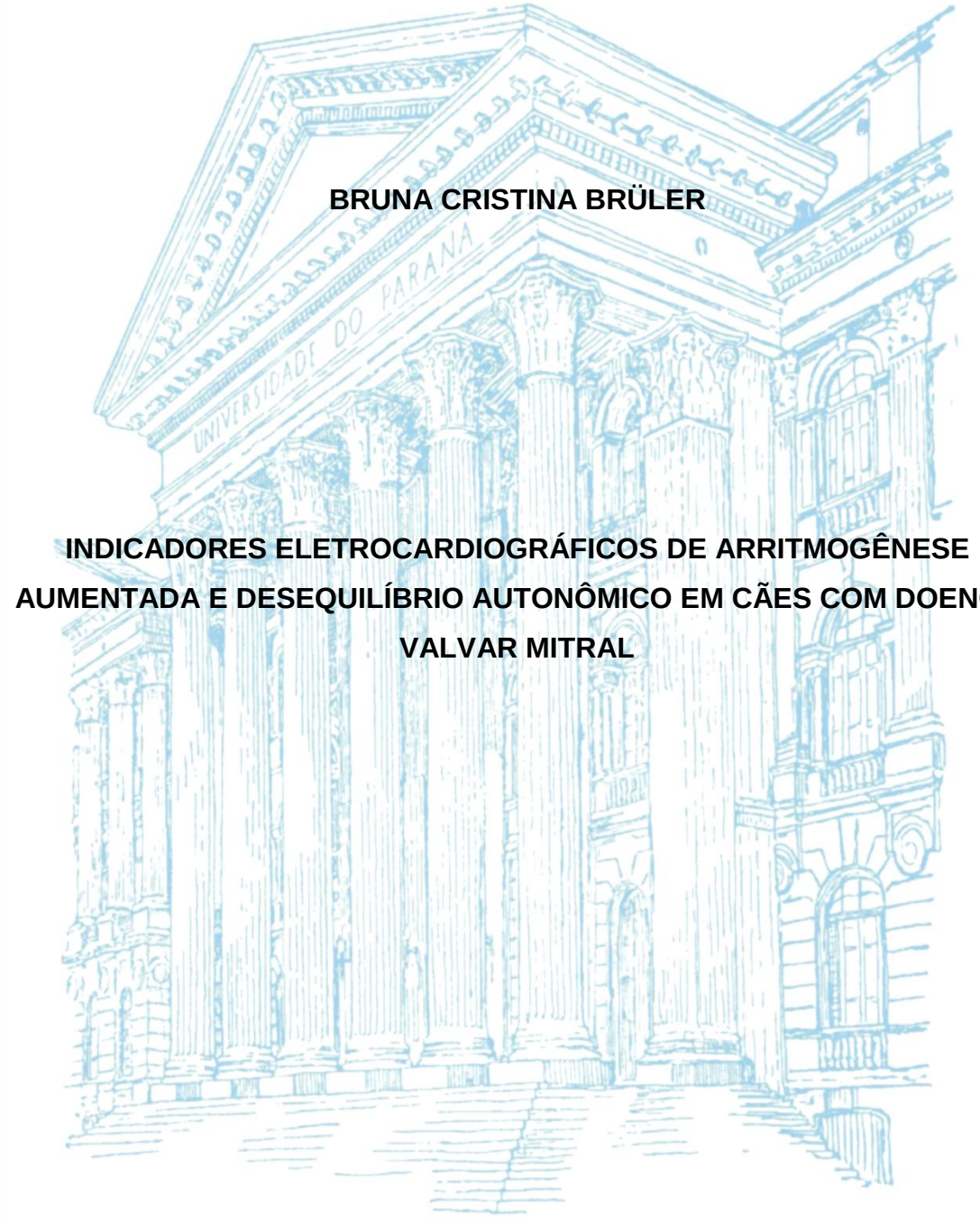
UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS



BRUNA CRISTINA BRÜLER

INDICADORES ELETROCARDIOGRÁFICOS DE ARRITMOGÊNESE AUMENTADA E DESEQUILÍBRIO AUTONÔMICO EM CÃES COM DOENÇA VALVAR MITRAL

CURITIBA
2017

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ELECTROCARDIOGRAPHIC SURROGATES OF INCREASED ARRHYTHMOGENESIS AND AUTONOMIC IMBALANCE IN DOGS WITH MITRAL VALVE DISEASE

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

Orientador: Prof. Dr. Marlos Gonçalves Sousa

CURITIBA

2017

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS

UFPR

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A Comissão Examinadora da Defesa da Dissertação intitulada **"INDICADORES ELETROCARDIOGRÁFICOS DE ARRITMOGÊNESE AUMENTADA E DESEQUILÍBRIO AUTONÔMICO EM CÃES COM DOENÇA VALVAR MITRAL"** apresentada pela Mestranda **BRUNA CRISTINA BRÜLER** declara ante os méritos demonstrados pela Candidata, e de acordo com o Art. 79 da Resolução nº 65/09–CEPE/UFPR, que considerou a candidata APROVADA para receber o Título de Mestre em Ciências Veterinárias, na Área de Concentração em Ciências Veterinárias.

Curitiba, 24 de março de 2017

Professor Dr. Marlos Gonçalves Sousa
Presidente/Orientador

Professor Dr. Flávio Ribeiro Alves
Membro

Professora Dra. Roberta Carareto
Membro

AGRADECIMENTOS

A Deus, por tornar tudo possível.

Aos meus pais José Otávio e Siomara, por todo amor e apoio durante essa jornada, e ao irmão Rafael, que mesmo longe se faz presente.

Ao professor Marlos não somente pela orientação, mas pelo seu papel como educador, amigo e inspiração. E aos professores do programa de pós-graduação da UFPR pela contribuição fundamental neste percurso, de aluna pra mestre.

À equipe do Laboratório de Cardiologia Comparada do Hospital Veterinário UFPR, pelo aprendizado, paciência e companheirismo sem medida.

Aos amigos feitos durante o mestrado, e a todos os outros conservados durante a vida, por estarem presentes nos melhores e piores momentos. E por transformarem essa e tantas outras batalhas em algo leve, bem humorado e feliz.

A todos, meu sincero e emocionado OBRIGADA!

“Nobody made a greater mistake than he who did nothing because he could do only a little.”

— Edmund Burke

RESUMO

Nesta dissertação foram estudados índices obtidos a partir de traçados eletrocardiográficos convencionais, com o objetivo de avaliar o desequilíbrio autonômico e os mecanismos de arritmogênese aumentada, ambos já reconhecidamente envolvidos com a progressão da doença mitral em cães. Para tanto, este trabalho foi subdividido em introdução e três capítulos. Inicialmente, procurou-se esclarecer sobre os aspectos etiológicos e fisiopatológicos da doença, dando ênfase para a ativação crônica de mecanismos compensatórios e desenvolvimento de arritmogênese, assim como seu papel no que tange à evolução e prognóstico. No primeiro capítulo, investigou-se o uso do índice de tônus vasovagal (VVTI), um indicador não convencional de atividade parassimpática, e seu comportamento nos diferentes estágios da doença mitral. Resultados mostraram que o referido índice é capaz de diferenciar cães em estágios diferentes da doença, no que diz respeito ao remodelamento cardíaco e à presença de sintomatologia clínica atribuída à insuficiência cardíaca congestiva. O segundo capítulo está centrado na avaliação do prejuízo no equilíbrio autonômico que acompanha a progressão da insuficiência valvar mitral. Para tanto, empregou-se uma manobra vagal amplamente descrita para demonstrar a resposta parassimpática fisiológica e, à medida que a insuficiência mitral se agrava, a atenuação dessa resposta. O estudo mostrou que é possível identificar indivíduos com doença cardíaca avançada com base na resposta individual ao reflexo óculo-cardíaco. Não obstante, os resultados obtidos ajudam a compreender a complexa desregulação autonômica na síndrome insuficiência cardíaca congestiva. Finalmente, o terceiro capítulo foca no estudo do prolongamento e da instabilidade do intervalo eletrocardiográfico QT com vistas à identificação do estado de arritmogênese aumentada na degeneração válvula mitral. O estudo mostrou que prolongamento e instabilidade não só estão presentes, como se intensificam conforme a doença progride, sendo os índices estudados capazes de identificar remodelamento cardíaco e arritmias ventriculares. Os achados deste estudo reforçam a presença de distúrbios de repolarização na doença valvar mitral e chamam a atenção para o potencial prognóstico dos índices ligados à instabilidade do QT, haja vista a constatação de que o aumento de tais parâmetros está associado com menor sobrevida do cão com doença valvar mitral.

Palavras-chave: eletrocardiografia, sistema nervoso autônomo, insuficiência mitral, intervalo QT, arritmia

ABSTRACT

The aim of this study was to evaluate the autonomic imbalance and mechanisms of increased arrhythmogenesis, obtained through conventional electrocardiographic tracings, which are known to be involved in the progression of mitral disease in dogs. For this purpose, this work was subdivided in introduction and three independent chapters. Initially, an attempt was made to clarify the etiological and pathophysiological aspects of the disease, emphasizing the chronic activation of compensatory mechanisms and the development of arrhythmogenesis, as well as its role in disease evolution and prognosis. In the first chapter, we investigated the use of the vasovagal tonus index (VVTI), an unconventional indicator of parasympathetic activity, and its behavior in different stages of mitral disease. The results showed that this index is capable of differentiating dogs at different stages of the disease, regarding cardiac remodeling and the presence of clinical symptomatology attributed to congestive heart failure. The second chapter is centered on the assessment of the impairment in autonomic balance that accompanies the progression of mitral valve insufficiency. For this purpose, a widely described vagal maneuver was used to demonstrate the physiological parasympathetic response and, as mitral regurgitation increases, the attenuation of this response. The study showed that it is possible to identify individuals with advanced heart disease based on individual response to the oculo-cardiac reflex. Nonetheless, the results obtained help to understand the complex autonomic dysregulation in congestive heart failure syndrome. Finally, the third chapter focuses on the study of the prolongation and instability of the electrocardiographic QT interval with the intent to identifying the state of increased arrhythmogenesis in mitral valve degeneration. The study showed that prolongation and instability are not only present, but also intensify as the disease progresses, and the indices studied are capable of identifying cardiac remodeling and ventricular arrhythmias. The findings of this study reinforce the presence of repolarization disorders in mitral valve disease and draw attention to the potential prognostic values of the indexes related to QT instability, given that the increase in these parameters is associated with lower survival of the dog with Mitral valve disease.

Key-words: electrocardiography, autonomic imbalance, mitral insufficiency, QT interval, arrhythmias

SUMÁRIO

INTRODUÇÃO

Myxomatous mitral valve disease (MMVD), also known as endocardiosis, is frequently responsible for mitral regurgitation in dogs, and represents the most common acquired heart disease in that species (Atkins et al, 2009). Although many causes have been speculated, it is most often a primary condition, being more frequent in small to medium size dogs (Freed et al., 2002). The existence of breeds particularly predisposed to the disease, such as the Cavalier King Charles Spaniel, Dachshunds, Miniature Poodles and Schnauzers, support the hypothesis of a determinant hereditary component. Researches carried out in attempt to identify genes associated to disease predisposition suggest a polygenic inheritance, with variable penetrance (Meurs et al, 2010). Males are around 1.5 times more frequently affected than females, in addition to presenting earlier onsets and faster disease progression (Atkins et al., 2009). From anatomopathological studies, it has been observed that dogs develop MMVD with age, which makes this disease very similar micro and macroscopically to mitral valve prolapse in people (Pedersen et al., 2000). With the exception of the Cavalier King Charles Spaniel and other possible genetically predisposed breeds, MMVD is uncommon in young dogs. However, it has a prevalence of over 90% in dogs of 10 years or older (Atkins et al., 2009). The progression of mitral valve disease is variable, resulting in assymptomatic patients for life, or in dogs evolving to death in a few months due to congestive heart failure (CHF) (Borgarelli et al, 2008).

MMVD is characterized by an abnormal protrusion of the mitral valve into the left atrium, resulting from the continuous deposition of glycosaminoglycans in the leaflets and tendon chordae (Freed et al., 2002). Little is known about the molecular mecanisms involved in the pathophysiology of the disease, but studies indicate that, similar to what happens in valve disease in human beings, increased levels of serotonin (5HT) signaling may be present. A research proved that predisposed dogs and/or dogs with naturally-occurring mitral valve disease have significantly increased levels of circulating 5-HT when compared to healthy subjects (Arndt et al., 2009). Another investigation that focused on the genomic expression of the mitral valve tissue also identified inflamatory cytokines and growing transforming factor beta as important contributors to the patophysiology of the disease (Oyama et al., 2006). In a histologic level, MMVD turns the originally thin and translucent valvar leaflets into

thick and opaque structures that evolve into nodules and deformation (Philip, 2012). The thickening of theses leaflets, along with the changes in colagenous fibers of the cordoalia due to senility, reduce the ability of the valve to resist to the mechanical stress imposed during systole, resulting in non-cooptation of the leaflets and retrograde blood flow, from the ventricle to the atrium (Whitney, 1974). The mitral valve is damaged alone in approximately 62% of the cases, while both atrial-ventricular valves are involved in 33% of the cases. Interestingly, the isolated degeneration of the tricuspid valve is considered uncommon (Atkins, 2009). Due to the variable progression of the disease, clinically healthy animals may be diagnosed during routine physical examination, by means of the auscultation of a systolic apical murmur. As the damage in the leaflets progresses, mitral insufficiency may become clinically overt (Borgarelli et al., 2008).

In MMVD, blood regurgitated to the left atrium causes a decrease in the volume ejected by the ventricle at each systole (systolic volume), minimizing the amount of blood ejected per minute (cardiac output). In order to avoid significant decreases in blood pressure, neurohormonal compensatory mechanisms are activated, which include an increase in sympathetic tone and parasympathetic withdrawal, activation of the renin-angiotensin-aldosterone system (RAAS) and vasopressin secretion (ADH). These mechanisms are effective in maintaining homeostasis in situations of acute hypotension and hypovolemia. However, in chronically affected hearts, the constant activation of these mechanisms leads to the clinical syndrome known as congestive heart failure (CHF), which is characterized by the inability of the heart to maintain cardiac output, resulting in inadequate blood distribution and abnormal fluid congestion in the organic systems (Ware, 2001).

According to the consensus statement of the American College of Veterinary Internal Medicine (ACVIM), MMVD may be classified into five stages. Stage A represent patients at high risk of developing mitral degeneration, however, without a cardiac murmur during auscultation. Stage B1 represents patients with cardiac murmur but no clinical signs. Also, these patients have no cardiac remodeling observed on radiographic and echocardiographic examinations. Along with the cardiac murmur, stage B2 dogs exhibit an enlarged heart (cardiac remodeling) on radiographic and echocardiographic exams, but clinical signs of CHF are still not present. On the other hand, stage C patients have clinical signs of CHF, which tend to respond to the conventional medical therapy, while stage D dogs are in terminal

stages of the disease, which make them refractory to the standard medical therapy (Atkins et al., 2009)

Compensatory mechanisms, working together and independently, allow the animal with MMVD to antagonize the decrease of blood pressure caused by the constant mitral regurgitation. This characteristic explains why affected dogs remain asymptomatic for months to years (Borgarelli et al, 2008). However, the chronic activation of these mechanisms plays an important role in the progression of the disease. The constant activation of the sympathetic nervous system and suppression of its parasympathetic counterpart result in a sustained increase in resting heart rate, which shortens the diastolic period and increases oxygen consumption by the myocardium, reducing cardiac output and resulting in hypoxia-mediated cellular death. In addition, augmented peripheral vascular resistance results in increased after-load, which rises cardiac energy expenditure. The consequences include a reduced cardiac output and an increase in mitral regurgitation MR volume (Ramírez et al, 2001). Advanced mitral insufficiency induces cardiac remodeling, which is characterized by dilation of the left atrium and ventricle, eccentric myocardial hypertrophy and changes in the intercellular matrix (Bonagura and Schober, 2009). Remodeling, along with an increase in resting heart rate, can lead to cardiac arrhythmias, especially those of atrial and junctional origin (Verheule et al., 2003).

The term arrhythmia (or dysrhythmia) refers to irregular heart rhythm. The pathophysiological causes of arrhythmias include abnormal automaticity of the pacemaker cells, pacemaker migration from the sinoatrial node to other areas of the heart, conduction blockages that interrupt or delay the normal electrical propagation throughout the heartconduction, abnormal pathways of impulse conduction, and spontaneous formation of a pulse in a non-specialized heart area (Tilley, 2008). In dogs with MMVD, supraventricular extrasystoles and supraventricular tachycardia are the rhythm disturbances most commonly diagnosed. Other alterations that may also be observed are atrioventricular block (AVB) in various degrees, sinus arrest and ventricular arrhythmias (Tilley, 1992). Crosara et al. (2010), in a study that recruited 36 dogs with MMVD, concluded that the presence of arrhythmias is a common finding in those patients regardless of clinical condition. In addition, it was documented that atrial dilatation is directly related to the exacerbation of supraventricular arrhythmias. Ventricular arrhythmias, however, were significantly more frequent in dogs with clinical MMVD. Finally, they showed that rhythm

disturbances in MMVD animals rarely result in clinical signs, such as fatigue or syncope, which leads to sub-diagnosing.

Although clinical signs resulting from rhythm disturbances are uncommon in dogs with MMVD, there is a concern about the incidence of ventricular arrhythmias in these animals because of the increased risk of sudden death and chronic damage due to decreased cardiac output (Crosara et al., 2010). In people, it has been speculated that arrhythmias may play a significant role in clinical symptoms and may be responsible for the slightly increased risk of sudden death in MVP patients. Electrocardiographic evidence of conduction disturbances such as unstable repolarization dynamics are a known risk factor for arrhythmia (Berger et al., 1997). The elucidation of the electrophysiological events underlying rhythm disorders may provide surrogate markers that are able to predict the occurrence of life-threatening arrhythmias in dogs with advanced MMVD.

Due to the high incidence of mitral valve disease in the canine population, as well as its variable progression rate, surrogates of morbidity and mortality are warranted. The existence of a resource that is able to predict when (or if) dogs with the benign form of the disease will decompensate, would allow to answer frequent questions of the owners regarding survival time, besides optimizing the treatment. Although there is still no ideal indicator capable of accurately predicting the occurrence of CHF, studies indicate heart rate variability (HRV) as a simple and reliable tool (Haagstrom et al, 2009). HRV is a measure of autonomic function used to assess the physiological phenomenon of change in heart rate, which is mediated by cardiac autonomic tone (Stein et. al. 1994). Decreased HRV values are a common finding at advanced stages of CHF, and numerous studies have acknowledged its use as a prognostic surrogate in a series of cardiac conditions. Cardiac biomarkers, such as serum natriuretic peptides, and echocardiographic indices of remodeling (left atrium / aorta ratio) are also considered promising resources (Reynolds et al, 2012). Nonetheless, the ideal indicator of morbidity and mortality remains undiscovered (Sargent et al., 2015)

In this dissertation, we studied potentially useful indices of autonomic imbalance and impaired ventricular repolarization dynamics obtained from conventional ECG tracings. These indices may serve as indicators of morbidity, as well as aid in predicting the development of ventricular arrhythmias in dogs with different stages of MMVD.

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CHAPTER 1 - Vasovagal tonus index in dog with myxomatous mitral valve disease[1]

ABSTRACT.- The vasovagal tonus index (VVTI) is an useful and assessable index, obtained from standard ECG recordings, that is used to estimate heart rate variability (HRV), and may provide valuable information regarding the likelihood of progression into congestive heart failure (CHF). In this paperwork, we investigated how the vasovagal tonus index (VVTI) behaves in dogs with naturally-occurring myxomatous mitral valve disease (MMVD) Electrocardiographic (ECG) recordings and echocardiographic data of 120 patients diagnosed with MMVD were reviewed. The VVTI was calculated from twenty consecutive RR intervals for each dog enrolled in the study. Lower VVTI values were found in MMVD patients in American College of Veterinary Internal Medicine (ACVIM) stage C compared with stages B1 and B2. Values were also lower in patients with severe cardiac remodeling. When a cut-off value of 6.66 is used, VVTI was able to discriminate MMVD patients in stage C from B1 and B2 dogs with a sensitivity of 70 per cent and a specificity of 77 per cent. MMVD dogs in which VVTI is lower than 6.66 are 30% more likely to develop congestive heart failure (CHF).
INDEX TERMS: heart rate variability, prognosis, autonomic nervous system, electrocardiography, congestive heart failure

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[1]Received on .....
Accepted for publication in .....
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RESUMO.- [Índice de tônus vasovagal em cães com doença mixomatosa da valva mitral.] O índice de tônus vasovagal (ITVV) é uma ferramenta útil e acessível, obtida por meio de traçados eletrocardiográficos convencionais (ECG), utilizada para calcular a variabilidade da frequência cardíaca (VFC), podendo também fornecer informações valiosas referentes à probabilidade de desenvolvimento de insuficiência cardíaca congestiva (ICC). Neste trabalho, foi investigado como o ITVV se comporta em cães com degeneração mixomatosa da valva mitral (DMVM) de ocorrência natural, ECGs e exames ecocardiográficos de 120 pacientes diagnosticados com DMVM foram avaliados. O ITVV foi calculado a partir de 20 intervalos RR consecutivos para cada cão envolvido. Valores menores de ITVV foram encontrados em pacientes em estágio C de doença mitral pela classificação do American College of Veterinary Internal Medicine (ACVIM), comparado com pacientes em estágio B1 e B2. Valores também foram menores em pacientes com remodelamento cardíaco importante. Quando um valor de corte de 6,66 foi usado, o ITVV foi capaz de distinguir pacientes em estágio C de B1 e B2 com uma sensibilidade de 70 por cento e uma especificidade de 77 por cento. Cães com DMVM cujo ITVV é menor que 6,66 são 30% mais propensos a evoluírem para ICC.
TERMOS DE INDEXAÇÃO: variabilidade da frequência cardíaca, prognóstico, sistema nervoso autônomo, eletrocardiografia, insuficiência cardíaca congestiva

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[1] Written in accordance with the guidelines of Pesquisa Veterinária Brasileira, available at *www.pvb.com.br*

INTRODUCTION

The ability of the heart to adapt to various conditions by means of change in heart rate (HR) is mediated by cardiac autonomic tone (Pomeranz et al. 1985). Patterns of increased and decreased HR are a signal of good adaptation and efficient autonomic mechanisms, and reflect a healthy heart (Saul et al. 1988). This physiological phenomenon can be assessed by a measure of autonomic function known as heart rate variability (HRV) (Stein et al. 1994). Therefore, an increase in sympathetic tone is followed by a decrease in HRV, while an increase in parasympathetic tone results in increased HRV(Montano et al. 2001).

Analyses of beat-to-beat changes in HR provide sensitive and early information of impaired cardiac function. Decreased HRV values are a common finding in initial stages of congestive heart failure (CHF) even before clinical signs become overt (Task Force 1996), and numerous medical and veterinary studies have acknowledged that certain measures of autonomic function may act as a prognostic surrogate in various structural cardiovascular conditions (Stein et al 1994, Calvert et al. 1998, Nolan et al. 1998, Lahiri et al. 2008). HRV indexes can be obtained by linear methods of either time or frequency domain analysis, with a few unconventional nonlinear methods also described in people (Vanderlei et al. 2009). In most cases, values of HRV are preferably obtained by 24-hour Holter monitoring, which is time-consuming and may be laborious to apply in daily clinical practice. In this scenario, the vasovagal tonus index (VVTI) stands out as an useful time domain indicator of HRV obtained from the standard electrocardiographic data, which does not require any specific equipment for its calculation, besides a conventional ECG recorder. It is mainly influenced by the parasympathetic tone, whose applicability has been recognized in previous studies (Häggstrom 1996, Doxey and Boswood 2004).

In this prospective cross-sectional observational study, we sought to investigate if VVTI is able to identify remodeling, as well as CHF, in dogs with naturally occurring mitral valve disease.

MATERIAL AND METHODS

Dogs were recruited for this study among patients attending routine cardiological evaluation in a veterinary hospital. All procedures were conducted in accordance with the institutional Animal Use Committee guidelines, under the protocol 039/2015. Three-minute ECG tracings were acquired using a computer-based ECG recorder (ECGP V6, TEB – Brazilian Electronic Technology Ltda., São Paulo, SP, Brazil) and echocardiography was carried out using an ultrasonography system (MyLab 30 – Esaote, Genova, Italy) equipped with a 5.0 MHz and a 7.5 MHz phased array transducers (P240 and P023 reference – Esaote, Genova, Italy).

In order to be included in the study, dogs needed to be diagnosed with MMVD at any stage, based on echocardiographic criteria of impaired valvar anatomy and function (Chetboul et al. 2012). Dogs with evidence of any congenital or acquired cardiac disease other than degenerative mitral valve disease were excluded from the study, along with patients with relevant systemic conditions or undergoing chronic medical therapy. After a positive diagnosis, three-minute ECG tracings were acquired for each patient. Bad quality recordings and ECG tracings where arrhythmias prevented a continuous run of 20 R-R intervals of sinus rhythm were excluded from the study, along with ECG tracings of patients being given any antiarrhythmic drug when the recording was obtained. Finally, dogs visibly frightened and agitated, along

with dogs that would not allow stress-free mechanical contentions were also excluded from the study.

ECG recordings were reviewed and the first 20 consecutive R-R intervals in which cardiac rhythm was of sinus origin were used to calculate VVTI for each patient. The index was obtained by calculating the natural logarithm of the variance of the 20 measured R-R intervals, as described by the equation VVTI = NL [VAR (R-R1 – R-R20)], where NL: natural logarithm, VAR: variance. Indexes of congestion and function obtained by echocardiographic examination, such as left atrium to aorta ratio (LA/Ao), body weight-indexed left ventricular internal diameter in diastole and systole (BW-indexed LVd and BW-indexed LVs), wall stress index in diastole and systole (WSId and WSId), fractional shortening (FS%), mitral E wave velocity (mitral E), mitral E-to-A ratio (mitral E/A), isovolumic relaxation time (IVRT) and mitral E-to-IVRT ratio (E/IVRT) were also documented for each dog on the study. All indexes were measured by the same investigator, as to avoid interinvestigator discrepancy.

For statistical purposes, the dogs were divided into three groups based on left atrial remodeling, according to Ljungvall et al. (2005). LA/Ao<1.4, 1.4<LA/Ao<1.8; LA/Ao>1.8). LA and Ao diameters were measured at right parasternal window using short axis images at the very last frame before aortic valve opening, and left ventricular volumes and diameters in systole and diastole were assessed by M-mode, also in short axis images. (Chetboul et al. 2012). Also, another classification was based on the stage of mitral valve disease (B1, B2 and C) proposed by the American College of Veterinary Internal Medicine (Atkins et al. 2009), which depended on the animal history concerning clinical signs attributable to CHF, as well as the echocardiographic proof of left atrial overload.

All data underwent the Shapiro-Wilk normality test. Either an analysis of variance followed by Tukey´s multiple comparison test or the Kruskal-Wallis test followed by Dunn's test was used to investigate differences in VVTI in the studied population. Either Pearson's or Spearman's test was used to assess whether correlations existed between VVTI and heart rate (HR), age and body-weight, as well as between VVTI and the echocardiographic parameters. Also, receiver operating characteristic (ROC) curves were constructed to investigate sensitivity and specificity of VVTI to differentiate MMVD dogs with remodeled hearts from those without remodeling, as well as those with overt clinical signs from the asymptomatic animals. All analyses were performed using the software GraphPad Prism (Version 5.0 - San Diego, CA, USA) using default settings. For all analyses, the level of significance was defined as $P<0.05$.

RESULTS

One hundred and twenty client-owned dogs diagnosed with MMVD were recruited at the end of the study. Several breeds were represented, and the age and body-weight of the animals varied from 6-18 years and 2.3-15.5 kg, respectively. Poodles (33/120) and Miniature Pinschers (17/120) were overrepresented in the study population.

No correlation was found between VVTI and body-weight ($P=0.1113$), but a significant negative correlation was documented between VVTI and HR ($P<0.0001$), as well as between VVTI and age ($P=0.0491$).

The three groups of dogs divided according to left atrial size held 40 animals each, and differences regarding age ($P=0.0598$) and body-weight ($P=0.2232$) did not exist between groups. VVTI values based on LA/Ao size are shown in Table 1. A significant difference between group LA/Ao>1.8 and the two groups in which LA/Ao was lower than 1.8 was documented ($P<0.05$). The lowest VVTI values were found in

dogs with LA/Ao>1.8 whereas the highest values corresponded to the animals with LA/Ao<1.4 (Figure 1-A).

When the dogs were divided in accordance with the ACVIM consensus statement, 40, 60 and 20 animals were assigned into groups B1, B2 and C, respectively. Again, no difference was found regarding age (P=0.1532) or body-weight (P=0.6307) between these three groups. Table 2 brings VVTI values distributed within the groups. When VVTI values of patients in groups B1, B2 and C were compared, a significant difference between group C and groups B1 and B2 was identified (P<0.05). The lowest VVTI values were found in group C, while the highest values corresponded to group B1 (Figure 1-B).

The relationship between VVTI and cardiac rhythms during ECG recording was also investigated. The identification of the rhythms was done according to heart rate and regularity of the QRS complexes. A sequence of sinus beats, with regular intervals and rate within normal for age and breed was classified as sinus rhythm (SR). Sinus beats where R-R intervals presented a variation of 10% or higher between consecutive beats was classified as sinus arrhythmia (SA). Finally, a sequence of sinus beats with increased heart rate regarding age and breed was classified as sinus tachycardia (ST) (Tilley 1992). VVTI calculated for dogs with SA was significantly different from that calculated for animals presenting either SR or ST. The lowest VVTI values were found in the ST group and the highest values corresponded to the SA group (Figure 1-C).

When it comes to echocardiographic indices of congestion and function, no correlation was found between VVTI and BW-indexed LVd (R: -0.0919; P=0.3440), BW-indexed LVs (R: -0.0634; P=0.5144), WSIs (R: -0.0362; P=0.6943) and FS% (R: -0.1043; P=0.2570). On the contrary, a significant correlation was observed between VVTI and mitral E wave (R:-0.5377; P<0.0001), mitral E/A (R:-0.4164; P<0.0001), IVRT (R:0.3539; P=0.0006), E/IVRT ratio (R:-0.5511; P<0.0001), WSId (R:-0.1931; P=0.0346), and LA/Ao (R:-0.4366; P<0.0001) (Figure 2).

The ROC curve constructed to investigate sensitivity and specificity to differentiate dogs with remodeled hearts from those without remodeling found an AUC of 0.6802 (95% CI: 0.5815 to 0.7789; P=0.0013) (Figure 3-A). To differentiate asymptomatic dogs from those with CHF, an AUC of 0.7778 (95% CI: 0.6574 to 0.8981; P<0.0001) was observed (Figure 3-B). VVTI cut-off values and its respective sensitivity, specificity, and positive likelihood ratio were calculated and are shown in Table 3.

DISCUSSION AND CONCLUSIONS

In this prospective study, lower VVTI values were found in MMVD dogs in stage C as compared to patients in stages B1 and B2. Values <7.385, <6.660 or <5.525 are associated with a positive likelihood ratio of 1.82, 3.04 or 10.0, respectively, which translates into a dog having a slight (0-15%), moderate (15-30%) or large (45%) increase in the probability of being symptomatic (stage C) than asymptomatic (either stage B1 or B2). Therefore, the lower the VVTI becomes the more suggestive that an asymptomatic MMVD patient may evolve into CHF. This finding is extremely valuable for clinical decision making, and is in accordance with previously reported data that suggests parasympathetic withdrawal in CHF syndrome (Eckberg et. al. 1971, Oliveira et. al. 2012). Left atrial enlargement is associated with the progression of MMVD, and the degree of dilation is recognized as a prognostic surrogate (Borgarelli et al. 2008, Reynolds et. al. 2012). Because this study documented the lower VVTI values in patients with LA/Ao >1.8 as compared to dogs with smaller left atriums, VVTI might also be considered a surrogate for CHF. This

finding is supported by the results of a retrospective study regarding multiple cardiac conditions, where the referred index has proven to be the most useful discriminatory parameter for detection of heart failure among a variety of selected laboratory and electrocardiographic parameters (Boswood et al. 2006).

Conventional HRV measures have been studied in people with heart disease, and were found to be an excellent predictor of adverse outcome (Saul et al. 1988, Nolan et. al. 1998, Lahiri et al. 2008). Time-domain heart rate variability parameters have also shown to have prognostic potential for determining the severity of MMVD in dogs (Rasmussen et al. 2012, Oliveira et al. 2012). These measures, however, usually hold the disadvantage of requiring a 24 hour Holter monitoring.

In our study, we sought to investigate if an unconventional and straightforward measure of HRV, obtained by the analyses of only 20 consecutive RR intervals, might be reliable in differentiating MMVD dogs in stages B1, B2, and C. Our results suggest that VVTI can discriminate MMVD patients in stage C from B1 and B2 dogs with a sensitivity of 70 per cent and a specificity of 77 per cent when a cut-off value of 6.66 is to be used.

Previous studies have acknowledged the feasibility of the VVTI as a measure of HRV in dogs with cardiac disease. Doxey and Boswood (2004) analyzed its diagnostic potential for CHF in 92 dogs of several breeds and different heart conditions. In that study, VVTI was shown to be a good diagnostic tool for CHF with a sensitivity of 100 per cent and a specificity of 83.6 per cent when a cut-off value of 6.75 was used. Boswood et al, 2006 analyzed the effects of heart disease, heart failure and diuresis on nine selected laboratory and echocardiographic parameters in dogs with multiple heart conditions, and observed that the VVTI decreased with the onset of CHF, proving to be the most useful discriminatory parameter for this syndrome among the tested. Also, Pereira et al. (2008) studied the index's applicability in dogs with dilated cardiomyopathy (DCM) and found the index to be a moderately accurate diagnostic tool for the assessment of severity of heart failure in DCM patients. When using a cut-off value of 7.59, a good sensitivity of 88.7 per cent was calculated, but the specificity of 62.5 per cent was considered low. When it comes to MMVD, Häggstrom et al. (1996) investigated the index in Cavalier King Charles spaniels, which are known for the rapid progression of MMVD into CHF. In their study, VVTI was similar in healthy and early staged subjects, but the index became significantly lower in CHF patients. Interestingly, our study is the first one to evaluate VVTI applicability in the general population of dogs with MMVD, exclusively.

Also, this investigation documented significant correlations between VVTI and some of the studied echocardiographic parameters. A moderate negative relationship was found between the VVTI and mitral E (R:-0.54) and between VVTI and E/IRVT (R:-0.55). A weak negative relationship was found between the VVTI and mitral E/A (R:-0.42), and VVTI and LA/Ao (R:-0.44). A weak positive relationship was documented between VVTI and IVRT (R: 0.35). As reported before, decreased HRV values are commonly observed during the initial stages of CHF even before clinical signs become evident (Task Force, 1996). In severe cases of mitral valve regurgitation, volume overload leads to left atrial and ventricular remodeling, both associated with clinical onset (Reynolds et al, 2012). Many echocardiographic indices tend to become altered when a still asymptomatic MMVD dog progresses into symptomatic CHF. In this study, the significant correlation found regarding the echo indices of congestion and function is supportive of VVTI being lower as CHF becomes more severe.

The negative correlation between VVTI and heart rate is likely ascribed to the role played by the parasympathetic tone in VVTI, therefore producing higher values when slower rates and irregular rhythms are present. VVTI is a time-domain method of analysis and, being acquired over a short period of time, provides information about high-frequency variations in heart rate, which are predominantly a result of vagal influences (Doxey and Boswood, 2004). This also explains the higher values of VVTI in patients presenting sinus arrhythmia when compared to dogs presented with sinus rhythm and sinus tachycardia. The influence of the autonomic nervous system on the heart is dependent on information from baroreceptors, chemoreceptors, atrial and ventricular receptors, among others. Therefore, several factors can interfere with HRV, including exercise and physical and mental stress, respiration, blood pressure regulation, thermoregulation, and influence of the renin-angiotensin system (Stein et al. 1994). Even though these uncontrolled conditions during ECG recording may increase HR, the lower VVTI found in animals exhibiting sinus tachycardia may reflect high sympathetic and low parasympathetic outflows, commonly found in severe cases of MMVD (Häggstrom et al. 1996). As for the negative correlation documented between VVTI and age, this is mostly likely due to the late onset and progressive nature of MMVD, usually becoming clinically relevant in animals of higher ages.

An important limitation involving the use of VVTI is the impossibility of calculation when a consecutive 20-beat recording of sinus rhythm is lacking. In severe stages of cardiac remodeling, atrial fibrillation and ventricular premature complexes are a common finding during ECG recordings, which invalidate the index (Verheule et al. 2003). In our study, arrhythmias did not preclude the calculation of the index in the population of dogs, but we do recognize that it might be a limitation in the clinical setting. Also, being the VVTI an index influenced mainly by the parasympathetic tone, external influences in heart rate, including stress, fear and agitation, may result in low values in healthy animals. For this reason, it is encouraged that ECG recordings be acquired in a quiet and stress-free environment. Finally, the lack of attention in this study regarding possible effects of treatment over the index, as documented by Boswood et al. 2006, may need to be taken in consideration.

In conclusion, this study supports the use of VVTI as a nonconventional index of HRV that may provide valuable information when assessing the severity of MMVD and the likelihood of a patient progressing into CHF. Dogs with VVTI <7.425 are slightly more likely to have remodeled hearts (around 15% increase in probability) as compared to those in which VVTI is higher, while those with VVTI <5.830 are moderately more likely to have dilated cardiac chambers (35-45% increase in probability). Also, MMVD dogs in which VVTI is lower than 6.66 have a moderate increase of 30% in the probability of evolving into CHF. Knowing how this index behaves in this particular canine heart disease may also be useful in studies aimed at predicting CHF in human beings with MVP.

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TABLES AND FIGURES

Table 1. Descriptive statistics of vasovagal tonus index (VVTI) calculated for dogs with myxomatous mitral valve disease (MMVD) and divided into groups in accordance with left atrial size.

	LA/Ao			P (Kruskal-Wallis)
	<1.4 (n=40)	1.4-1.8 (n=40)	>1.8 (n=40)	
Minimum	5.710	1.370	2.250	<0.0001
25% Percentile	6.798	6.898	5.250	
Median	8.560 [a]	7.880 [a]	6.330 [b]	
75% Percentile	9.285	8.888	7.358	
Maximum	10.51	10.82	9.900	
Coefficient of variation	16.99%	22.51%	25.43%	

Table 2. Descriptive statistics of vasovagal tonus index (VVTI) calculated for dogs with myxomatous mitral valve disease (MMVD) and divided into stages in accordance with the consensus statement proposed by the American College of Veterinary Internal Medicine (ACVIM).

	Stage			P (Kruskal-Wallis)
	B1 (n=40)	B2 (n=60)	C (n=20)	
Minimum	5.710	1.370	2.250	<0.0001
25% Percentile	6.798	6.533	4.890	
Median	8.560 [a]	7.340 [a]	5.555 [b]	
75% Percentile	9.285	8.735	7.293	
Maximum	10.51	10.82	9.350	
Coefficient of variation	16.99%	22.51%	29.28%	

Table 3. Cut-off values and their corresponding sensitivity and specificity indices when using vasovagal tonus index (VVTI) to differentiate dogs with remodeled and normal hearts, and asymptomatic dogs from those presenting overt clinical signs.

	VVTI cut-off	Sensitivity (95% CI)	Specificity (95% CI)	Likelihood ratio (+)
Normal vs. Remodeled Hearts	**< 5.830**	22.50 (13.91 to 33.21)	97.50 (86.84 to 99.94)	9.00
	< 6.220	30.00 (20.26 to 41.28)	95.00 (83.08 to 99.39)	6.00
	< 6.860	41.25 (30.35 to 52.82)	72.50 (56.11 to 85.40)	1.50
	< 7.095	50.00 (38.61 to 61.39)	70.00 (53.47 to 83.44)	1.67
	< 7.425	60.00 (48.44 to 70.80)	65.00 (48.32 to 79.37)	1.71
Asymptomatic vs. Symptomatic Dogs	**< 5.525**	50.00 (27.20 to 72.80)	95.00 (88.72 to 98.36)	10.00
	< 6.220	55.00 (31.53 to 76.94)	85.00 (76.47 to 91.35)	3.67
	< 6.660	70.00 (45.72 to 88.11)	77.00 (67.51 to 84.83)	3.04
	< 7.035	75.00 (50.90 to 91.34)	66.00 (55.85 to 75.18)	2.21
	< 7.385	80.00 (56.34 to 94.27)	56.00 (45.72 to 65.92)	1.82

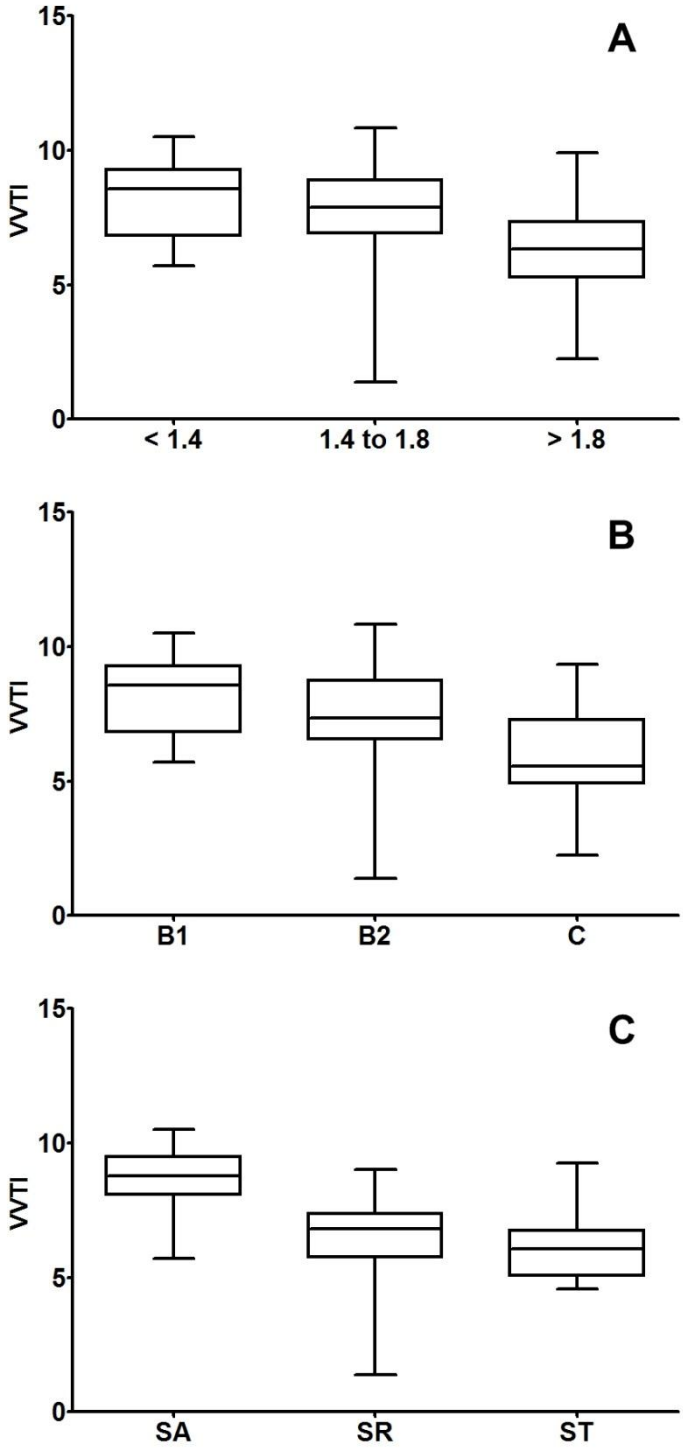

Figure 1 – Box plot depicting the medians, interquartile ranges and amplitude of vasovagal tonus index (VVTI) in dogs with myxomatous mitral valve disease (MMVD), subdivided in accordance with (A) left atrial remodeling, (B) disease stages and (C) cardiac rhythm during ECG recording. SA: sinus arrhythmia; SR: sinus rhythm; ST: sinus tachycardia.

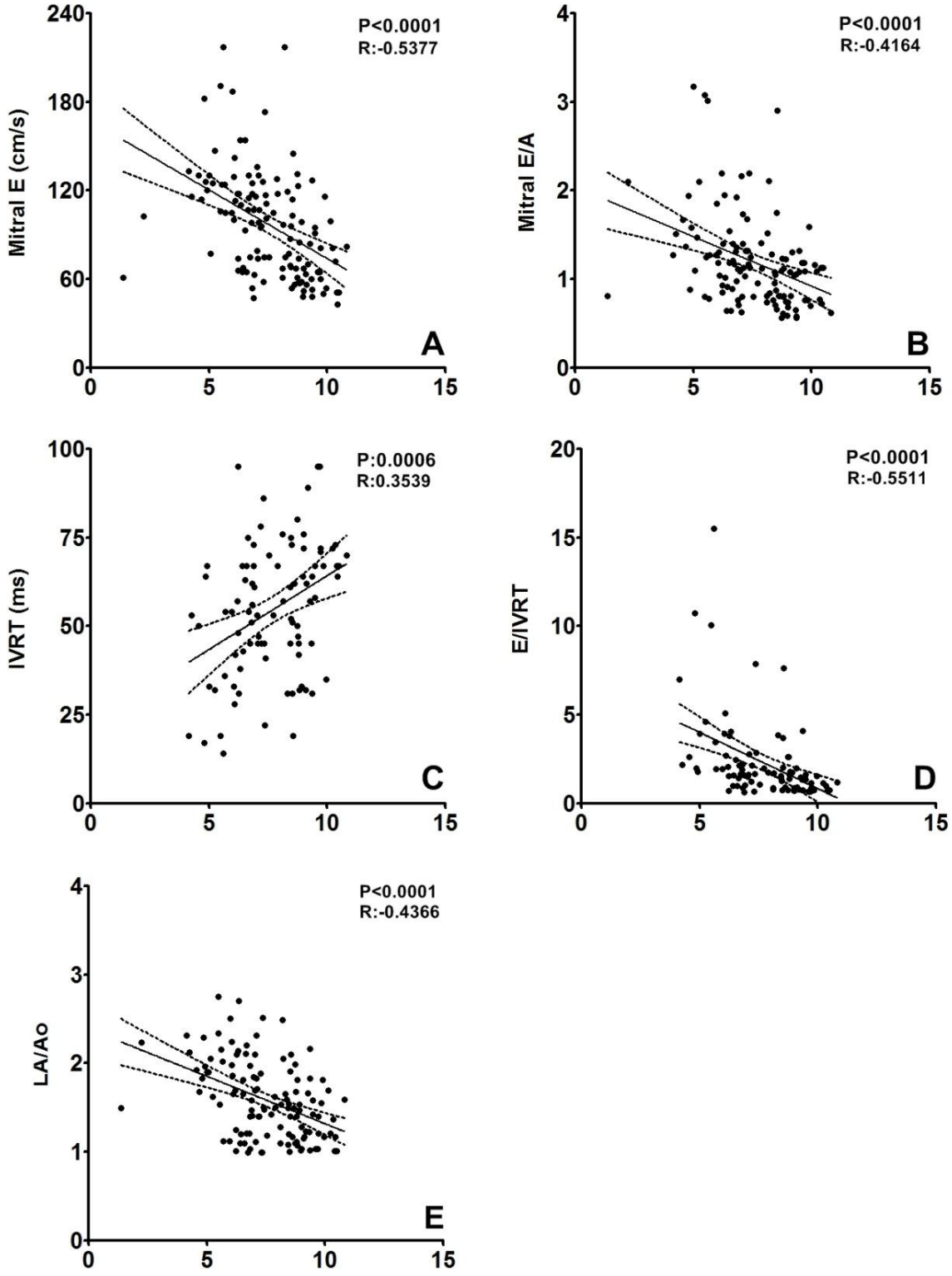

Figure 2 – Scatter plots depicting significant correlations between vasovagal tonus index (VVTI) and mitral E peak velocity (A), mitral E-to-A ratio (B), isovolumic relaxation time (IVRT) (C), E-to-IVRT ratio (D) and left atrium-to-aorta ratio (E). Bestfit lines and 95% confidence band are shown**.**

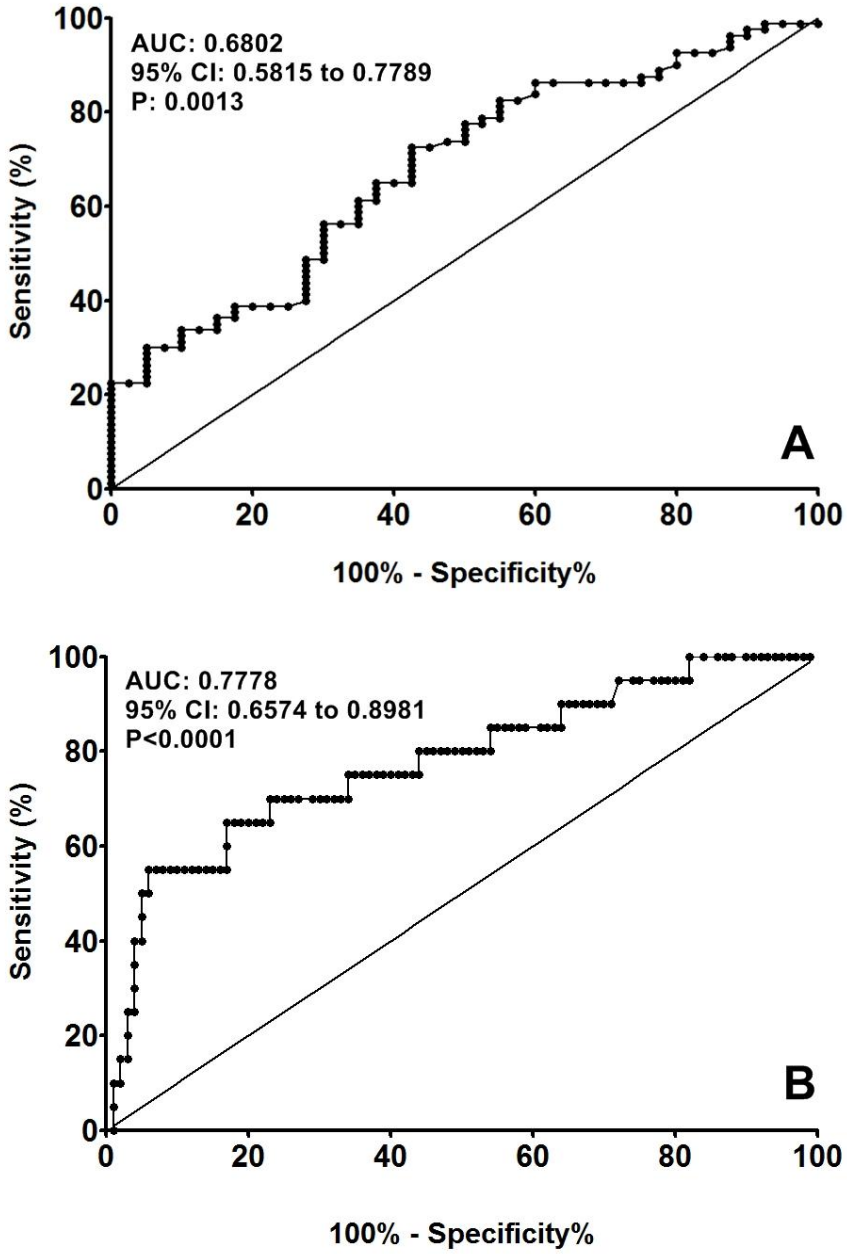

Figure 3 – Receiver operating characteristic curves constructed to investigate sensitivity and specificity of vasovagval tonus index (VVTI) to differentiate myxomatous mitral valve disease (MMVD) dogs with remodeled hearts from those without remodeling (A), as well as those with overt clinical signs from the asymptomatic animals (B).

CHAPTER 2 - Characterization of the Heart Failure-Induced Autonomic Imbalance by Means of the Response to a Vagal Maneuver in a Naturally-Occuring Model of Valve Insufficiency in Dogs[2]

ABSTRACT

Congestive heart failure is characterized by reduced heart rate variability. Although parasympathetic withdrawal and sympathetic activation have previously been documented, a vagal maneuver was never used to assess the autonomic impairment associated with disease progression. In this study, we investigated the cardiac autonomic control in a naturally-occurring model of mitral insufficiency by means of individual responses to oculocardiac reflex. Indices of heart rate variability (MRR, SDNN, RMSSD and VVTI) were calculated from 5-minute ECG tracings obtained before and after ocular compression. After compression, a significant increase in SDNN, RMSSD and VVTI was documented in healthy control animals. Also, an increase in these indices was shown in asymptomatic dogs with remodeled hearts, but no variation was seen in symptomatic animals. While only VVTI percent change (before-after) was different between controls and diseased dogs, all other parameters showed a tendency to behave differently in the symptomatic group. Our results documented a CHF-dependent autonomic dysfunction in a model of valve insufficiency.

KEYWORDS

Parasympathetic withdrawal, sympathetic activation, congestive heart failure, autonomic nervous system, oculocardiac reflex

INTRODUCTION

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[2] Written in accordance with the guidelines of Journal of Comparative Physiology, available at *http://www.springer.com/life+sciences/animal+sciences/journal/359*

Heart rate (HR) is determined by the rate of sinoatrial (SA) nodal discharge and autonomic tone influence (Hamlin, 1999). Therefore, parasympathetic and sympathetic nervous impulses are responsible for decreases and increases in HR, respectively (Montano et al, 2001). When vagal centers in the medulla oblongata are stimulated, acetylcholine binds to receptor sites present in the SA node, decreasing the discharge rate. Similarly, the stimulation of sympathetic centers in the medulla produces norepinephrine that binds to β1 receptors sites at the SA node, increasing heart rate (Hamlin, 1999). The autonomic influence to the heart determines the adaptive circadian variations of HR, which is a result of the prevailing balance of the autonomic impulses in the SA node (Montano et al, 2001).

The oculocardiac reflex is the heart's physiological response to digital compression of the eyeballs. Described in 1908 by Ashner and Dagnini, it consists of a vagal maneuver obtained through the indirect stimuli of the ophtalmic branch of the trigeminal nerve, causing negative chronotropic and inotropic responses in healthy subjects. The reflex, primarily described in human beings, has recently been characterized in dogs and rabbits (Giannico et. al, 2014).

Congestive heart failure (CHF) is a syndrome to which most cardiovascular disorders tend to evolve in advanced conditions. In CHF, as a result of structural, neural and electrophysiological remodeling, heart rate undergoes a sustained increase in response to autonomic imbalance (Tomaselli et al, 1999). Due to a slight fall in systemic arterial pressure caused by the failing heart, vagal restraint on heart rate is reduced, from either decreased stretching or diminished function of arterial baroreceptors. The levels of circulating cacthecolamines are also elevated in heart failure syndrome, which in turn increase sympathetic activation (Amorim et al, 1981).

This dysregulated autonomic tone leads to increases in resting HR, which is known to interfere in the prognosis of CHF (Floras et al., 2009).

Sustained sympathetic activation and parasympathetic withdraw have been characterized in numerous cardiovascular diseases, and the imbalance is considered an aggravating factor in circulatory failure (Nolan et al, 1998; Cohn et. al, 1984). Numerous measures of autonomic function have been proposed, especially due to their value in determining prognosis (Saul et al, 1988). Through surgical and pharmacological means, many researches have focused on documenting the precise mechanisms of impaired autonomic function in CHF (Ogawa et al, 2007; Sosunov et. al, 2001;Eckeberg et al, 1971). Nevertheless, the exact role played by the autonomic imbalance in the progression of cardiac diseases, or vice versa, remains unclear. Therefore, in this investigation we sought to evaluate the reliability of a novel way to assess the level of autonomic imbalance in CHF patients. Dogs with a naturally-occurring degenerative mitral valve disease were recruited, and their individual response to the oculocardiac reflex was used as a surrogate for the autonomic cardiac control.

MATERIAL AND METHODS

Dogs recruited for this prospective transversal observational study were selected among patients admitted for regular cardiac evaluation at a veterinary teaching facility. All procedures were previously approved by the institutional Animal Use Committee, and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

In order to be included in the study, the diagnosis of naturally-occurring mitral insufficiency was required, which was based on the echocardiographic criteria of

impaired valvar anatomy and function (Chetboul and Tissier, 2012). Dogs with echocardiographic evidence of any congenital or acquired cardiac disease other than degenerative mitral valve disease were excluded from the study, along with patients with history of intra-thoracic or abdominal tumors, ophthalmologic disorders, such as glaucoma, cataract and ulcerated corneas, as well as brachycephalic dogs. Once recruited, the dogs were subdivided based on two criteria: cardiac morphology and clinical history concerning signs attributable to CHF. In this regard, three groups were created to best describe the study population: No Remodeling (NR), Remodeled but still Asymptomatic (RA) and Remodeled and Symptomatic (RS) patients. Also, healthy animals lacking signs of valvular dysfunction were recruited as controls. All echocardiograms were carried out by experienced veterinary cardiologists using an ultrasonography system (MyLab 30 – Esaote, Genova, Italy) equipped with 5.0 MHz and 7.5 MHz phased array transducers (P240 and P023 reference – Esaote, Genova, Italy).

Once enrolled into the study, ECG tracings were acquired for each patient, with the dog positioned in right lateral recumbency and maintained in position by gentle physical restraint. Electrodes were attached to the skin and wet with alcohol to improve electrical conduction. The left arm and right arm electrodes were placed at the elbows, while the left leg and right leg electrodes were placed at the stifles. The electrodes used were toothless alligator-types to reduce discomfort. ECG was performed continuously and uninterrupted for five minutes. During the last minute, manual ocular compression was applied by exerting continuous digital force using the thumb over the superior eyelid in both eyes together, until the run of five minutes was over. To avoid interinvestigator discrepancy, ocular compressions were made by the same investigator, which was blinded to the patient's clinical condition, in all

dogs. Bad quality recordings and ECG tracings where arrhythmias prevented a continuous run of 20 R-R intervals of sinus rhythm were not used in the study

Once the recording was over, the ECG tracing was used to calculate the mean RR interval (MRR), the standard deviation of the RR intervals (SDNN), the root mean square of the successive differences in RR intervals (RMSSD) and the vasovagal tonus index (VVTI). The calculation of these indices were based on the following equations:

MRR = $\bar{I} = \frac{1}{N-1} \sum_{n=2}^{N} I(n)$

SDNN = $\sqrt{\frac{1}{N-1} \sum_{n=2}^{N} [I(n) - \bar{I}]^2}$

RMSSD = $\sqrt{\frac{1}{N-2} \sum_{n=3}^{N} [I(n) - I(n-1)]^2}$

VVTI = NL [VAR (R-R1 – R-R20)]

Each index was calculated twice for every tracing, the first one before and one the other one twenty seconds after the beginning of ocular compression. Also, echocardiographic indices of congestion and cardiac function were also documented. These included the left atrium-to-aorta ratio (LA/Ao), body weight-indexed left ventricular internal diameter in diastole and systole (BW-indexed LV_d and BW-indexed LV_s), wall stress index in diastole and systole (WSI_d and WSI_s), fractional shortening (FS%), mitral E wave velocity (mitral E), mitral E-to-A ratio (mitral E/A), isovolumic relaxation time (IVRT) and mitral E-to-IVRT ratio (E/IVRT), for posterior correlation.

All data underwent the Shapiro-Wilk normality test. Either an analysis of variance followed by Tukey´s multiple comparison test or the Kruskal-Wallis test followed by Dunn’s test was used to investigate differences between groups. Later, either the Mann-Whitney or the T test was used to compare pre and post OCR data

for each index, in every group. The percent change of every surrogate using the variation between pre and post OCR values was also analyzed, and the Kruskal-Wallis test followed by Dunn's test was used to compare this data among groups. Finally, either the Pearson's or the Spearman's test was used to assess whether correlations existed between the percent changes of each index and the echocardiographic parameters of congestion and cardiac function. All analyses were performed using the software GraphPad Prism (Version 5.0 - San Diego, CA, USA) using default settings. For all analyses, the level of significance was defined as $P<0.05$.

RESULTS

Sixty-eight client-owned dogs were recruited for the study. Several breeds were represented, and the age and body-weight of the animals ranged 1-16 years and 2.5-32 kg, respectively. Beagles (8/68), Dachshunds (6/68) and Miniature Pinschers (5/68) were overrepresented in the study population. No difference was found between groups regarding weight ($P=0.3943$), but the animal's age was considered different between the control and the RS group ($P=0.0074$). Descriptive statistics of the studied population is summarized on Table 1.

Sixteen healthy dogs were used as controls, while 31, 10 and 11 animals were assigned into groups NR, RA and RS, respectively. The box plots in Figure 1 demonstrate how the OCR interfered with the surrogates of autonomic balance in all dogs regardless of cardiac condition. Interestingly, a significant decrease was found to exist between groups when comparing the values of all four parameters documented during ocular compression. For every index, the symptomatic group differed statistically from control and NR groups. Also, in the NR group, ocular

compression significantly increased SDNN (from 49.7 to 87.3), RMSSD (from 289.7 to 506.4) and VVTI (from 8.1 to 8.9), while in healthy controls, an increase was documented in VVTI (from 8.1 to 8.9). No significant variation, however, was seen in remodeled groups.

When the before-after percent change was analyzed, a significant difference between groups was only documented for VVTI (P=0.0419). Interestingly, in spite of the absence of significant changes, the percent change of the other surrogates showed an obvious tendency to behave in the opposite way in the symptomatic group when compared to the asymptomatic and control dogs, as shown in Figure 2.

In regards to echocardiographic indices of congestion and function, weak negative correlations were found between SDNN and WSIs (R: -0.2508), LA/Ao (R: -0.3558) and Mitral E (R: -0.2714), as well as between RMSSD and BSA-indexed LVs (R: -0.2695), WSIs (R: -0.3188) and LA/Ao (R: -0.2810). VVTI was the index with most significant correlations, as shown in in Figure 3. On the contrary, no correlation existed between MRR and the echocardiographic data.

DISCUSSION

Congestive heart failure is known to be characterized by autonomic imbalance, which results in sustained elevation and diminished variability of heart rate (Stein et al, 1994). In this study, we sought to characterize such autonomic imbalance by means of a reduced response to a vagal maneuver in a naturally-occurring model of mitral valve insufficiency. The oculocardiac reflex, first described in people by Ashner and Dagnini (1908), is one of the peripheral subtypes of the trigeminal cardiac reflexes, and an important cause of profound bradycardia during eye surgery, especially in pediatric procedures (Espahbodi et al., 2015). Also, a

recent study that investigated the OCR in dogs demonstrated that manual compression of the eyeballs, either together or individually, results in significant decreases in heart rate in that species (Giannico et al, 2014).

Experimental evidence of increased sympathetic and reduced vagal tone have encouraged the development of quantitative markers of autonomic activity (Task Force, 1996). Heart rate variability (HRV) is an important measure of autonomic tone defined as the fluctuation time between normal sinus beats (RR intervals) (Vanderlei et. al, 2009). Although assessing the autonomic nervous system is not simple, HRV measurements have proved to be clinically useful, as analyses of beat-to-beat changes in HR provide sensitive and early information of impaired cardiac function, even before clinical signs become overt (Task Force, 1996). HRV indices are normally obtained by linear methods of either time or frequency domain analysis (Vanderlei et al., 2009). The time-domain analysis consists of statistical calculations based on normal RR intervals. In this research, we used the mean of all RR intervals (MRR), the standard deviation of all normal RR intervals (SDNN), the root mean square of the successive differences in RR intervals (RMSSD) and the vasovagal tonus index (VVTI), which have all been used to assess autonomic dysfunction in dogs (Lopez-Alvarez et. al, 2014; Oliveira et al., 2012; Calvert et al, 1998; Häggström, 1996), as well as in human beings (Takase, 2010; Vanderlei et al., 2009; Lahiri et al., 2008 Task Force, 1996). However, this investigation is the first to assess how a vagal maneuver interferes with these parameters. While SDNN is influenced by the short-term high frequency variations and low frequency components, reflecting total autonomic activity, only high frequency variations, which are normally related to parasympathetic influence, are thought to play a role in the other three parameters (Task Force, 1996).

In our study, the symptomatic group responded differently when undergoing the digital ocular compression as compared to every other group. The lack of response to the OCR in RS group dogs, as demonstrated by the absence of significant changes in all four parameters, is in accordance with the parasympathetic withdrawal in heart failure patients, primarily described by Eckberg et. al (1971). Since then, this information has been sustained by many studies that assessed autonomic function in CHF in various species (Schwartz et al., 2011, Floras et. al, 2009; Little et. al, 2005). More importantly, this finding illustrates not the lack of vagal activity, but rather the diminished response to parasympathetic stimuli in these patients. Also, the lack of significant difference between the symptomatic and the remodeled but asymptomatic group for MRR and VVTI supports the theory that some grade of autonomic imbalance in cardiovascular disorders precedes its clinical evidence (Task Force, 1996).

When it comes to the before-after analyses within a group, the parameters documented in the RS group not only appeared to respond less to the oculocardiac reflex, but rather behaved the exact opposite way, showing a clear tendency to increase with the vagal maneuver. This interesting finding might be attributable to the previously mentioned parasympathetic withdrawal together with a subtle sympathetic activation due to discomfort related to eyeball compression, which is likely irrelevant in dogs with a still preserved vagal response. However, the other and more intriguing explanation is that, in addition to parasympathetic withdrawal, patients in CHF are also presented with an increased sustained sympathetic activation (Schwartz et al., 2011, Floras et. al, 2009; Ishie et al., 1998). This hypothesis becomes clearer when considering the percent variation of the surrogates obtained for each group of our study. The exact opposite behavior of the symptomatic group is most likely a result of

sustained sympathetic tone, rather than transitional sympathetic activation. In addition, the clear similarity between the NR and RA groups and the control healthy group, as opposed to the RS dogs, suggests that, unlike parasympathetic withdrawal, which appears to be present at initial stages, the sustained sympathetic activation is more evident in overtly affected patients. Similar findings have been previously documented in people in CHF (Cohn et.al, 1984; Binkley et la, 1991)

The significant correlation between the before-after percent variation of the surrogates of cardiac autonomic regulation and the echocardiographic indices of congestions and function supports the theory that individual response to OCR diminishes with the progression of cardiac disease. In severe cases of mitral valve regurgitation, volume overload leads to left atrial and ventricular remodeling, both of which are associated with the clinical onset (Reynolds et al, 2012). Many echocardiographic indices tend to become altered when a still asymptomatic mitral insufficient dog progresses into symptomatic CHF (Chetboul et. al, 2012). Therefore, the significant correlations found in this study are likely supportive of decreased parasympathetic role as CHF becomes overt. This finding is in accordance with data previously documented in people (Binkley et al., 1991; Eckberg et. al, 1971), dogs (Little et al., 2005) and rats (Nihei et al., 2005).

An important limitation of this research is the impossibility to control external factors that potentially interfere with autonomic tone, such as stress and fear. Most dogs enrolled in this study were calm and quiet during the procedure, and appeared to tolerate the ocular compressions. However, some animals appeared more reluctant to physical restraint and more uncomfortable by the end of the maneuver, causing an intensification of sympathetic tone.

In this study, dogs with naturally-occurring mitral valve insufficiency were used as a model of heart disease leading to CHF, with the purpose of investigating autonomic imbalance. The vagal maneuver, represented by a manual compression of the eyeballs, was a reliable technique to demonstrate the deterioration of cardiac autonomic regulation as mitral insufficiency progresses towards congestive heart failure. Dogs in congestive heart failure were shown to develop signs of parasympathetic withdrawal and an increased sustained sympathetic activation. These findings contrast with both the asymptomatic and control dogs, in which lack of parasympathetic response to ocular compression was not observed. Although many questions remain regarding the exact influence of autonomic limbs in cardiovascular disorders, we believe this research based on the response to a simple vagal maneuver helps to shred a light in how the autonomic nervous system behaves in congestive heart failure syndrome.

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TABLES AND FIGURES

Table 1: Descriptive statistics (either mean/SD or median/IQR) of the recruited population subdivided in accordance with the echocardiographic and clinical criteria in healthy controls, and mitral insufficient dogs with no cardiac remodeling (NR), remodeled hearts but still asymptomatic (RA) or remodeled heart and symptomatic (RS).

	Control (n=16)	NR (n=31)	RA (n=10)	RS (n=11)	P
Age (years)	2.2 (1.5-8.0) ^{A}	11.0 (9.0-13.0) ^{B}	11.0 (8.5-12.0) ^{B}	13.0 (12.0-14.2) ^{B}	<0.0001*
Sex (F/M)	10/6 (63%/34%)	18/13 (58%/42%)	7/3 (70%/30%)	5/6 (45%/55%)	-
Weight (kg)	9.0 (9.0-17.0)	9.7 (5.3-13.7)	8.6 (5.8-13.6)	7.1 (5.1-9.0)	0.1028
BSA indexed LVd (mm/m^2)	62.5 (15.3) ^{A}	67.3 (17,2) ^{A}	75.7 (14.9) ^{AB}	90.6 (17.0) ^{B}	0.0003
BSA indexed LVs (mm/m^2)	36.2 (24.7-42.6)	34.4 (29.3-39.8)	40.7 (34.6-46.3)	41.7 (37.0-49.9)	0.0906*
WSId	3.8 (1.4)	3.6 (0.9)	4.1 (0.9)	4.1 (1.0)	0.4128
WSIs	1.3 (0.3)	1.3 (0.4)	1.3 (0.7)	1.3 (0.4)	0.9102
FS (%)	43.2 (5.9) ^{A}	46.9 (8.8) ^{AB}	43.2 (10.0) ^{AB}	51.9 (6.5) ^{B}	0.0327
LA/Ao	1.2 (1.1-1.3) ^{A}	1.1 (1.0-1.2) ^{A}	1.6 (1.4-1.7) ^{B}	2.1 (1.9-2.3) ^{B}	<0.0001*
Mitral E (cm/s)	82.8 (19.1) ^{A}	66.4 (10.7) ^{B}	92.5 (18.6) ^{A}	151.9 (30.5) ^{C}	<0.0001
Mitral E/A	1.4 (1.0-1.6) ^{AB}	0.9 (0.7-1.2) ^{A}	1.1 (0.8-1.4) ^{A}	2.3 (1.6-3.2) ^{B}	<0.0001*
IVRT (ms)	58.0 (40.2-56.2)	51.0 (43.0-60.5)	51.0 (41.5-51.2)	35.0 (17.5-49.5)	0.1514*
E/IVRT	1.5 (1.1-2.4) ^{A}	1.3 (1.1-1.6) ^{A}	1.7 (1.4-2.2) ^{AB}	3.9 (2.5-9.6) ^{B}	<0.0001*

BSA indexed LVd: body surface area indexed left ventricular internal diameter in diastole; BSA indexed LVs: body surface area indexed left ventricular internal diameter in systole; WSId: wall stress index in diastole; WSIs: wall stress index in systole; FS: fractional shortening; LA: left atrium; Ao: aorta; E: E wave; A: A wave; IVRT: isovolumic relaxation time *Non parametric analysis

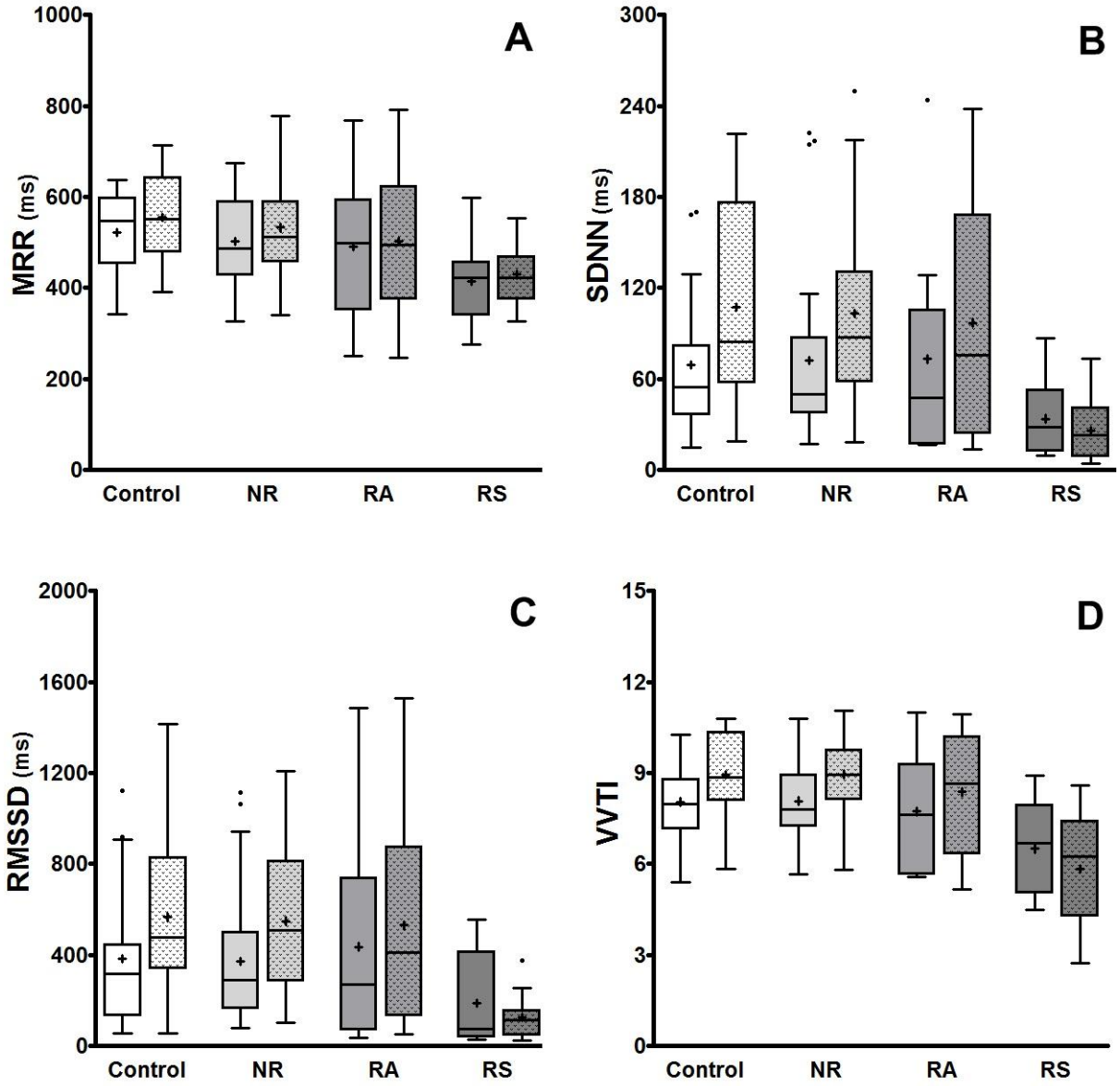

Fig 1 - Box-plots depicting the effect of oculocardiac reflex (OCR) on (A) mean RR interval (MRR), (B) the standard deviation of the RR intervals (SDNN), (C) the root mean square of the successive differences in RR intervals (RMSSD) and (D) the vasovagal tonus index (VVTI), which were used as surrogates for autonomic control in healthy control dogs and mitral insufficient dogs with no cardiac remodeling (NR), remodeled hearts but still asymptomatic (RA) or remodeled heart and symptomatic (RS). Dashed boxes represent the parameters recorded after OCR. Outliers are shown. ("+" represents de mean).

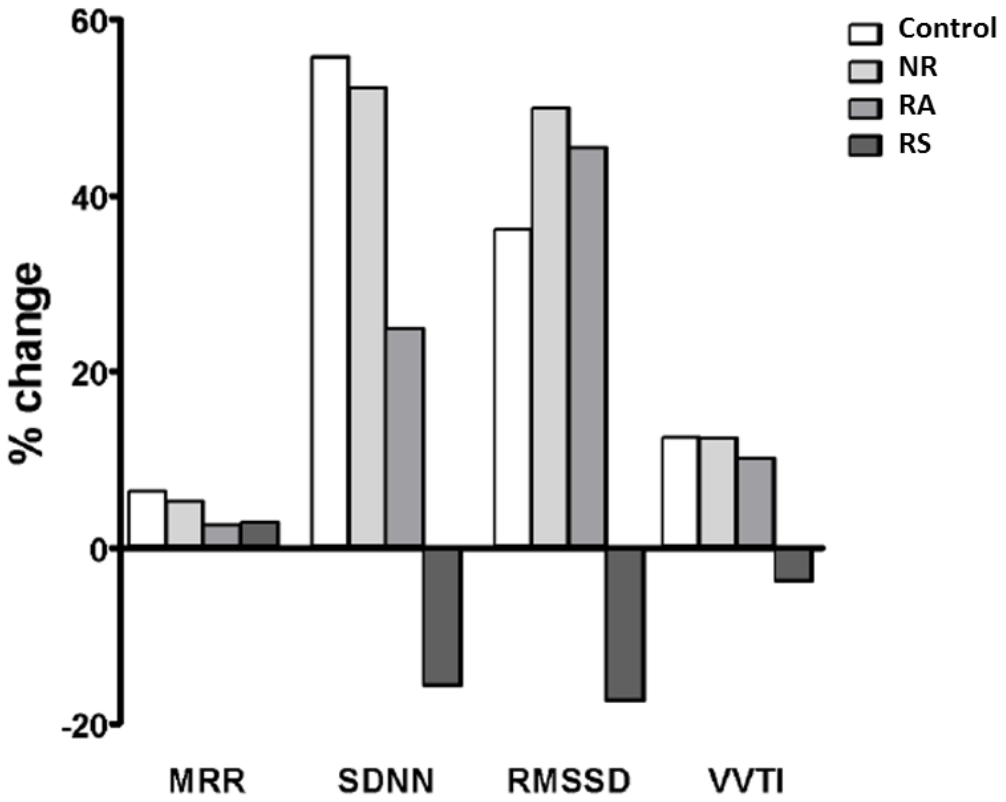

Fig 2 - Median percent change (before-after) of the surrogates used to assess autonomic balance in healthy control dogs and mitral insufficient dogs with no cardiac remodeling (NR), remodeled hearts but still asymptomatic (RA) or remodeled heart and symptomatic (RS) undergoing manual ocular compression to induce the oculocardiac reflex.

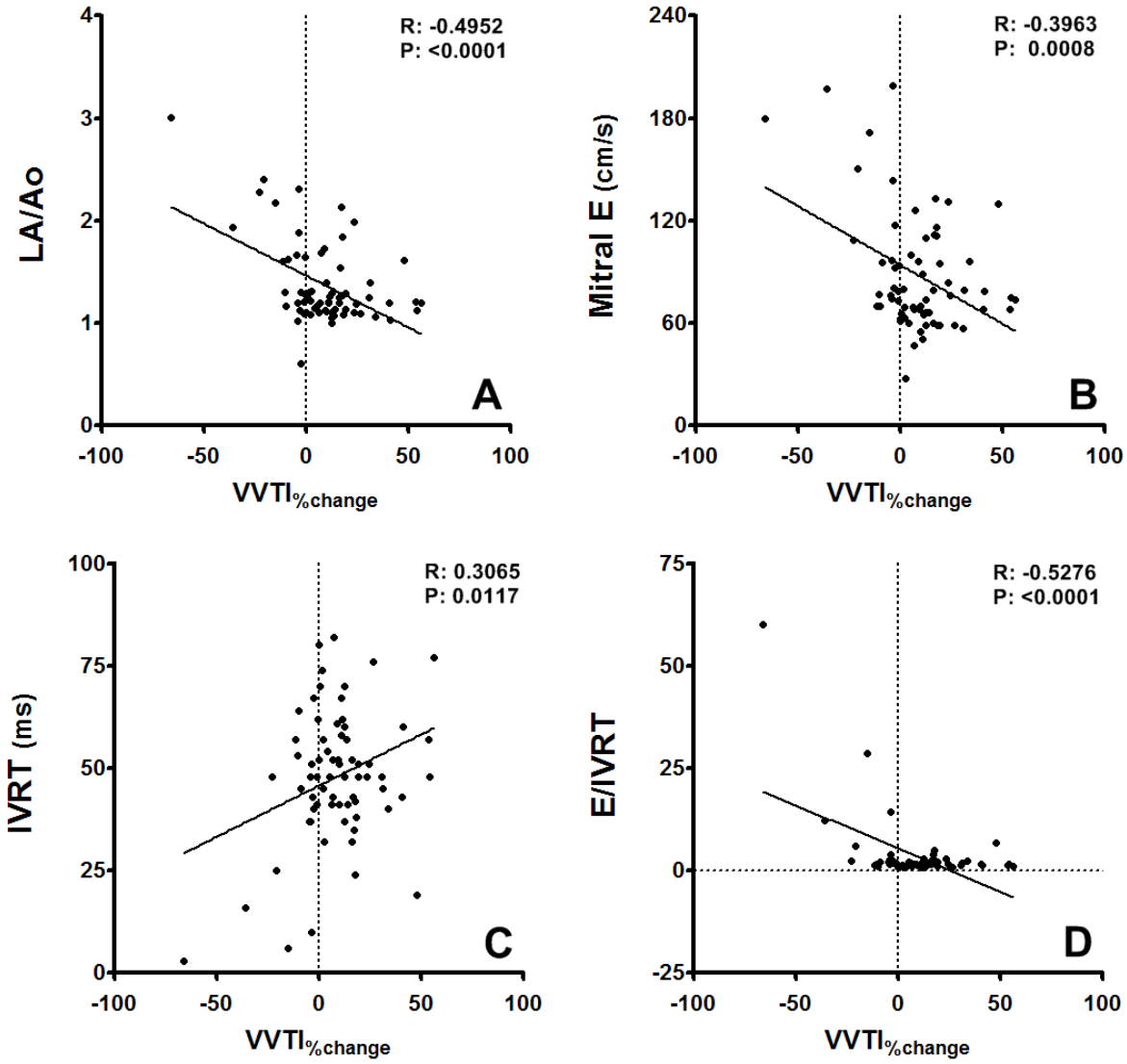

Fig 3 – Scatter plots depicting the significant correlations obtained between the percent change in VVTI and (A) the left atrium-to-aorta ratio, (B) mitral E wave, (C) isovolumic relaxation time and (D) mitral E wave-to-isovolumic relaxation time ratio.

CHAPTER 3 - QT Instability, an Indicator of Augmented Arrhythmogenesis, Increases With The Progression of Mitral Valve Disease in Dogs[3]

ABSTRACT

Objectives: To investigate the occurrence of prolongation and/or instability of the QT interval in dogs with naturally-occurring mitral insufficiency, and to assess whether these findings are associated with an increased predisposition to augmented ventricular arrhythmogenesis.

Animals: 167 dogs diagnosed with myxomatous mitral valve disease that met study inclusion criteria.

Methods: Echocardiographic data and 5-minutes ECG tracings were gathered. Fifity consecutive QT intervals measured from sinus beats were recorded for each dog. Both heart rate-corrected and uncorrected QT intervals were used to calculate the average QT (QTa) and QT variance (QTv), as well as total instability (TI), short-term instability (STI), and long-term instability (LTI) using a previously reported technique. Analysis of variance and Kruskal-Wallis test followed by Tukey and Dunns test, respectively, were used to investigate differences between disease stages in the studied population, and Spearman's test was used to assess existing correlations of such indices and the echocardiographic data. Sensitivity and specificity of QTa, QTv, TI, STI and LTI in identifying arrhythmias and remodeling was calculated. Follow-up from referring veterinarians was obtained by questionnaire, and progression and survival were analyzed using Kaplan-Meier curves.

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[3] Written in accordance with the guidelines of Journal of Veterinary Cardiology, available at *https://www.elsevier.com/journals/journal-of-veterinary-cardiology/1760-2734/guide-for-authors*

Results: An increase related to disease progression was documented for every index studied, with significant difference between B1 and B2 dogs for QTa, and between B1 and C dogs for all the rest. The studied also demonstrated that QTa and STI were the indices that best distinguished remodeled from dilated hearts, and ventricular arrhythmias from only sinus rhythms, respectively. Dogs with QTa >310 and STI >7.9 were shown to be slightly (15%) and moderately (30-45%) more prone to developing ventricular arrhythmias, respectively. A QTa >300 was able to discriminate B1 from B2/C with a sensitivity of 61% and specificity of 70%. The use of QT intervals corrected for heart rate lead to more significant results, as opposed to results obtained from uncorrected QT intervals. All indices in exception of LTI showed prognostic value, with increases significantly relating to all-cause mortality.

Conclusion: Quantification of beat-to-beat QT instability clearly demonstrated changes in short-term, long-term and total instability. Stage C of MMVD dogs are presented with higher STI, which results in a 3.71 times increase in probability of developing ventricular arrhythmias when values over 7.9 are reached. Increased indices of QT prolongation and instability are significantly related to mortality and may be useful as prognostic surrogates in MMVD patients.

KEYWORDS: arrhythmias, electrocardiography, repolarization, congestive heart failure, prognosis

Abbreviations

ACVIM	American College of Veterinary Internal Medicine
Ao	aorta
BW	bodyweight
CHF	congestive heart failure
FS%	fractional shortening
IVRT	isovolumic relaxation time
LA	left atrium
LTI	long-term instability
LVd	left ventricle in diastole
LVs	left ventricle in systole
MMVD	myxomatous mitral valve disease
MVP	mitral valve prolapse
PVC	premature ventricular complexes
QTa	average QT
QTc	corrected QT
QTv	QT variance
QTVI	QT variability index
ROC	receiver operating characteristic
STI	short-term instability
TI	total instability
WSId	wall-stress index in diastole
WSIs	wall-stress index in systole

INTRODUCTION

Sudden cardiac death, frequently caused by ventricular arrhythmias, is an important concern in cardiovascular medicine, since it is responsible for nearly half of the mortality in patients with structural cardiac diseases [1]. Although implantable cardioverter defibrillators are currently the most effective therapy for patients at risk of dying suddenly [2], a precise method to identify patients that would benefit from prophylactic implantation remains undiscovered [3]. The elucidation of the electrophysiological events underlying rhythm disorders may provide surrogate markers that are able to predict the occurrence of life-threatening arrhythmias in these patients, in order to improve prevention and/or optimize clinical and surgical approach [4].

The QT interval is the electrocardiographic representation of the total ventricular activity, and is affected by repolarization disorders [5]. Unstable repolarization dynamics has been hypothesized to play a role in the generation of ventricular arrhythmias, which first led Berger and colleagues [6] to create a non-invasive measure of repolarization known as QT variability index (QTVI). Due to their promising results regarding arrhythmia risk stratification in ischemic and non-ischemic dilated cardiomyopathy, QTVI has since been applied to a wide variety of subjects with cardiovascular disease. Also, it has inspired the development of novel methods aimed at assessing the risk of augmented arrhythmogenesis and sudden cardiac death [7,8].

Prolongation and increased variability of the QT interval have both been described in a series of cardiac conditions [9,10,11,6]. In mitral valve prolapse (MVP), the most common valvular disease in human beings, especially in the young, repolarization abnormalities have been documented, with prolongation and dispersion of the QT interval increasing as the disease progresses [12,13]. Although MVP is a predominantly benign condition, sudden death is a significant complication, due to a probable arrhythmogenic underlying mechanism. The etiology of these arrhythmias seems multifactorial, including anatomical substrate (myocardial stimulation through prolapsed leaflets and ventricular dilation) and a disrupted autonomic nervous system [14].

When it comes to veterinary medicine, myxomatous mitral valve disease (MMVD) is the most common cause of mitral regurgitation in dogs, and also represents the most frequent acquired cardiac disease in that species [15]. Due to macro and microscopic similarities, as well as an analogous progression, MMVD in dogs has been proposed as a spontaneous animal model of MVP [16]. Researches

involving electrocardiographic recordings have documented that the occurrence of ventricular arrhythmias increases significantly with the progression of the disease, being most commonly diagnosed in dogs in congestive heart failure (CHF) as compared to those in the earlier stages of the disease [17]. Prolongation of the QT interval is described in dogs with ventricular arrhythmias, particularly in those under treatment with class I or III antiarrhythmics, such as sotalol and quinidine [5]. Also, increases in QT interval and QT variability have been identified in dogs with heart failure in a model of pacing-induced tachycardia [18]. Nonetheless, the prolongation and instability of the QT interval in dogs with naturally-occurring mitral valve disease is yet to be documented.

With this in mind, in this study the authors hypothesized that symptomatic MMVD dogs would exhibit an augmented instability of QT interval, which would make them more prone to developing ventricular arrhythmias. Therefore, the purpose of this study was three-fold: 1) To investigate whether dogs with MMVD develop a prolongation and/or instability of the QT interval in accordance with the progression of the disease; 2) To assess how the indices of QT instability relate to ventricular arrhythmias and the echocardiographic indicators of cardiac remodeling and function; 3) To evaluate the role of QT instability indices as prognostic surrogates for mortality and the development of cardiac-related clinical signs in dogs with naturally-occurring mitral valve disease.

ANIMALS, MATERIAL AND METHODS

Dogs recruited for this prospective cross-sectional observational study were selected among patients admitted for regular cardiac evaluation at a veterinary teaching facility. All procedures were previously approved by the institutional Animal

Use Committee, and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

In order to be included in the study, the diagnosis of MMVD at any stage was required, which was based on clinical history and the echocardiographic criteria of impaired valvar anatomy and function [19]. Dogs that lacked valvar commitment and those with echocardiographic evidence of any congenital or acquired cardiac disease other than MMVD were excluded from the study, along with patients undergoing antiarrhythmic therapy at time of diagnosis. Some echocardiographic parameters, including left atrium-to-aorta ratio (LA/Ao), body weight-indexed left ventricular internal diameter at end-diastole and end-systole (BW-indexed LVd and BW-indexed LVs), wall stress index in diastole and systole (WSId and WSId), fractional shortening (FS%), mitral E wave peak velocity (mitral E), mitral E-to-A ratio (mitral E/A), isovolumic relaxation time (IVRT) and mitral E-to-IVRT ratio (E/IVRT) were also recorded for each dog for posterior correlation with the QT parameters. All echocardiograms were carried out by experienced veterinary cardiologists using an ultrasonography system (MyLab 30 – Esaote, Genova, Italy) equipped with 5.0 MHz and 7.5 MHz phased array transducers (P240 and P023 reference – Esaote, Genova, Italy).

After the selection, ECG tracings were acquired for each patient using a computer-based ECG recorder (ECGP V6, TEB – Brazilian Electronic Technology Ltda., São Paulo, SP, Brazil). The dogs were placed in right lateral recumbency and maintained in position by gentle physical restraint. The electrodes attached to the skin were toothless alligator-types to reduce discomfort, and alcohol was applied to improve electrical conduction. The left arm and right arm electrodes were placed at

the elbows, while the left leg and right leg electrodes were placed at the stifles. ECG was performed continuously and uninterrupted for five minutes.

ECG tracings were reviewed, and 50 consecutive QT intervals obtained from sinus beats were obtained for each dog. Whenever ectopic ventricular depolarizations occurred, they were ignored and the QT interval was then obtained from the following sinus beat. To avoid inter-observer discrepancy, QT intervals were measured by the same investigator, who was also blinded to the patient's heart condition determined by echocardiography and clinical history. Once the 50 QT intervals were obtained, they were corrected for heart rate using the method described by Bazzet [20]. Following, both corrected and uncorrected QT intervals were used to calculate the average QT (QTa) and QT variance (QTv). Also, we calculated the total instability (TI), the short-term instability (STI), and the long-term instability (LTI) using the technique proposed by Van der Linde and colleagues [7] to quantitatively assess QT instability by means of a Poincaré plot. The calculation of these indices was based on the following equations:

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

$$TI_n = M(TIn)$$

$$LTI_n = \left|Rcg(x) - ((\cos\theta \; x \; QT_{n+1}) - (\sin\theta \; x \; QT_n))\right|$$

$$LTI = M(LTIn)$$

$$STI_n = \left|Rcg(y) - ((\sin\theta \; x \; QT_{n+1}) - (\cos\theta \; x \; QT_n))\right|$$

$$STI = M(STIn)$$

Where cg represents the center of gravity of the data cluster, and *Rcg* the rotated centers. These parameters were previously obtained from the following equations:

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

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

$$Rcg(x) = (\cos\theta \; x \; cg(x)) - (\sin\theta \; x \; cg(y))$$
$$Rcg(y) = (\sin\theta \; x \; cg(x)) + (\cos\theta \; x \; cg(y))$$

Briefly, the morphological properties of the plot were used to determine the indices, based in the distribution of the points in the data cluster, the width being a measure of the STI, the length a measure of the LTI and a width-and-length-dependent parameter the measure of TI. An example of the Poincare plot is represented in Figure 1.

For statistical purposes, the dogs were divided in accordance with the stage of MMVD (B1, B2 and C) proposed by the consensus statement of the American College of Veterinary Internal Medicine [21], which depended on clinical signs attributable to CHF, as well as the echocardiographic evidence of cardiac remodeling. All data underwent the Shapiro-Wilk normality test. Data obtained from uncorrected and heart rate corrected QT intervals were compared. Either an analysis of variance followed by Tukey´s multiple comparison test or the Kruskal-Wallis test followed by Dunn's test was used to investigate differences between disease stages in the studied population. Spearman's test was used to assess whether correlations existed between QT indices and heart rate, age, body weight and body surface area, as well as between the indices and the echocardiographic surrogates of congestion and function. Receiver operating characteristic (ROC) curves were constructed to investigate sensitivity and specificity of QTa, QTv, TI, STI and LTI to differentiate MMVD dogs with and without previously recognized ventricular arrhythmias, and to distinguish patients with dilated hearts from those with no remodeling. Finally, Kaplan-Meier curves and the log-rank test were used to assess the prognostic value of QT indices concerning the development of clinical signs attributable to congestive

heart failure (for B1 and B2 dogs) and the all-cause mortality (for all dogs). All analyses were performed using the software GraphPad Prism (version 5.0 - San Diego, CA, USA) using default settings. For all analyses, the level of significance was defined as $P<0.05$.

RESULTS

In this investigation, we sought to assess QT instability in dogs with MMVD by means of a mathematical technique in which sequential QT intervals are used to calculate how unstable the electrical activity of the ventricular myocardium is. One-hundred-and-sixty-seven client-owned dogs were recruited at the end of the study. Several breeds were represented, and the age and body-weight of the animals ranged from 6-18 years and 2.7-36 kg, respectively. Sixty-six, 64, and 37 animals were classified as stages B1, B2 and C, respectively. No difference existed between stages regarding weight ($P= 0.2286$) and age ($P=0.721$).

A significant difference was documented between B1 and C and B2 and C for QTv, TI and STI when using both QT and QTc, as shown in Figure 2. When using corrected QT intervals, QTv increased from 131.1 (B2) to 265.7 (C). A significant increase between the same groups was also documented for TI (14.27 to 18.78) and STI (6.98 to 10.54). No difference between groups was found for QTa and LTI when using uncorrected QT intervals, but significant difference was found between B1 and C (QTa and LTI) and B2 and C (QTa) when using QTc. LTI increased from 9.27 (B1) to 12.05 (C), and stage C dogs had a significantly higher QTa (321.3) when compared to stage B2 (302.7). For QTa, a difference between B1 (287.0) and B2 (302.7) was also observed when using QTc, as shown in Figure 2-A. When only the visual characteristics of the Poincare plot are taken into consideration, it is also clear

that QT instability increases with the progression of MMVD, as shown in Figure 3. Considering all analyses, statistic differences were more significant when using the heart rate-corrected QT intervals. In general, stage C dogs presented significantly higher values for most of the analyzed parameters when compared to stage B1 and B2 dogs.

Only the indices calculated from QT intervals corrected for heart rate were used in the correlation tests. All five indices showed moderate positive correlations with heart rate, and every index but QTa showed weak negative correlations with body weight. QTa was the only index that correlated significantly with age (R: 0.2128). Weak but significant correlations were found to exist between QTa, QTv, STI and TI and the described echocardiographic parameters except for BW-indexed LVs. A moderate positive correlation was found between LA/Ao and QTa (R: 0,4294). LTI was the index that correlated less with the echocardiographic parameters, only weakly doing so with LA/Ao, BW-indexed LVd, WSId, WSIs and FS%. Significant correlations and their respective Spearman correlation coefficients are shown in Table 2. Scatter plots depicting the most significant correlations (R >0.25) are shown in Figure 4.

Again, only the heart rate corrected QT intervals were used to construct the ROC curves. Areas under the curve (AUC) >0.5 were obtained when using QTa and STI to differentiate dogs with and without ventricular arrhythmias at time of diagnosis, and their corresponding curves are shown in Figures 5-A and 5-B. QTa had an AUC of 0.713 (P=0.0348), while STI showed and AUC of 0.6992 (P=0.0455) Also, to distinguish dogs with remodeled hearts from those without remodeling, an AUC >0.5 was documented for each of the five QT indices. Of note, QTa (AUC=0.7121; P<0.0001) and QTv (AUC= 0.6799; P<0.0001) were the indices that best

discriminated B1 from B2/C dogs, and the ROC curves for these parameters are shown in Figures 5-C and 5-D. The cut-off values for these surrogates and their respective sensitivity, specificity, and positive likelihood ratio when used to detect the presence of ventricular arrhythmias and cardiac remodeling are shown in Tables 3 and 4, respectively.

Concerning the development of clinical signs attributable to congestive heart failure, none of the calculated indices were able to predict the time needed for B1 and B2 dogs to become overtly symptomatic. On the other hand, the increase in all indices but LTI was associated with increased all-cause mortality. For instance, QTa, when a cut-off value of 302 is applied, reflects in a median difference of 105 days in survival time, with a hazzard-ratio of 0.4296. Similary, STI reflects a median difference of 128.5 with a hazard-ratio of 0.4986 when a cut-off value of 7.5 is applied. The results of the prognostic study are shown on Table 5 and the Kaplan-Meier curves obtained for the significant findings are also shown on Figure 6.

DISCUSSION

Studies regarding MMVD in dogs have shown an increase in ventricular arrhythmias with the progression of the disease, although precise mechanisms are yet to be underlined [17]. In our study, we demonstrated that prolongation and instability of the QT interval is not only present in these dogs, but increases with the disease progression. This finding is most likely involved in the generation of ventricular arrhythmias. In the normal heart tissue, myocyte repolarization time is uniform at a given rate, and autonomic modulation and occasional changes in electrolyte levels cause modest predictable effects on the action potential. In chronic conditions such as heart failure, however, there is an increase in beat-to-beat

variability in action potential duration, resulting from increased membrane instability, steepening of the restitution relationship between the QT and diastolic interval, and increased ratio of sympathetic to parasympathetic tone [22]. Intermittent prolongation of the QT interval promotes the opportunity for a premature depolarization to arise, block in one area, and conduct in an adjacent region. The result is an increased risk for reentrant arrhythmias, particularly ventricular fibrillation [23].

A few methods for measuring repolarization instability have been described in literature, most of them using the standard deviation of QT intervals. The length and width of a Poincaré plot have been described as a weighted combination of low- and high-frequency power, which can also be used for calculating instability of QT intervals [24]. In a study by Van der Linde et al [7], 32 Beagle dogs were given increasing doses of a potentially arrhythmogenic anestheti. Their results were superior in detecting prolongation and instability of the QT interval, demonstrating a higher precision still in pre-arrhythmic states as compared to other methods aimed at quantifying Poincaré plots, which have been used to conventionally determine heart rate instability [25,26] or ventricular repolarization [27].

Increased heart rate is known to have negative correlation with QT intervals, which may disguise prolongation and instability. Since dogs in the advanced stages of the disease tend to have increased heart rate owing to autonomic imbalance [28], the use of the QT interval corrected for heart rate seems more proper, as the results of this study have confirmed. When using corrected QT intervals for calculation, QTa, QTv, TI, STI and LTI all pointed to a significant difference between stages B1 and C regarding the potential to developing arrhythmia.

Even though it may seem obvious, this study was able to demonstrate objectively that both prolongation (QTa) and instability of the QT interval increase

with the progression of MMVD. Stage C dogs are significantly more prone to ventricular arrhythmias, in agreement with the findings of Crosara et. al [17]. Also, our study shows that, as it happens with the frequency of ventricular arrhythmias, QT instability is higher in advanced stages, and repolarization disorders may actually play a role in the development of these arrhythmias in symptomatic patients. Also, the significant difference between B1 and B2 found for QTa may indicate that prolongation of the QT interval precedes instability, therefore being the first indicator of repolarization disorder in an otherwise asymptomatic animal. Similar findings have been described in people with MVP. In a Holter study of autonomic profile and QT dynamicity, Digeos-Hasnier et al. [12] found that prolongation of the QT interval appears precociously in MVP patients, even in non-symptomatic stages, when compared to a control group. This finding increases the similarities and straightens the role of dogs with MMVD as a naturally occurring animal model of MVD.

In regards to the echocardiographic data, the identification of significant correlations with the QT instability indices supports the hypothesis that prolongation and instability of the QT interval increases with disease progression. In severe cases of mitral valve regurgitation, volume overload leads to left atrial and ventricular remodeling, both of which are associated with the onset of clinical signs attributable to cardiac disease [29]. The significant correlations with indices of congestion and function, especially the moderate positive correlation between QTa and LA/Ao (a surrogate with known prognostic value in MMVD), are supportive of repolarization disorders increasing as clinical signs become more severe [29].

Concerning sensitivity and specificity, an AUC >0.5 was found for QTa and STI when the indices were used to discriminate dogs with and without ventricular arrhythmias. Dogs with QTa >310 (sensitivity:56%/specificity:71%), and STI >7,9

(sensitivity:67%/specificity:65%) were shown to be slightly (15%) and moderately (30-45%) more prone to developing ventricular arrhythmias, respectively. Since QTa is the index that best reflects prolongation of the QT interval, which precedes instability and clinical evidence as this study has shown, QTa may be useful as an early indicator of repolarization disorders in still asymptomatic patients. As the disease progresses and prolongation of the QT interval evolves to instability, STI seems to be the most effective index to identify patients in more advanced stages most likely to develop ventricular arrhythmias. As shown in the odds ratio column in Table 4, MMVD dogs with STI> 7.9 are 3.7 times more likely to present ventricular arrhythmias. These parameters, to the author's knowledge, are the first electrocardiographic indices with predictive value for ventricular arrhythmias in dogs with myxomatous mitral valve disease.

Also, QTa was the index that best discriminated MMVD dogs with remodeled and non-remodeled hearts. A QTa >300 was able to discriminate B1 from B2/C with a sensitivity of 61% and specificity of 70%, with dogs being 3.6 times more likely to show cardiac dilatation. Although echocardiography is the gold standard for evaluation of cardiac anatomy and function, the existence of indices of remodeling obtainable from routine electrocardiographic tracings may aid in clinical approach when echocardiography may be unavailable.

Finally, another important result of this study was the significant correlations found between most of the studied indices and all-cause mortality. Diagnosis and staging of MMVD, with the evolution and increasing accessibility to echocardiography, has become more and more precise. Prognosis, on the other hand, due the variable nature of disease progression, is still a challenge [30]. Up to date, there is no ideal way of telling if or when an asymptomatic patient with mitral

disease will develop CHF, nor how long will it live after that. In this matter, surrogates with prognostic value have gained attention, with LA/Ao, heart rate variability and NT-proBNP serum levels standing out as the most reliable tools [29,31]. In this study, we have shown that indices of QT instability, which are easily calculated from standard ECG tracings, may also aid in prognosis, offering complementary information for determining mortality in affected dogs.

An important limitation of this research relates to the indices being obtained from regular ECG recordings. As we know, paroxysmal ventricular arrhythmias may lead to false negative diagnosis when based in five-minute tracings. The use of a 24-hour Holter monitoring would certainly result in the identification of a higher number of animals with ventricular arrhythmias, which might have increased sensitivity and specificity of the studied parameters. Also, the use of Holter monitoring with automated analysis based on a pre-determined QT duration criteria to quantify variability, as proposed by Berger et al. [6] ensures reproducibility. The method involving the Poincaré plot used in this study requires a smaller amount of data, enabling manual beat-to-beat measuring, which may vary between observers. Although the QT intervals were measured by the same investigator in order to avoid inter-observer discrepancy, consistent reproducibility of the method requires more studies. Another possible limitation was the use of sinus beats that followed ventricular ectopic beats in the formulas. Although premature ventricular contractions (PVCs) were excluded from the analysis of QT variability, QT dynamics after PVCs is poorly understood. It is speculated, however, that premature ventricular contractions (PVC) can alter the QT interval within several consecutive cardiac cycles [32]. Finally, QT duration was measured only on lead II and, therefore, might carry errors, as

measurement of the heart vector on a single ECG lead is never completely reliable [33].

CONCLUSIONS:

Both prolongation and instability of the QT interval increase with progression of mitral valve disease in dogs, and are likely to play a role in the development of ventricular arrhythmias in these patients. QTa and STI are, up to date, the only parameters obtained from conventional ECG tracings with predictive value for ventricular arrhythmias in MMVD dogs. Also, the indices of QT instability may serve as prognostic surrogates for mortality.

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TABLES AND FIGURES

Table 1 - Characteristics of the dog population enrolled in the study. Either mean (SD) or median (IQR) are shown. Dogs were divided in accordance with the disease stages proposed by the consensus statement of the American College of Veterinary Internal Medicine.

	MMVD Stage			P
	B1	B2	C	
N	66	64	37	-
Age (years)	11 (6-18)	12 (7-18)	12 (7-18)	0.7210
Sex (F/M)	42/24 (64%/36%)	31/33 (48%/52%)	21/16 (57%/43%)	-
Body weight (kg)	7.7 (2.7-36.0)	7.3 (3.3-16.5)	7.2 (2.9-15.0)	0.2286
BW-indexed LVd (mm/kg)	64.9 ± 17.9 [A]	77.6 ± 20.2 [B]	98.5 ± 20.1 [C]	<0.0001*
BW-indexed LVs (mm/kg)	37.9 (32.9-46.3) [A]	40.1 (34.7-51.3) [AB]	49.7 (40.1-58.3) [B]	0.0052
WSId	3.8 (3.1-4.3) [A]	4.3 (3.3-5.2) [AB]	5.0 (3.9-5.8) [B]	0.0011
WSIs	1.4 (1.2-1.7)	1.4 (1.2-1.9)	1.5 (1.1-1.9)	0.8252
FS (%)	45.1 ± 8.8 [A]	47.3 ± 9.8 [AB]	50.9 ± 9.3 [C]	0.0110*
LA/Ao	1.1 (1.1-1.2) [A]	1.6 (1.5-1.8) [B]	2.1 (1.9-2.4) [C]	<0.0001
Mitral E (cm/s)	66.5 (56.7-74.2) [A]	96.0 (75.0-116.0) [B]	132.8 (101.4-171.3) [C]	<0.0001
Mitral E/A	1.1 (1.1-1.3) [A]	1.1 (0.9-1.3) [A]	1.6 (1.2-2.3) [B]	<0.0001
TRIV (ms)	60 (48-72) [A]	57.0 (44.0-61.5) [A]	33.0 (19.0-48.5) [B]	<0.0001
E/TRIV	1.1 (0.8-1.6) [A]	1.7 (1.3-2.1) [B]	4.1 (2.6-7.6) [C]	<0.0001

BW-indexed LVd: body weight-indexed left ventricular internal diameter at end-diastole; BW-indexed LVs: body weight-indexed left ventricular internal diameter at end-systole; WSId: wall stress index at end-diastole; WSIs: wall stress index at end-diastole and systole; FS: fractional shortening; LA/Ao: left atrium-to-aorta ratio; Mitral E: mitral E wave peak velocity (mitral E); Mitral E/A: mitral E-to-A ratio; IVRT: isovolumic relaxation time; E/IVRT: mitral E-to-IVRT ratio. (*) parametric analyses

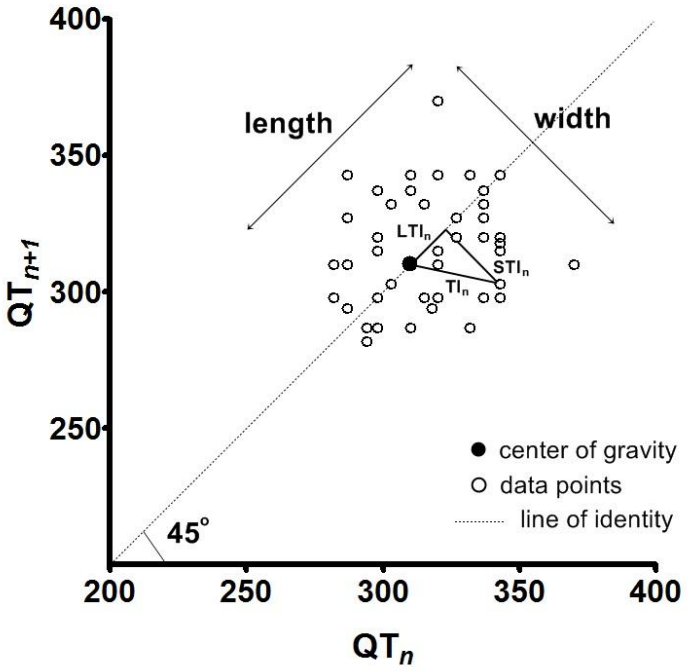

Figure 1 - A schematic example of the Poincaré plot and the quantification of QT instability based on the distances of a cluster of 50 QT intervals to the center of gravity as proposed by Van der Linde and colleagues (2005). The rationale behind the determination of total instability (TI), long-term instability (LTI) and short-term instability (STI) is the distance of each individual point to either the center of gravity or the identity line. In this example, not all data points are seen because some are exactly the same.

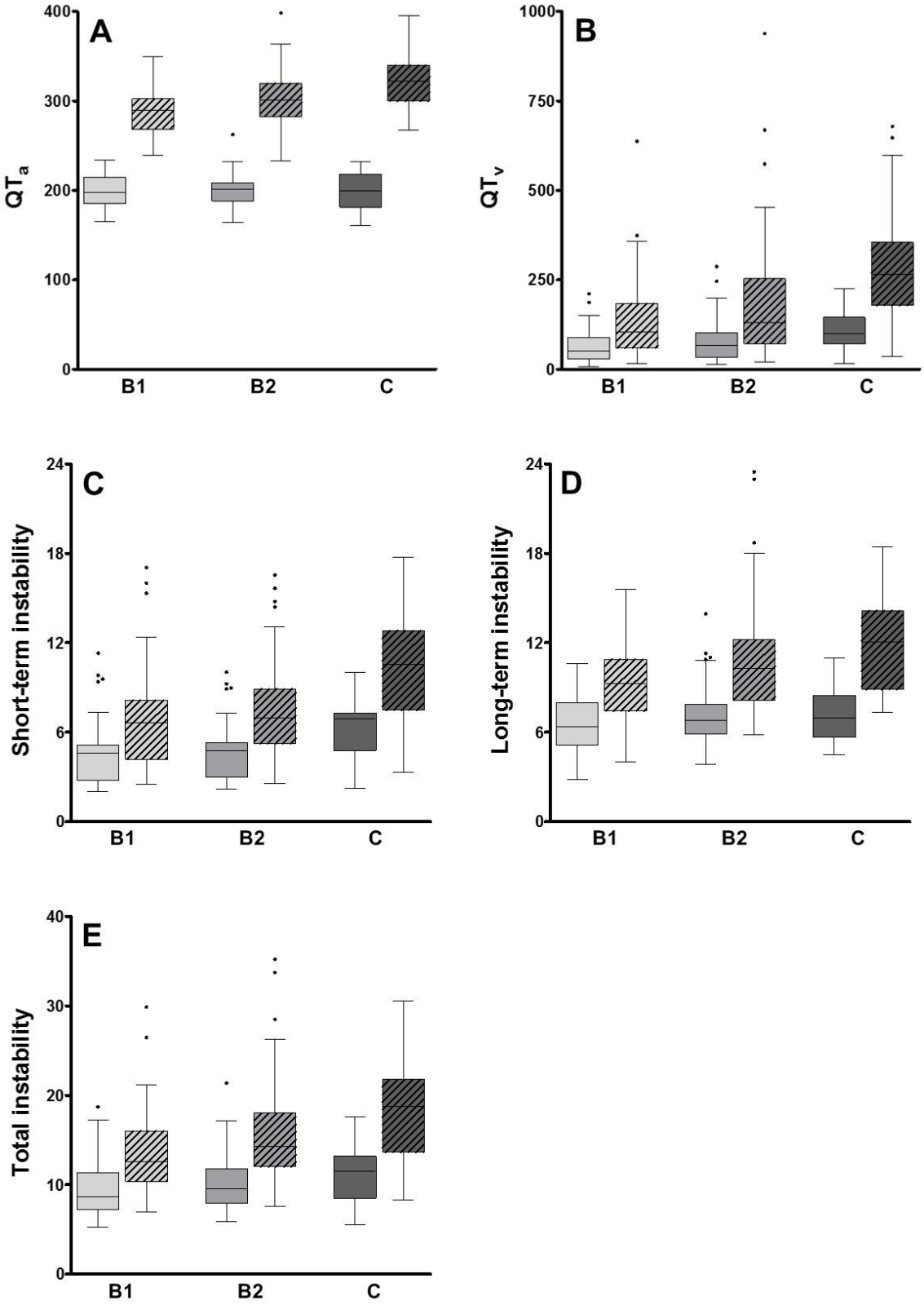

Figure 2 - Box plots depicting the medians, interquartile ranges and amplitude of (A) average QT, (B) QT variance, (C) short-term instability, (D) long-term instability and (E) total instability in dogs in different stages of myxomatous mitral valve disease. Dashed boxes represent the results calculated from heart rate-corrected QT intervals. Outliers are shown.

Table 2 - Significant correlations documented between between QT instability parameters and the echocardiographic indices of congestion and function.

	QTa	QTv	TI	LTI	STI
BW-indexed LVd	R: -0.1615 P: 0.0469	R: 0.2712 P: 0.0007	R: 0.2068 P: 0.0106	-	R: 0.2631 P: 0.0011
BW-indexed LVs	R: -0.1984 P: 0.0143	-	-	R: -0.1779 P: 0.0283	-
WSId	-	R: 0.1556 P: 0.0452	-	-	R: 0.2111 P: 0.0063
WSIs	-	R: -0.2214 P: 0.0040	R: -0.1839 P: 0.0173	R: -0.1617 P: 0.0368	R: -0.2010 P: 0.0092
FS%	-	R: 0.2462 P: 0.0013	R: 0.1731 P: 0.0252	-	R: 0.2296 P: 0.0028
LA/Ao	-	R: 0.2271 P: 0.0032	R: 0.1944 P: 0.0118	-	R: 0.1849 P: 0.0168
Mitral E	-	R: 0.1965 P: 0.0112	R: 0.1642 P: 0.0345	-	R: 0.2059 P: 0.0078
Mitral E/A	-	R: 0.1567 P: 0.0452	-	-	R: 0.1606 P: 0.0405
IVRT	-	R: -0.2271 P: 0.0224	-	-	R: -0.3266 P: 0.0009
E/IVRT	-	R: 0.2065 P: 0.0393	-	-	R: 0.2883 P: 0.0036

QTa: average QT interval; QTv: QT variance; TI: total instability; STI: short-term instability; LTI: long-term instability; BW-indexed LVd: body weight-indexed left ventricular internal diameter at end-diastole; BW-indexed LVs: body weight-indexed left ventricular internal diameter at end-systole; WSId: wall stress index at end-diastole; WSIs: wall stress index at end-diastole and systole; FS%: fractional shortening; LA/Ao: left atrium-to-aorta ratio; Mitral E: mitral E wave peak velocity (mitral E); Mitral E/A: mitral E-to-A ratio; IVRT: isovolumic relaxation time; E/IVRT: mitral E-to-IVRT ratio.

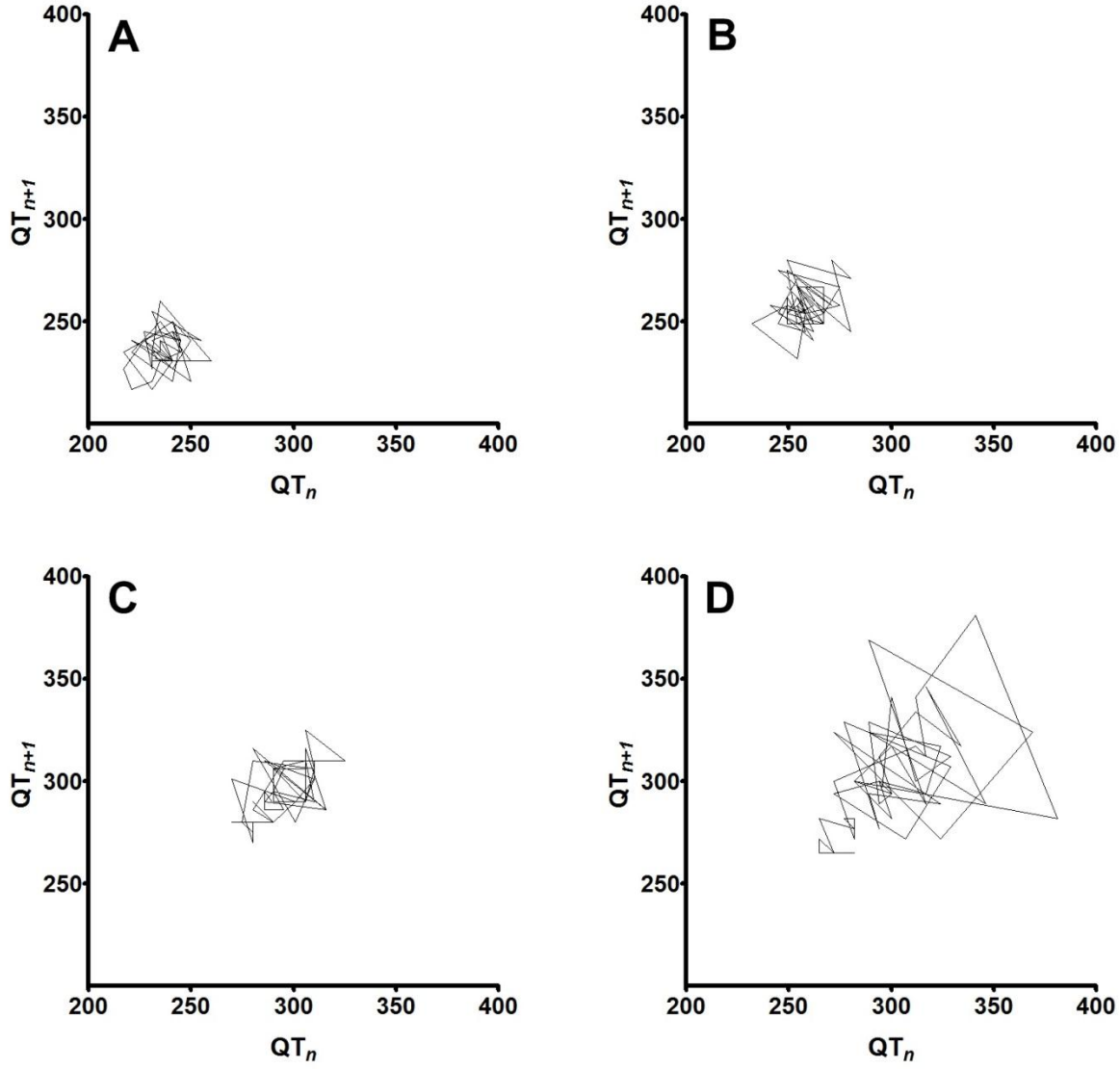

Figure 3 – Modified Poincare plots in which a line is connecting the 49 combinations of 50 successive QT intervals to depict QT instability of four different patients, in different stages of MMVD. (A) B1 dog with a total instability of 12.1; (B) B2 patient with total instability of 14.3; (C, D) C and D dogs with total instability of 18.1 and 27.8, respectively.

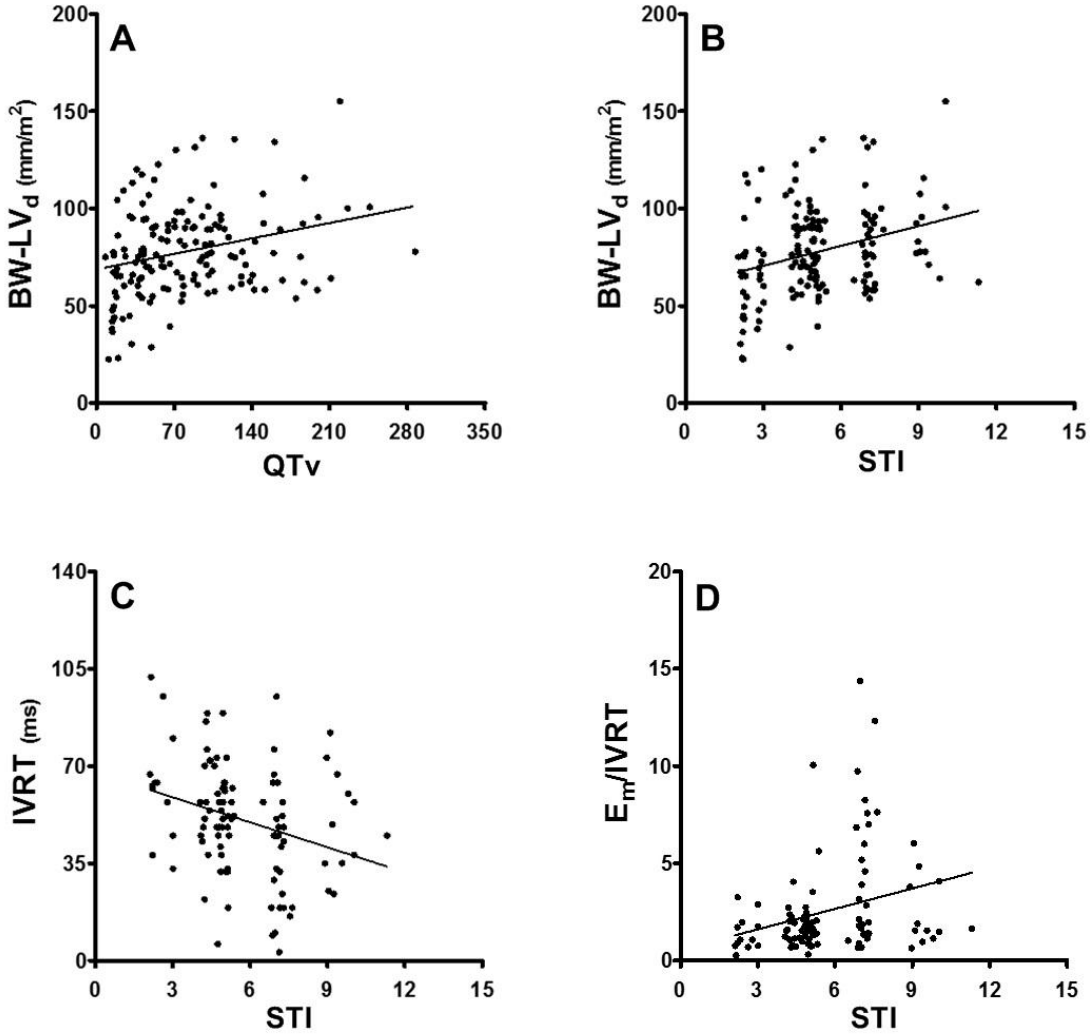

Figure 4 – Scatter plots depicting the most significant correlations (R >0.25) obtained between (A) body weight-indexed left ventricular internal diameter at end-diastole and the QT interval variance (QTv); (B) body weight-indexed left ventricular internal diameter at end-diastole and the short-term instability (STI) of QT interval; (C) isovolumic relaxation time (IVRT) and the STI of QT interval; and (D) mitral E wave peak velocity-to-isovolumic relaxation time ratio and the short-term instability of QT interval. Linear regression lines are shown.

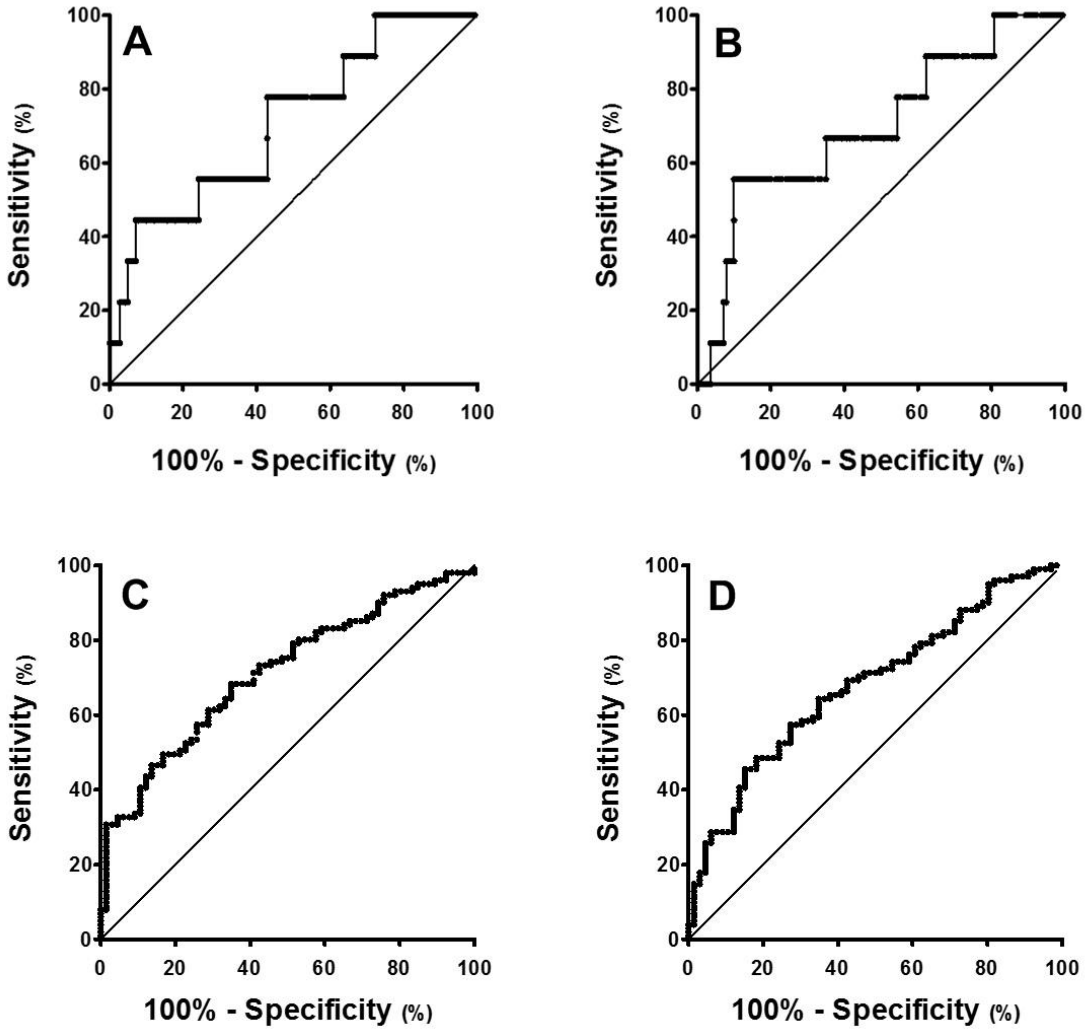

Figure 5 - Receiver operating characteristic (ROC) curves constructed to investigate the sensitivity and specificity of the (A) average QT and (B) short-term instability to differentiate myxomatous mitral valve disease (MMVD) dogs with and without ventricular arrhythmias. ROC curves constructed to assess sensitivity and specificity of the (C) average QT interval and (D) QT variance in differentiating MMVD dogs with either remodeled (stages B2 and C) or non-remodeled (stage B1) hearts.

Table 3 – Cut-off values, sensitivity, specificity, positive predictive value (PPV), post-test probability, accuracy and odds ratio obtained when using either the average QT (QTa) or the short-term instability (STI) to differentiate myxomatous mitral valve disease (MMVD) dogs presenting only sinus rhythms from MMVD dogs presenting any ventricular arrhythmia.

	Cut-off	Sensitivity	Specificity	PPV	Post-test probability	Accuracy	Odds Ratio
QTa	**>297.0**	0.78	0.49	0.09	8.97	51.01	3.40
	>300.0	0.78	0.57	0.10	10.45	58.39	4.67
	>305.0	0.56	0.66	0.10	9.62	65.77	2.47
	>310.0	0.56	0.71	0.11	11.11	70.47	3.13
STI	**>6.7**	0.78	0.46	0.08	8.43	47.65	2.95
	>7.9	0.67	0.65	0.11	10.91	65.10	3.71
	>9.0	0.56	0.73	0.12	11.63	71.81	3.36
	>11.0	0.56	0.85	0.19	19.23	83.22	7.08

QTa: heart-rate corrected average QT interval; STI: short-term instability.

Table 4 –Cut-off values, sensitivity, specificity, positive predictive value (PPV), post-test probability, accuracy and odds ratio obtained when using either the average QT (QTa) or the QT variance (QTv) to differentiate myxomatous mitral valve disease (MMVD) dogs with remodeled (stages B2 and C) and non-remodeled (stage B1) hearts.

	Cut-off	Sensitivity	Specificity	PPV	Post-test probability	Accuracy	Odds Ratio
QTa	**>292.0**	0.74	0.55	0.71	71.43	66.47	3.46
	>295.0	0.73	0.58	0.73	72.55	67.07	3.72
	>300.0	0.61	0.70	0.76	75.61	64.67	3.66
	>302.0	0.57	0.74	0.77	77.33	64.07	3.89
QTv	**>107.0**	0.71	0.53	0.70	69.90	64.07	2.80
	>115.0	0.68	0.58	0.71	71.13	64.07	2.93
	>130.0	0.64	0.62	0.72	72.22	63.47	2.96
	>165.0	0.56	0.73	0.76	76.00	62.87	3.45

QTa: heart rate corrected QT interval; QTv: heart rate-corrected QT variance.

Table 5 – Data obtained from the prognostic study. QT indices were investigated as prognostic surrogates concerning the development of clinical signs attributable to congestive heart failure and all-cause mortality.

	Criteria	Cut-off	Result (days)	Hazard ratio	95% CI	P
Development of Clinical Signs Due to CHF	**QTa**	< 302	362.0	0.5322	0.2677 to 1.058	0.0720
		≥ 302	225.0			
	QTv	< 162	266.0	0.7495	0.3772 to 1.4890	0.4104
		≥ 162	234.0			
	STI	< 7.5	225.0	0.7915	0.3879 to 1.6150	0.5204
		≥ 7.5	250.0			
	LTI	< 10.0	373.0	0.5674	0.2906 to 1.1080	0.0968
		≥ 10.0	215.5			
	TI	< 14.0	324.0	0.5526	0.2715 to 1.1250	0.1019
		≥ 14.0	206.0			
All-cause Mortality	**QTa**	< 302	279.0	0.4296	0.2426 to 0.7607	0.0038
		≥ 302	174.0			
	QTv	< 162	276.5	0.4840	0.2696 to 0.8691	0.0271
		≥ 162	174.0			
	STI	< 7.5	276.5	0.4986	0.2746 to 0.9052	0.0222
		≥ 7.5	148.0			
	LTI	< 10.0	257.5	0.9418	0.5583 to 1.5890	0.8223
		≥ 10.0	177.0			
	TI	< 14.0	276.5	0.5657	0.3248 to 0.9854	0.0442
		≥ 14.0	174.0			

QTa: average QT interval; QTv: QT variance; STI: short-term instability; LTI: long-term instability; TI: total instability. All parameters were calculated based on heart rate-corrected QT intervals.

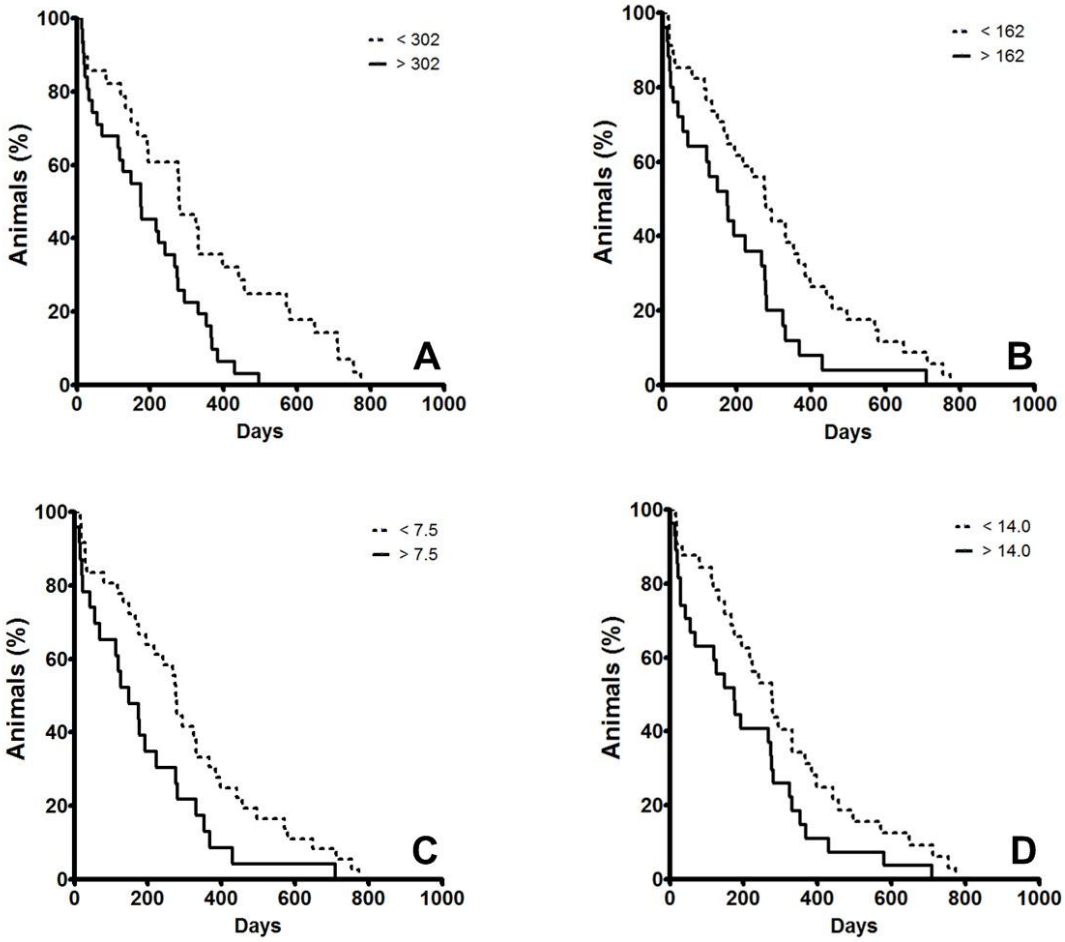

Figure 6 – Kaplan-Meier curves constructed to investigate whether indices derived from QT interval would serve as prognostic surrogates for mortality. A lower (A) heart rate-corrected average QT (QTa), (B) QT variance, (C) short-term instability and (D) total instability were significantly associated with reduced all-cause mortality in dogs with myxomatous mitral valve disease.

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ANEXOS

1- Aprovação pelo Comitê de Ética do Setor de Ciências Agrárias da Universidade Federal do Paraná

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

CERTIFICADO

Certificamos que o protocolo número 039/2015, referente ao projeto "**Instabilidade do intervalo QT em cães com doença valvar mitral**", sob a responsabilidade de **Bruna Cristina Brüler** – 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 nº 11.794, de 8 de Outubro, de 2008, do Decreto nº 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, em reunião de 27/05/2015

CERTIFICATE

We certify that the protocol number 039/2015, regarding the project "**Instability of the QT interval in dogs with mitral valve disease**", under **Bruna Cristina Brüler** 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 nº 11.794, of 8 October, 2008, of Decree nº 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), in session of 05/27/2015

Curitiba, 27 de Maio de 2015.

Ananda Portella Félix
Presidente CEUA-SCA

Simone Tostes de Oliveira Stedile
Vice-Presidente CEUA-SCA

Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias - UFPR