-to-aorta ratio
LVIDd	- diastolic left ventricular internal diameter
LVIDs	- systolic left ventricular internal diameter
MMVD	- myxomatous mitral valve disease
NLVIDd	- normalized diastolic left ventricular internal diameter
NLVIDs	- normalized systolic left ventricular internal diameter
PTFV ₁	- P-wave terminal force in V ₁ derivation

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1 INTRODUCTION

The P-wave represents the depolarization activity of both atria. In people, the P-wave is usually biphasic in the right precordial lead (V_1). The terminal part of the P-wave in lead V_1 represents left atrial electrical activity. The product of the duration and amplitude of this P-wave terminal portion is called the P-wave terminal force in lead V_1 (PTFV₁), a parameter that has been extensively used in people as the main criterion for inference of left atrial enlargement. In people, other electrocardiographic markers, such as notched P-waves and prolonged P-wave duration, are considered to have a similar predictive value to PTFV₁ (Hopkins and Barrett, 1989; Meek and Morris, 2002; Hancock et al., 2009).

In 1964, Morris et al. first presented PTFV₁ as an electrocardiographic marker of left-sided valvular lesions that could be used to estimate the severity of valvular disease. Additionally, they documented PTFV₁ in idiopathic myocardial hypertrophy, hypertensive vascular disease and subaortic stenosis, and PTFV₁ was proposed as a diagnostic surrogate for left-sided heart diseases as a whole (Morris et al., 1964). Over the years, there have been numerous studies assessing PTFV₁ as a predictor of left atrial size and function in people with structural heart diseases, such as mitral or aortic valve disease (Hopkins and Barrett, 1989; Hazen et al., 1991; Elbey et al., 2012; Win et al., 2015). PTFV₁ has also been investigated in other cardiovascular diseases, i.e. myocardial infarction, atrial fibrillation and ischemic stroke as a predictor of clinical deterioration, development of related diseases, relapse, or even cardiac death (Lui et al., 2013; Kamel et al., 2015; Tanoue et al., 2016; Goda et al., 2017). Currently, PTFV₁ is regarded as the main electrocardiographic marker of left atrial abnormalities in people (Hancock et al., 2009).

Human Cardiology uses the Wilson precordial lead system, which positions lead V_1 at the level of the right fourth sternal margin. Using that position, the P-wave is usually diphasic, while right ventricular depolarization is reflected by a deep negative S wave (Rajaganeshan et al., 2008). In veterinary medicine, there have been various proposed precordial lead systems with the right precordial lead positioned mainly at the level of the fifth intercostal space. Positioning in the fifth intercostal space

produces a tall R wave with a short S wave (Takahashi and Kita, 1964; Detweiler and Patterson, 1965; Kraus et al., 2002). Recently, a new location for V_1 has been proposed in dogs, at the level of the costochondral junction of the first intercostal space. In this position, there is a negative P-wave with a R/S ratio <1 for most patients regardless of the thoracic conformation of the dog. Consequently, characteristics of lead V_1 at the first costochondral junction in dogs would resemble the lead V_1 characteristics in people (Santilli et al., 2019).

In veterinary medicine, the most widely used electrocardiographic markers of atrial morphology and electrophysiology are P-wave duration and amplitude (Tilley et al., 2008; Santilli et al., 2018). Other P-wave parameters have been investigated in animals, including P-wave dispersion (Noszczyk-Nowak et al., 2011; Dittrich et al., 2018), P-wave mean electrical axis, Macruz index and P-wave area (Soto-Bustos et al., 2017). However, at least to our knowledge, there are no major studies investigating PTFV₁ in veterinary medicine, despite its importance in analysis of left-sided heart diseases in people.

1.1 OBJETIVES

The aim of this study was four-fold: (1) to investigate whether PTF may be identified in healthy adult dogs and in dogs with different stages of MMVD; (2) to evaluate whether a correlation exists between PTF and echocardiographic surrogates of cardiac remodeling and function; and (3) to compare the performance of PTF with other P-wave indices, including P-wave duration, P-wave area, Cornell product, and P-wave axis; (4) to determine which location of lead V_1 provides the most reliable identification of PTF.

2 ANIMALS, MATERIALS AND METHODS

2.1 ANIMALS

This prospective cross-sectional observational study included a control group and a group of dogs with MMVD at different stages. The latter were classified according to the consensus statement proposed by the American College of Veterinary Internal Medicine (ACVIM) (Keene et al., 2019). Dogs in the control group were considered healthy based on an unremarkable history, physical examination, 9-lead electrocardiogram and transthoracic echocardiography. Dogs in the study group were recruited at a Veterinary Teaching Facility and underwent a thorough history and physical examination. Laboratory examinations included at least: a complete blood count, serum biochemistry and urinalysis. Additionally, three-view thoracic radiographs were obtained as required by the resident in charge. Transthoracic echocardiography and 9-lead electrocardiography were obtained at least once in every patient to confirm MMVD as the main morbidity.

Dogs meeting exclusion criteria: comorbidities that could potentially alter echocardiographic or electrocardiographic parameters (such as endocrine and neoplastic diseases), local or systemic infections, moderate or severe hepatic disease, were excluded from the study. Likewise, dogs in both control and MMVD groups were excluded if they showed: presence of congenital heart diseases, any degree of aortic insufficiency, or heart diseases that could directly or indirectly affect the mitral valve (dilated cardiomyopathy, infective endocarditis). Similarly, dogs with arrhythmias that could alter P-wave morphology (atrial premature complexes, atrial fibrillation, junctional complexes, junctional tachycardia, atrial standstill), as well as dogs taking antiarrhythmic drugs (classes 1 to 4), were excluded from the study.

2.2 ECHOCARDIOGRAPHY

A single operator performed all echocardiographic examinations using an ultrasound machine¹ equipped with 4-2, 8-3 and 12-4 MHz phased array transducers and simultaneous electrocardiographic recording. All dogs were gently restrained in lateral recumbency during the echocardiographic examination. Qualitative evaluation

¹ Philips Affiniti 50

of mitral valve thickness and presence of mitral regurgitation was made from left parasternal four-chamber apical and right parasternal long axis four-chamber views. Pulsed wave Doppler evaluation of transmitral flow (E wave, A wave and E/A ratio) at the opening of the mitral valve and tissue Doppler evaluation (E' wave, A' wave and E'/A' ratio) at the septal or lateral mitral annulus insertion were performed from the left parasternal apical four-chamber view. MMVD was diagnosed on the presence of a thickened mitral valve with some degree of regurgitation, and sometimes mitral valve prolapse. Using right parasternal short axis images, the M-mode edge-to-edge technique was used to measure the septal and free wall thickness and the left ventricular diameter in both systole and diastole. Fractional shortening and ejection fraction were also calculated. Three M-mode measurements were obtained and averaged for each parameter. Left atrium-to-aorta ratio (LA/Ao) was obtained at the first frame after aortic valve closure as previously described (Rishniw and Erb, 2000). Diastolic and systolic left ventricular internal diameter dimensions (LVIDd and LVIDs, respectively) were normalized using the formulae $LVIDd(cm)/(BW(kg))^{0.294}$ and $LVIDs(cm)/(BW(kg))^{0.315}$ (NLVIDd and NLVIDs, respectively) (Cornell et al., 2004).

2.3 ELECTROCARDIOGRAPHY

Electrocardiographic evaluation² was performed with the patient in right lateral recumbency with four leads in the frontal plane and three unipolar leads recording simultaneously from three different positions for V₁. The first unipolar lead was located in the first intercostal space at the level of the costochondral junction (1CCJ); the second unipolar lead was positioned in the third intercostal space at the level of the costochondral junction (3CCJ); while the third unipolar lead was located in the fifth intercostal space at the level of the sternochondral junction (5ECJ). A three-minute ECG recording was performed in each dog. PTFV₁ was calculated as the algebraic product of the amplitude in millivolts (mV) and duration in milliseconds (ms) of the deflection formed by the prolongation of the second half of the P-wave at the three precordial V₁ locations used in this study. For every patient, five PTFV₁ values

² TEB (Tecnologia Eletrônica Brasileira) ECGPC V3.2

(ms.mV) were calculated and averaged for each V_1 positioning. In patients in which a wandering pacemaker was documented, the three highest and the three lowest P-wave amplitudes were measured and then averaged to obtain a single P-wave amplitude at each V_1 positioning. Whenever varying P-wave durations were observed, five P-wave duration values were measured and averaged to obtain the duration value for each position of the V_1 lead. The final P-wave duration and amplitude for each V_1 lead position was used to calculate the P-wave voltage-duration product and P-wave area. The P-wave voltage-duration product was calculated as the algebraic product of P-wave amplitude and duration (Molloy et al., 1992), while P-wave area was obtained using the formula: [P-wave amplitude x 1/2P-wave duration] (Zeng et al., 2003). The P-wave axis was calculated for each patient from P-wave amplitudes documented in leads I and aVF (Li et al., 2014). Lastly, mean heart rate, heart rhythm and QRS mean electrical axis were recorded for every patient. Prior to measurements, ECG tracings were enlarged using the zoom option for better visualization of electrocardiographic waves.

2.4 STATISTICAL ANALYSIS

Normality of all groups of data was assessed using the Shapiro Wilk test. Data was expressed as median with interquartile range. In order to make comparisons between two groups of data, the T test was used for parametric values, while the Mann-Whitney test was used for non-parametric values. Similarly, to make comparisons between three or more groups of data, the one-way ANOVA test was used for parametric data, whereas the Kruskal-Wallis test was used for non-parametric data. Tukey's post-hoc test was used after ANOVA test and Dunn's post-hoc test after Kruskal-Wallis analysis. Correlation analyses were performed with Pearson test for parametric data and Spearman test for non-parametric data. Cut-off values with the best combination of sensitivity and specificity values were obtained for the five electrocardiographic parameters evaluated in this study using receiver operator curve (ROC) analysis. Chi-square test was used to assess association between the frequency of detection of PTF and V_1 lead location. In all statistical

analyses, the significance was set at 0.05. Statistical tests were performed with commercially available software³.

3 RESULTS

A total of 47 healthy dogs (25 female and 22 male) with a median age of four years (IQR, 3) and median weight of 12 kg (IQR, 10.1), and 74 MMVD dogs (41 female and 33 male) with median age of 11 years (IQR, 4) and a median weight of 6.8 kg (IQR, 6.5) were enrolled in the study. A variety of breeds were represented, including: Yorkshire terrier (n=14), Poodle (n=10), Dachshund (n=8), Shih-tzu (n=7), Whippet (n=5), German Shepherd (n=4), Lhasa Apso (n=4), Chihuahua (n=3), Miniature Pinscher (n=3), Schnauzer (n=3), Border Collie (n=2), French Bulldog (n=2), Pug (n=2), Shetland Sheepdog (n=2), as well as Akita, Beagle, English Cocker Spaniel, Golden Retriever, Labrador Retriever, Mallinois, Maltese and Spitz (one each). There were also 44 mixed-breed dogs. Pure-breed dogs included 15 brachymorphic, 40 mesomorphic and 22 dolichomorphic animals. Table 1 shows the characteristics of the studied population. Sinus arrhythmia was the predominant cardiac rhythm (30 healthy and 60 MMVD dogs), followed by sinus tachycardia (8 healthy and 9 MMVD) and sinus rhythm (1 healthy and 5 MMVD dogs).

As shown in Table 2, frequency of detection of PTF was similar at the three V_1 intercostal locations for control dogs, whilst for MMVD patients, frequency of detection was better at 1CCJ and 3CCJ locations when compared to the 5ECJ location ($p=0.0024$). The polarity of P-wave is shown in Table 3. In both 1CCJ and 3CCJ locations, the P-wave was negative for almost all dogs. This meant that PTF was the positive deflection at the end of the negative P-wave (Figure 1). When the 5ECJ location was used, although most P-waves were positive, some other P-wave forms were also documented (negative, biphasic and mixed). Despite heart rate, age and sex did not affect PTF, the results obtained from the 1CCJ location were correlated with body weight ($r= 0.2397$; $p=0.0397$).

³ Prism 8 for Windows 64-bit. Version 8.3.0 (538). GraphPad Software, LLC.

Results of all P-wave parameters obtained from healthy and MMVD dogs (B1, B2 and C+D) are shown in Table 4. All ECG parameters in the control and B1 groups had statistical difference between at least one V_1 location ($p < 0.05$). Conversely, B2 and C+D groups showed no statistical difference between the three V_1 locations for any ECG parameter, except for PTF ($p > 0.05$).

Sensitivity and specificity analysis were performed to investigate whether P-wave indices would differentiate healthy and diseased dogs (Table 5). For 1CCJ and 3CCJ locations, the higher the cut-off value of P-wave duration, P-wave area and Cornell product, the higher the specificity and the lower the sensitivity. Conversely, these three ECG parameters at the 5ECJ location showed decreasing cut-off values as the specificity increased and the sensitivity decreased.

To differentiate control from MMVD dogs, the highest areas under the curves were calculated for P-wave area or Cornell product obtained from 3CCJ recordings (0.7234 and 0.7238, respectively; $p < 0.0001$). Nonetheless, P-wave duration obtained from the 3CCJ produced the best sensitivity-specificity combination (71.6% and 65.9%, respectively), while PTF and P-wave axis failed to differentiate control and MMVD dogs. With the exception of the P-wave axis, better results were found when P-wave indices were used to differentiate control from MMVD dogs with remodeling (B2, C+D). The highest areas under the curve values were calculated for P-wave area and Cornell product obtained from the 3CCJ (0.8249 and 0.8249; $p < 0.0001$), while the best combination of sensitivity and specificity was found for the latter (75 and 70.2%, respectively) (Figure 2). For PTF_{V_1} , the best AUC to differentiate control from remodeled MMVD dogs was obtained from 1CCJ (0.6720; $p = 0.0184$) and 3CCJ (0.6933; $p = 0.0081$) locations (Figure 2; Table 5).

Receiver operating characteristics curves were constructed to investigate the ability of P-wave parameters to differentiate MMVD dogs with and without left atrial remodeling (B1 vs. B2+C+D) as well as asymptomatic and symptomatic MMVD dogs (B1+B2 vs. C+D). These groups could only be distinguished using P-wave area (AUC=0.6733; $p = 0.0157$) or P-wave duration (AUC=0.6992; $p = 0.0050$) obtained from 3CCJ recordings for non-remodeled vs. remodeled MMVD dogs. The P-wave axis

produced the best AUC for distinguishing between asymptomatic and symptomatic dogs (AUC=0.661; $p=0.0270$).

Only P-wave duration and PTF correlated with echocardiographic surrogates of cardiac dilation and congestion. When measurements were made from 1CCJ ECG recordings in MMVD dogs, PTF was significantly correlated with LA/Ao ratio ($r=0.2700$; $p=0.0200$), LVIDd ($r=0.3803$; $p=0.0008$), NLVIDd ($r=0.3196$; $p=0.0055$) and E wave ($r=0.2798$; $p=0.0157$) (Figure 3). Likewise, PTF obtained from 3CCJ recordings also correlated significantly with LA/Ao ratio ($r=0.2345$; $p=0.0443$), LVIDd ($r=0.2743$; $p=0.0180$), NLVIDd ($r=0.2419$; $p=0.0379$) and E wave ($r=0.2829$; $p=0.0146$).

For P-wave duration, significant correlations existed with LVIDd ($r=0.4939$; $p<0.0001$), NLVIDd ($r=0.4067$; $p=0.0003$), LVIDs ($r=0.3820$; $p=0.0008$), NLVIDs ($r=0.3471$; $p=0.0024$), E wave ($r=0.2689$; $p=0.0205$) and E wave/IVTR ratio ($r=0.2630$; $p=0.0236$) when measurements were obtained from 1CCJ. When 3CCJ was used instead, P-wave duration correlated significantly with all echocardiographic parameters, i.e. LA/Ao ratio ($r=0.2384$; $p=0.0408$), LVIDd ($r=0.5113$; $p<0.0001$), NLVIDd ($r=0.4592$; $p<0.0001$), LVIDs ($r=0.4512$; $p<0.0001$), NLVIDs ($r=0.4219$; $p=0.0002$), E wave ($r=0.3614$; $p=0.0016$), and E wave/IVTR ratio ($r=0.3748$; $p=0.001$) (Figure 4). In contrast, when using 5ECJ ECG recordings, P-wave duration correlated significantly with only LVIDd ($r=0.3257$; $p=0.0052$) and LVIDs ($r=0.2695$; $p=0.0220$). For P-wave axis, significant correlations were identified with LA/Ao ratio ($r=0.2294$; $p=0.0493$), E wave ($r=0.2636$; $p=0.0232$) and E wave/IVRT ($r=0.2457$; $p=0.0349$). None of the other ECG parameters obtained from any V_1 location correlated with the echocardiographic parameters recorded in MMVD dogs.

4 DISCUSSION

The P-wave terminal force (PTF) obtained from lead V_1 ECG recordings is the most reliable electrocardiographic marker of left atrial morbidities in human cardiology (Hazen et al., 1991; Hancock et al., 2009). In this study, we investigated whether PTF might be identified in dogs and whether it correlates with

echocardiographic measurements of left atrial enlargement and left-sided congestion. Additionally, we studied four other electrocardiographic parameters: P-wave duration, P-wave area, P-wave Cornell product and P-wave axis.

In people, the precordial lead V_1 is characterized by a QRS complex of consistent rS pattern (R wave/S wave < 1). The deep S wave represents right ventricular depolarization (Levy et al., 1950). A recent study of the three dog morphotypes, showed that the locations in the first and third intercostal spaces at the level of the costochondral junction were the most similar to the precordial lead V_1 in people (Santilli et al., 2019). In our study, positioning the precordial lead V_1 in the first and third intercostal spaces usually resulted in negative P waves (Table 3), which is consistent with previous studies (Takahashi and Kita, 1964; Santilli et al., 2019). Notably, the P-wave in the precordial lead V_1 is biphasic in people, with the first wave positive and the second wave negative (Meek and Morris, 2002). This is a significant difference between species. Locating lead V_1 in the fifth intercostal space (the modified Lannek precordial lead system) tested the use of one of the most commonly used precordial lead system in dogs (Detweiler and Patterson, 1965; Tilley et al., 2008) to detect PTF. Consistent with findings of other studies (Takahashi and Kita, 1964; Santilli et al., 2019) the fifth intercostal space showed greater variation in P-wave morphologies (negative, positive, biphasic, mixed and absent) than 1CCJ and 3CCJ.

These inter and intra species variations in P-wave morphology can be understood by comparing the mean vector of atrial activation of man and dog and by analyzing the distribution of electrical potentials on the body surface of the dog. In man the direction of the mean vector of atrial activation follows an inferior, anterior and leftward pattern (Selvester and Haywood, 1968), whilst in the dog it has an inferior, posterior and leftward pattern (Santilli et al., 2018). In the dog the thoracic limit where the negative and positive electrical potentials are equal is called the transitional zone, and goes upward across the thorax from the left side at the level of the costochondral junction of the second rib, to the right side at the level of the costochondral junction of the fifth rib (Takahashi and Kita; 1964). The electrical potentials cranial to this border are negative, caudal ones are positive, and electrical

potential across this border tend to be biphasic. Consequently, P-wave and QRS complexes cranial to the transitional zone are predominantly negative in the dog (Takahashi and Kita; 1964). It is worth mentioning that previous research found that thoracic conformation, such as brachymorphism, influences P-wave measurements. P-wave amplitude and duration differed in brachymorphic dogs from those obtained from mesomorphic and dolichomorphic dogs at the 3CCJ and 5ECJ locations (Santilli et al., 2019).

According to experimental studies in dogs, the negative P-wave on precordial leads represents right atrial depolarization (Takahashi and Kita, 1964). Nonetheless, it is also stated that the final part of the P-wave represents left atrial depolarization in this species (Santilli et al., 2018). In people, PTF is the area between the baseline and the negative wave of the biphasic P-wave (Morris et al., 1964). Based on this information, in this study we characterized PTF_{V_1} as the positive deflection following the end of the negative P-wave in either lead V_1 location.

In people, Morris et al. (1964) found that PTF values of -1 mm (-0.1 mV) and 0.04 s (40 ms), i.e. -0.04 mm.s (4 mV.ms), had 92% sensitivity for the presence of left-sided valvular heart disease. Their study included heart diseases with varying degrees of insufficiency and stenosis of the mitral and/or aortic valves. In our study, none of the tested V_1 locations was able to accurately distinguish control from MMVD dogs in the logistic regression analysis (Table 5). However, when only dogs with left atrial enlargement were considered, the 1CCJ location offered an $AUC=0.6720$ ($p=0.0184$) for PTF_{V_1} values >1.49 mV.ms (best combination of sensitivity and specificity, 62.5% and 76.6%, respectively), while the 3CCJ location produced an $AUC= 0.6933$ ($p= 0.0081$) for PTF_{V_1} values >1.75 mV.ms (best combination of sensitivity and specificity, 62.5% and 74.47%, respectively). Interestingly, increasing the PTF cut-off value resulted in higher specificity and lower sensitivity. Studies of PTF in man also produced better specificities than sensitivities for detection of left atrial enlargement (Chirife et al., 1975; Hopkins and Barrett, 1989; Hazen et al., 1991). Remarkably, our study found that both the 1CCJ and 3CCJ locations reached their highest specificities when using a PTF cut-off >2 mV.ms.

LA/Ao ratio, LVID, NLVIDd and E wave correlated with PTF. In people, correlation values between left atrial size and PTFV₁ are also positive (Miller et al., 1983; Hopkins and Barrett, 1989; Elbey et al., 2012). In dogs with MMVD, LA/Ao ratio values >1.6 are indicative of left atrial enlargement (Rishniw and Erb, 2000) and predict cardiac mortality (Sargent et al., 2015). LVIDd and NLVIDd, which are surrogates for left ventricular dilatation (Cornell et al., 2004), also correlated with PTF. In dogs with MMVD, an NLVIDd ≥ 1.7 has been associated with rapid disease progression, development of congestive heart failure and cardiac-related death (Hezzell et al., 2012; Kim and Park, 2015; Sargent et al., 2015). Similarly, human patients with mitral insufficiency have increased PTF and LVIDd values (Elbey et al., 2012). A study in people with mitral regurgitation found LVIDd to be the only independent predictor of an accelerated increase of mitral regurgitation over time (Suri et al., 2011). The E wave is part of the mitral inflow profile and is influenced by a variety of factors, including ventricular systolic pressure, myocardial relaxation rate and left atrial pressure (Choong et al., 1988). In our study, E wave correlated with PTF. In dogs with MMVD, E wave velocity rises due to the increase in left atrial pressure secondary to mitral insufficiency (Kim and Park, 2015). In people, left atrial pressure correlated with P-wave terminal force (Boswood et al., 2016). Human studies showed that the frequency of detection of PTF was highest in patients with moderate and significant mitral regurgitation (Elbey et al., 2012).

The fact that PTFV₁ is the most reliable electrocardiographic marker of left-sided valvular lesions in man has a vectorial explanation. The axis of lead V₁ and the vector that originates the second half of P-wave are both in the horizontal plane and are almost parallel, which causes no negative terminal force in healthy people (Morris et al., 1964). Conversely, left-sided valvular lesions deviate the referred vector posteriorly, which causes a deep negative deflection at the end of P-wave in lead V₁ (Morris et al., 1964). In the frontal plane, there is no coincidence of any lead axis with the vector that represents left atrial depolarization (Morris et al., 1964).

The recently proposed 1CCJ location for precordial lead V₁ in healthy dogs was considered the most appropriate positioning since it evaluated the best right ventricular depolarization (small R wave, deep S wave), similar to readings from

precordial lead V_1 in people (Santilli et al., 2019). However, that research did not provide information on how parallel the vector of left atrial depolarization is to the axis of the 1CCJ location. To the author's knowledge, there is no information regarding how the vector of left atrial depolarization is shifted by left-sided valvular diseases in the dog to create PTF. Despite this, the higher PTF values documented in MMVD dogs with left atrial enlargement (increased LA/Ao ratio) in comparison to control dogs suggest the existence of a similar relationship between the 1CCJ lead axis and the vector of atrial depolarization in this species.

In veterinary (Tilley et al., 2008; Santilli et al., 2018) and human (Faggiano et al., 1997; Ariyaratnam et al., 2005) medicine, increased P-wave duration in the frontal leads is associated with left atrial overload and may coincide with left atrial enlargement. In this study, we evaluated P-wave duration in three locations of the precordial lead V_1 . P-wave duration was the best ECG surrogate for differentiation between controls and MMVD dogs with remodeling (Table 5). The best combination of sensitivity and specificity (95.83% and 65.96%, respectively) was documented from 3CCJ ECG recordings (P-wave duration >41.5 ; AUC=0.8027; $p<0.0001$). Another study found that a P-wave duration >40 ms in frontal lead II has a combined sensitivity and specificity of 68 and 64%, respectively, for detection of left atrial enlargement (Savarino et al., 2012). In our study, a correlation was indeed demonstrated between P-wave duration and LA/Ao ratio in MMVD dogs with dilated LA. Other studies have also demonstrated correlations between lead II P-wave duration and LA/Ao ratio in dogs (Savarino et al., 2012; Soto-Bustos et al., 2017).

When using Cornell product to differentiate healthy and MMVD dogs with dilated LA, the best combination of sensitivity and specificity was obtained from 3CCJ ECG recordings (table 5). Cornell product values >3.7 ms.mV were shown to identify left atrial enlargement (LA/Ao ≥ 1.6) with a 75% sensitivity and 70.2% specificity (AUC=0.8249; $p<0.0001$). In man, Cornell product applied to the QRS complex significantly improves sensitivity of detection of left ventricular hypertrophy over QRS duration or amplitude alone (Molloy et al., 1992) and is considered as an electrocardiographic criteria for the management of hypertensive patients (Williams et al., 2018).

P-wave area was also satisfactory in distinguishing control from remodeled MMVD dogs in our study. In people, P-wave area is considered a marker of left atrial abnormalities (Hancock et al., 2009; Weinsaft et al., 2014). A P-wave area >4 ms.mV was shown to accurately identify left atrial enlargement in patients with mitral stenosis. Moreover, a correlation was found between left atrial diameter and P-wave area (Zeng et al., 2003). In dogs with MMVD in this study, P-wave area (3CCJ) >2.2 ms.mV produced the best combination of sensitivity (62.5%) and specificity (85.11%) for detection of left atrial dilatation (AUC=0.8249; $p<0.0001$). Curiously, it did not correlate with any of the echocardiographic parameters. A previous study also found a difference between P-wave areas in healthy and MMVD dogs with left atrial enlargement. Similarly, that study reported a low correlation coefficient between P-wave area values and LA/Ao ratios (Soto- Bustos et al., 2017).

P-wave axis was the least useful ECG parameter for the purposes of this study. Even though it could be used to differentiate control and MMVD dogs as well as asymptomatic and symptomatic MMVD dogs, the calculated AUC were lower than those produced for other ECG parameters. Moreover, no correlations between P-wave axis and echocardiographic surrogates of cardiac remodeling were documented. Normal P-wave axis reference values vary between $+90^\circ$ to -20° for dogs (Santilli et al., 2018). In our study, P-wave axis values for all dogs were within that normal range (Table 4). Another study with MMVD dogs found a mild correlation between P-wave axis and LA/Ao ratio ($R=0.129$; $p<0.01$), but left atrial enlargement could not be identified using the P-wave axis. In comparison to our research, that study showed larger P-wave axis alterations, even though only B1 and B2 dogs were enrolled (Soto-Bustos et al., 2017). In contrast, P-wave axis $<30^\circ$ in people is regarded as highly specific for left atrial enlargement (Li et al., 2014), and studies in human patients with heart diseases showed a higher cardiac mortality among patients with abnormal P-wave axis (Tsao et al., 2008).

In medicine, a study which investigated electrocardiographic surrogates of left atrial dilatation obtained from precordial lead V_1 (P-wave area, P-wave duration, P-wave amplitude and P terminal force) found positive correlations with the left atrial area obtained by cardiac magnetic resonance imaging (Weinsaft et al., 2014).

Interestingly, studies that demonstrate the usefulness of P-wave parameters obtained from precordial leads recordings to investigate left atrial dilatation in dogs with MMVD are lacking in veterinary medicine. This makes our data unique and might facilitate the triage of dogs found to have a cardiac murmur at physical examination.

Electrocardiography has widely known limitations as a marker of cardiac morbidities, mainly due to its high specificity along with poor sensitivity in both people (Hazen et al., 1991; Tsao et al., 2008) and dogs (Noszczyk-Nowak et al., 2011; Soto-Bustos et al., 2017). In the present study, the five ECG parameters studied had those same limitations. This is not surprising since parameters such as: inter-atrial conduction abnormalities (Pettersson et al., 2014), intra-atrial volume and pressure (Kasser and Kennedy, 1969), and even levels of mental stress (O'Neal et al., 2017) may alter P-wave duration, amplitude, or both, notwithstanding the presence or absence of atrial enlargement.

5 LIMITATIONS

We acknowledge the limitations of this study. Firstly, the sample size of dogs with left atrial enlargement was smaller than that of stage B1 and control dogs. Likewise, the role played by thoracic conformation in the ECG parameters assessed in this study was not investigated. Also, we recognize that the positioning of precordial lead V₁ might have been imprecise in some patients due to either difficulty in palpating the ribs or a restless patient. More importantly, we cannot completely rule out the possibility that some electrocardiographic artifact might have been interpreted as a deflection. However, we made an effort to discard all electrocardiographic tracings of poor quality. Lastly, all measurements of the study were performed by an investigator not blinded to the echo diagnosis of each patient.

6 CONCLUSIONS

In dogs, P-wave terminal force can be best documented in the 1CCJ and 3CCJ locations for precordial lead V₁, and its polarity is opposite to the one described in

people. P-wave terminal force, P-wave duration, P-wave area and P-wave Cornell product obtained from lead V₁ ECG recordings may help identify dogs with left atrial enlargement secondary to MMVD.

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TABLES AND FIGURES

TABLE 1 Characteristics and echocardiographic parameters of all dogs included in the study.

	Control (n=47)	B1 (n=50)	B2 (n=8)	C+D (n=13+3)	p-value
Age	4 (5;2)	10 (12;8)	13.5 (14;10.5)	12 (14;11)	<0.0001
Sex (F/M)	25/22	28/22	3/5	10/6	-
Weight	12 (17.8;7.7)	7.3 (13.1;4.6)	6.6 (10.2;4.7)	5.4 (9.7;2.8)	0.0014
LA/Ao ratio	1.23 (1.33;1.14)	1.2 (1.29;1.12)	1.76 (1.91;1.64)	2.16 (2.52;1.94)	<0.0001
LVIDd	2.79 (3.36;2.35)	2.6 (3.13;2.17)	2.94 (3.66;2.86)	3.56 (4.16;2.80)	0.0079
NLVIDd	1.38 (1.47;1.3)	1.44 (1.56;1.35)	1.79 (2.05;1.58)	2.09 (2.21;1.87)	<0.0001*
LVIDs	1.72 (2.09;1.34)	1.50 (2.05;1.22)	1.59 (2.29;1.21)	1.73 (2.22;1.28)	0.6877
NLVIDs	0.79 (0.83;0.75)	0.79 (0.89;0.70)	0.84 (1.15;0.75)	1.07 (1.21;0.79)	0.0032
E-wave	73.20 (82.60;64.80)	65.55 (76.08;54.93)	108.0 (116.8;90.45)	135.0 (165.3;97.95)	<0.0001*
E-wave/IVRT	1.1 (1.3;0.91)	0.89 (1.20;0.74)	1.49 (1.62;1.27)	2.26 (3.36;1.80)	<0.0001

Values expressed as median (IQR). * Indicate parametric statistical analysis. F, female; M, male; LA/Ao ratio, left atrium-to-aorta ratio; LVIDd, diastolic left ventricular internal diameter; NLVIDd, normalized diastolic left ventricular internal diameter; LVIDs, systolic left ventricular internal diameter; NLVID, normalized systolic left ventricular internal diameter; E-wave, peak early diastolic mitral inflow velocity; E-wave/IVRT, peak early diastolic mitral inflow velocity to isovolumic relaxation time index. B1, B2, C and D indicate the stage of myxomatous mitral valve disease.

TABLE 2 Frequency of PTFV₁ detection at each V₁ location.

	1CCJ		3CCJ		5ECJ	
Control (n=47)	45	96%	45	96%	42	89%
B1 (n=50)	48	96%	46	92%	15	30%
B2 (n=8)	8	100 %	8	100 %	3	38%
C (n=13) ^a	12	92%	11	85%	6	50%
D (n=3) ^a	2	67%	3	100%	0	0%

^aP-wave terminal force (PTFV₁) was not recorded at 5ECJ in one patient. B1, B2, C and D indicate the stage of myxomatous mitral valve disease. 1CCJ: Precordial lead V₁ positioned in the first intercostal space at the level of the costochondral junction. 3CCJ: Precordial lead V₁ positioned in the third intercostal space at the level of the costochondral junction. 5ECJ: Precordial lead V₁ positioned in the fifth intercostal space at the level of the sternochondral junction.

TABLE 3 Frequency of P-wave polarity at each V₁ location.

	1CCJ	3CCJ	5ECJ
Control (n=47)	47N	43N, 4M	39P, 5N, 3M,
B1 (n=50)	50N	47N, 2P, 1M	33P, 7M, 6N, 4B
B2 (n=8)	8N	8N	5P, 2N, 1B
C (n=13) ^a	13N	13N	7P, 3B, 1M, 1N
D (n=3) ^a	3N	3N	2P

^aP-wave terminal force (PTF) was not recorded at 5ECJ in one patient. P-wave polarity: P=positive; N=negative; B=biphasic; M=mixed. B1, B2 C and D indicate the stage of myxomatous mitral valve disease. 1CCJ: Precordial lead V₁ positioned in the first intercostal space at the level of the costochondral junction. 3CCJ: Precordial lead V₁ positioned in the third intercostal space at the level of the costochondral junction. 5ECJ: Precordial lead V₁ positioned in the fifth intercostal space at the level of the sternochondral junction.

TABLE 4 P-wave parameters obtained in healthy and B1, B2 and C+D MMVD dogs using three different lead V₁ locations.

ECG parameter	lead V ₁	Control (n=47)	B1 (n=50)	B2 (n=8)	C+D (n=13+3)	p-value
PTF	1CCJ	1.32 (1.48; 1.04) ^a	1.34 (1.69; 0.99) ^a	1.58 1.63; 1.2) ^a	1.68 (2.21; 1.18) ^{ab}	0.1332
	3CCJ	1.48 (1.85; 1.08) ^a	1.52 (2.16; 1.19) ^a	1.68 (1.96; 1.35) ^a	2.04 (2.83; 1.38) ^a	0.0613
	5ECJ	0.00 (0.00; 0.00) ^b	0.00 (1.45; 0.00) ^b	0.00 (2.03; 0.00) ^a	0.00 (1.70; 0.00) ^b	0.4624
P-wave duration	1CCJ	45 (47; 41) ^a	46 (48.3; 42) ^a	49 (52.3; 44) ^a	47.00±4.75 ^a	0.0304*
	3CCJ	40 (45; 37) ^b	43 (46.3; 40) ^a	51.5 (53; 43) ^a	45.00±3.75 ^a	0.0002
	5ECJ	63 (73; 53) ^c	56.5 (65.5; 42.3) ^b	46 (63.5; 39) ^a	48.50±26.50 ^a	0.0036*
P-wave area	1CCJ	2.03 (2.59; 1.58) ^a	2.28 (2.93; 1.78) ^{ab}	2.54 (5.58; 1.39) ^a	2.84 (3.72; 2.20) ^a	0.0259
	3CCJ	1.58 (2.15; 1.17) ^b	2.00 (2.59; 1.56) ^b	2.19 (5.63; 1.75) ^a	2.37 (3.84; 1.92) ^a	<0.0001
	5ECJ	3.57 4.37; 2.52) ^c	2.45 (3.76; 1.59) ^a	2.63 (3.29; 1.40) ^a	2.30 (3.56; 1.47) ^a	0.0219
Cornell product	1CCJ	4.05 (5.17; 3.15) ^a	4.55 (5.86; 3.56) ^{ab}	5.07 (11.17; 2.77) ^a	5.68 (7.44; 4.40) ^a	0.0256
	3CCJ	3.15 (4.30; 2.34) ^b	4.00 (5.17; 3.12) ^a	4.37 (11.26; 3.49) ^a	4.73 (7.68; 3.84) ^a	<0.0001
	5ECJ	7.14 (8.73; 5.04) ^c	4.89 (7.52; 3.17) ^b	5.25 (6.58; 2.78) ^a	4.59 (7.12; 2.94) ^a	0.0217
P-wave axis	63.50 (68.7; 47.0)	50.8 (60.4; 39.2)	51.5 (64.2; 38.0)	58.4 (73.2; 48.6)	0.0045	

Different superscript letters imply statistical difference inside each column of three different V₁ locations. Data is expressed as median (IQR) for all ECG parameters. * Indicates a parametric statistical test. B1, B2 C and D indicate the stage of myxomatous mitral valve disease. 1CCJ: Precordial lead V₁ positioned in the first intercostal space at the level of the costochondral junction. 3CCJ: Precordial lead V₁ positioned in the third intercostal space at the level of the costochondral junction. 5ECJ: Precordial lead V₁ positioned in the fifth intercostal space at the level of the sternochondral junction.

TABLE 5 Sensitivity and Specificity of five ECG parameters to distinguish between healthy and MMVD dogs.

	ECG parameter	V ₁ lead location	AUC	95% CI	Cut-off value	Sensitivity (%)	Specificity (%)	p-value	
Healthy vs. MMVD (all)	PTF	1CCJ	0.5758	0.4739 to 0.6777	> 1.34	56.8	55.3	0.1611	
		3CCJ	0.6137	0.5137 to 0.7138	> 1.51	59.5	55.3	0.0354	
		5ECJ	0.5761	0.4558 to 0.6964	> 1.26	30.6	85.2	0.2449	
	P-wave duration	1CCJ	0.6173	0.5169 to 0.7177	> 45.50	56.8	61.7	0.0300	
		3CCJ	0.687	0.5893 to 0.7847	> 41.50	71.6	66.0	0.0005	
		5ECJ	0.68	0.5837 to 0.7762	< 60.50	65.3	63.8	0.0009	
	P-wave area	1CCJ	0.6318	0.5310 to 0.7327	> 2.04	73.0	53.2	0.0148	
		3CCJ	0.7234	0.6299 to 0.8169	> 1.61	79.7	59.6	<0.0001	
		5ECJ	0.6664	0.5689 to 0.7639	< 2.49	55.6	78.7	0.0022	
	Cornell product	1CCJ	0.6323	0.5314 to 0.7331	> 4.08	73.0	53.2	0.0144	
		3CCJ	0.7238	0.6303 to 0.8173	> 3.21	79.7	59.6	<0.0001	
		5ECJ	0.6665	0.5690 to 0.7640	< 4.98	55.6	78.7	0.0022	
	P-wave axis		0.6622	0.5615 to 0.7628	< 61.55	75.7	59.6	0.0027	
	Healthy vs. MMVD (remodeled)	PTF	1CCJ	0.6720	0.5282 to 0.8158	> 1.49	62.5	76.6	0.0184
			3CCJ	0.6933	0.5570 to 0.8295	> 1.75	62.5	74.5	0.0081
5ECJ			0.6052	0.4423 to 0.7681	> 1.26	36.4	85.2	0.209	
P-wave duration		1CCJ	0.7154	0.5875 to 0.8433	> 46.50	66.7	66.0	0.0031	
		3CCJ	0.8027	0.6995 to 0.9060	> 41.50	95.8	66.0	<0.0001	
		5ECJ	0.7408	0.6104 to 0.8712	< 55.50	68.2	70.2	0.0013	
P-wave area		1CCJ	0.6928	0.5518 to 0.8338	> 2.18	75.0	59.6	0.0082	
		3CCJ	0.8249	0.7300 to 0.9198	> 2.225	62.5	85.11	<0.0001	
		5ECJ	0.6954	0.5565 to 0.8342	< 2.98	72.7	61.7	0.0093	
Cornell product		1CCJ	0.6933	0.5523 to 0.8342	> 4.35	75.0	59.6	0.0081	
		3CCJ	0.8249	0.7300 to 0.9198	> 3.70	75.0	70.2	<0.0001	
		5ECJ	0.6958	0.5571 to 0.8346	< 5.95	72.7	61.7	0.0091	
P-wave axis			0.5802	0.4348 to 0.7257	< 61.55	66.7	59.6	0.2713	

MMVD: myxomatous mitral valve disease. Remodeled: dogs belonging to B2+C+D stages of MMVD with LA/Ao \geq 1.6. PTF: P-wave terminal force. 1CCJ: Precordial lead V₁ positioned in the first intercostal space at the level of the costochondral junction. 3CCJ: Precordial lead V₁ positioned in the third intercostal space at the level of the costochondral junction. 5ECJ: Precordial lead V₁ positioned in the fifth intercostal space at the level of the sternochondral junction.

FIGURE 1 Electrocardiographic tracings (lead V₁) in the dog. (A) Arrow indicates a P-wave followed by no terminal force. (B) Arrow indicates a P-wave followed by a visible terminal force (bluish area) of a dog with MMVD (stage B1). Letters P, R, S and T indicate four electrocardiographic waves of the sinus complex.

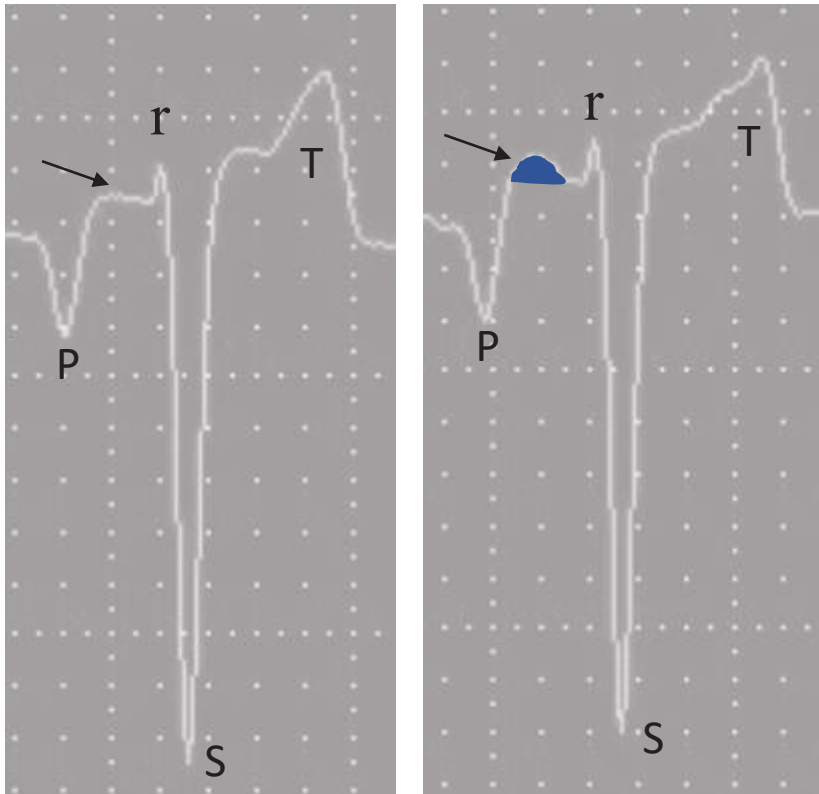


FIGURE 2 Receiver operating characteristic curves of P-wave terminal force (A-B), P-wave duration (C), P-wave area (D), P-wave Cornell product (E) and P-wave axis (F). (A,B,C,D,E) demonstrate sensitivity and specificity of P-wave parameters in differentiating healthy and MMVD dogs with remodeled LA (stages B2+C+D), while (F) involves the differentiation of healthy and MMVD dogs regardless of LA size. (A)

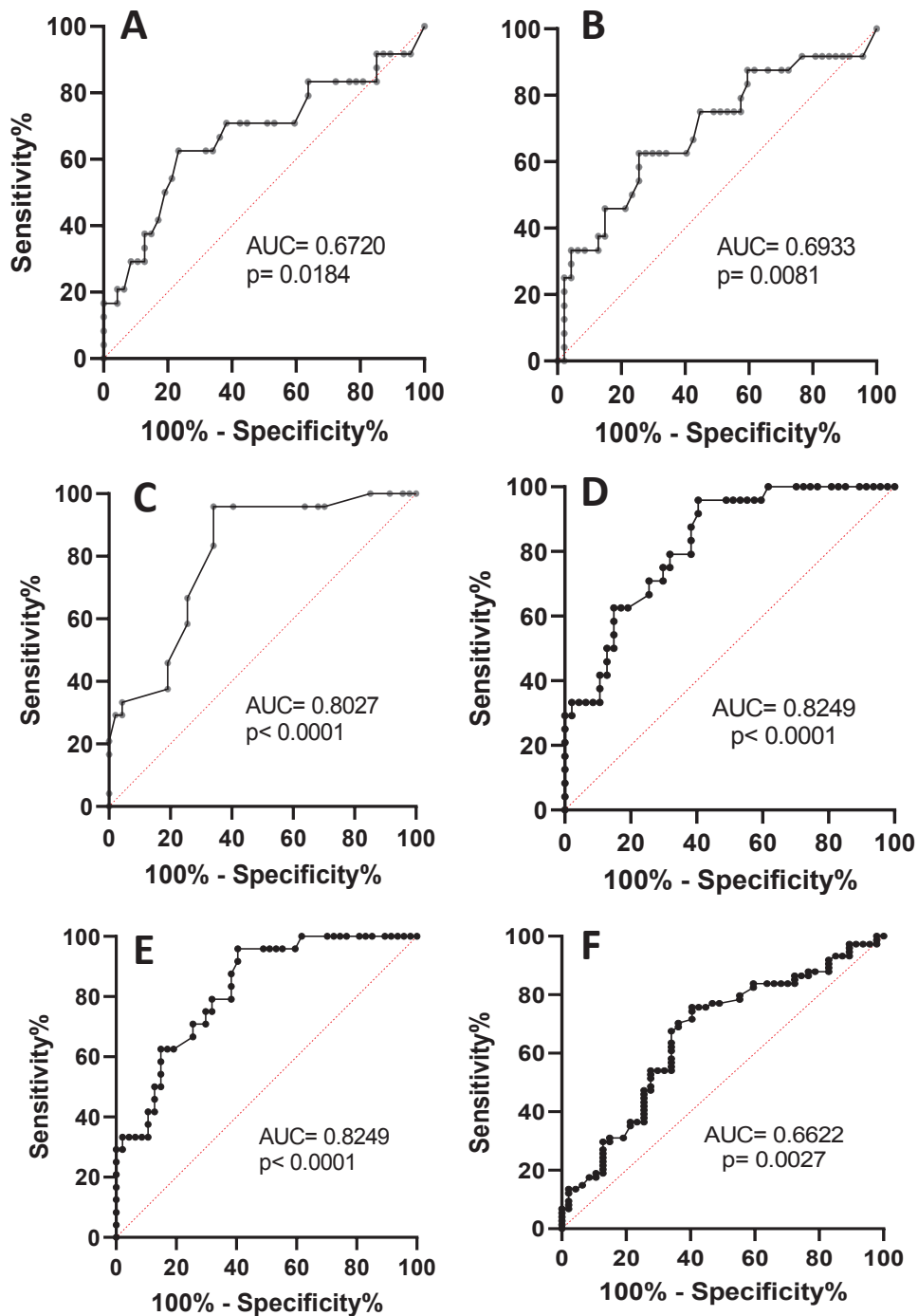


FIGURE 3 Correlations between P-wave terminal force and LA/Ao ratio (A), LVIDd (B), NLVIDd (C), and E-wave (D). ECG recordings were obtained at 1CCJ.

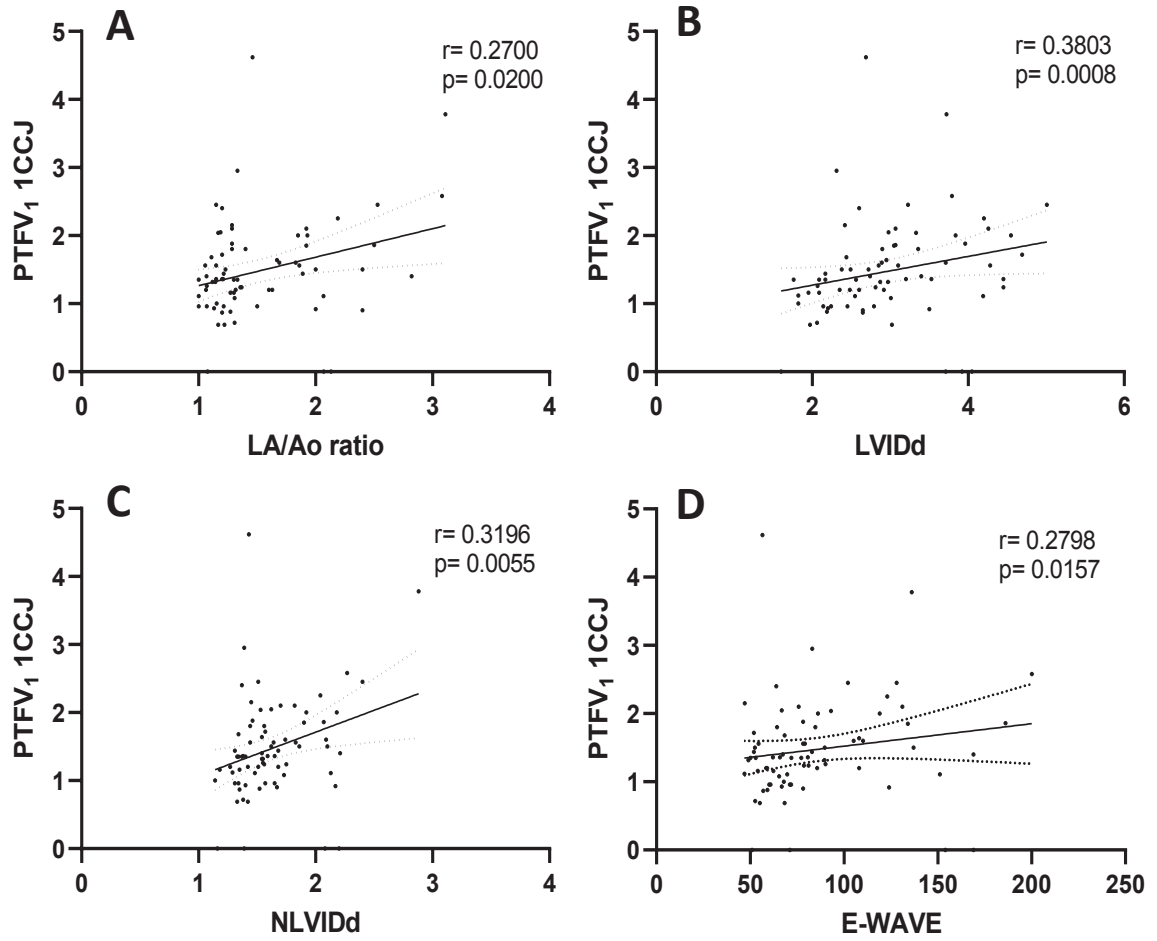
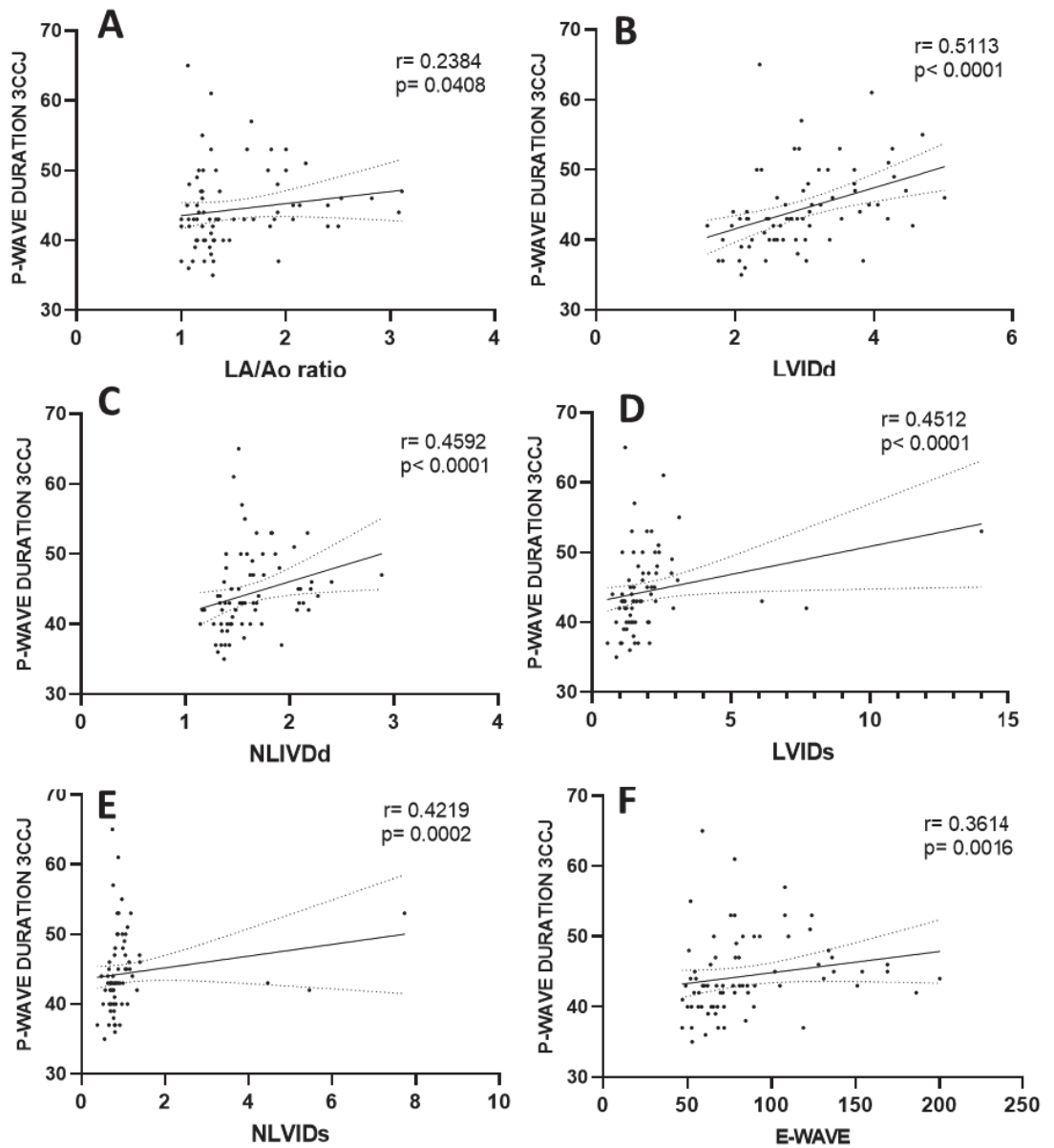


FIGURE 4 Correlations between P-wave duration and LA/Ao ratio (A), LVIDd (B), NLVIDd (C), LVIDs (D), NLVIDs (E), and E-wave (F). ECG recordings were obtained at 3CCJ.



ANEXO 1 Aprovação do estudo no Comitê de Ética do Setor de Ciências Agrárias da UFPR.



**UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS**

CERTIFICADO

Certificamos que o protocolo número 096/2018, referente ao projeto “**Força terminal da onda p na derivação V₁ em cães com doença mixomatosa da válvula mitral**”, sob a responsabilidade **Marlos Gonçalves Sousa** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino – encontra-se de acordo com os preceitos da Lei n° 11.794, de 8 de Outubro, de 2008, do Decreto n° 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 2 de invasividade, em reunião de 05/12/2018.

Vigência do projeto	Março/2019 até Novembro/2019
Espécie/Linhagem	<i>Canis familiaris</i> (cão)
Número de animais	250
Peso/Idade	Variável/Variável
Sexo	Macho e fêmea
Origem	Hospital Veterinário da Universidade Federal do Paraná, Curitiba, Paraná, Brasil

CERTIFICATE

We certify that the protocol number 096/2018, regarding the project “**P wave terminal force in lead V₁ in dogs with myxomatous mitral valve disease**” under **Marlos Gonçalves Sousa** supervision – which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law n° 11.794, of 8 October, 2008, of Decree n° 6.899, of 15 July, 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved by the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of the State of Paraná, Brazil), with degree 2 of invasiveness, in session of 05/12/2018.

Duration of the project	March/2019 until November/2019
Specie/Line	<i>Canis familiaris</i> (canine)
Number of animals	250
Wheight/Age	Variable /Variable
Sex	Male and Female
Origin	Veterinary Hospital of Federal University of Paraná, Curitiba, Brazil

Curitiba, 05 de dezembro de 2018

Chayane da Rocha
Chayane da Rocha

Coordenadora CEUA-SCA