UNIVERSIDADE ESTADUAL PAULISTA - UNESP

CÂMPUS DE JABOTICABAL

MARCADORES ELETROCARDIOGRÁFICOS DE ARRITMOGÊNESE AUMENTADA EM CÃES DA RAÇA BOXER

Elizabeth Regina Carvalho

Médica Veterinária

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Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – UNESP, câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutor em Medicina Veterinária (área de Clínica Médica Veterinária).

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Câmpus de Jaboticabal

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TÍTULO DA TESE: MARCADORES ELETROCARDIOGRÁFICOS DE ARRITMOGÊNESE AUMENTADA EM CÃES DA RAÇA BOXER

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Jaboticabal, 12 de março de 2019

DADOS CURRICULARES DO AUTOR

ELIZABETH REGINA CARVALHO – nascida em Curitiba, Paraná, em 31 de julho de 1991, filha de Sonia Maria Anelli e Antônio Carvalho. Graduou-se em Medicina Veterinária pela Universidade Federal do Paraná, câmpus de Curitiba, em 2013. Durante a graduação foi bolsista três vezes do Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, sob a orientação do Prof. Dr. Ricardo Guilherme D'Otaviano de Castro Vilani, em 2010/2011, 2011/2012 e 2012/2013. Ingressou no programa de residência em Anestesiologia Veterinária na Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP – câmpus de Botucatu sob a orientação do Prof. Dr. Stelio Pacca Loureiro Luna em março de 2014 e concluiu em fevereiro de 2016. Em julho de 2017 concluiu o curso de Mestrado em Medicina Veterinária, área de concentração em Clínica Médica Veterinária, junto à Faculdade de Ciências Agrárias e Veterinárias da UNESP câmpus de Jaboticabal, sob a orientação do Prof. Dr. Marlos Gonçalves Sousa e coorientação do Prof. Dr. Aparecido Antônio Camacho. Em agosto de 2017 ingressou no curso de Doutorado na mesma instituição, sob orientação do Prof. Dr. Marlos Gonçalves Sousa e coorientação do Prof. Dr. Aparecido Antônio Camacho.

“Transportai um punhado de terra todos os dias e fareis uma montanha”

Confúcio

À minha mãe, pela minha vida e amor incondicional.

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APRESENTAÇÃO

A presente tese de Doutorado faz parte das obrigações para obtenção do título de Doutora em Medicina Veterinária, área de Clínica Médica, pelo Programa de Pós-Graduação em Medicina Veterinária da Faculdade de Ciências Agrárias e Veterinárias da UNESP câmpus de Jaboticabal. A tese está dividida em capítulos para melhor compreensão. O capítulo 1 (um) compreende as considerações gerais, e objetivos do trabalho; o capítulo 2 (dois) é um artigo científico já publicado, trata-se de revisão de literatura sobre a Cardiomiopatia Arritmogênica do Ventrículo Direito no Boxer; o capítulo 3 (três) é o segundo artigo científico, submetido para publicação, a respeito da análise de sobrevida em Boxers com arritmias ventriculares; e o capítulo 4 (quatro) é o terceiro artigo científico, também já submetido para publicação, sobre os marcadores eletrocardiográficos de arritmogênese em Boxers e no grupo controle.

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CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto intitulado **"Marcadores eletrocardiográficos de arritmogênese aumentada em cães da raça Boxer"**, protocolo nº 012277/17, sob a responsabilidade do Prof. Dr. Marlos Gonçalves Sousa, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 03 de Agosto de 2017.

Vigência do Projeto	20/09/2017 a 01/09/2019
Espécie / Linhagem	Canina
Nº de animais	Mínimo de 40 (estudo retrospectivo)
Peso / Idade	Acima de 4 anos de idade
Sexo	Ambos os sexos
Origem	Cães de tutores, atendidos no Serviço de Cardiologia do H.V.da FCAV

Jaboticabal, 03 de agosto de 2017.

Profª Drª Lizandra Amoroso
Coordenadora – CEUA

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MARCADORES ELETROCARDIOGRÁFICOS DE ARRITMOGÊNESE AUMENTADA EM CÃES DA RAÇA BOXER

RESUMO – A cardiomiopatia arritmogênica do ventrículo direito (CAVD) é uma doença miocárdica hereditária observada no Boxer adulto, caracterizada pela substituição dos cardiomiócitos do ventrículo direito (VD) por tecido fibroadiposo, gerando áreas propensas à formação de arritmias ventriculares, que podem culminar em morte súbita. Devido aos avanços no mapeamento genético em cães domésticos, pode-se concluir que os Boxer são acometidos pela CAVD de maneira similar ao que ocorre em seres humanos, e, portanto, são considerados um modelo animal natural para o estudo da CAVD humana. Em seres humanos, diversos estudos com marcadores eletrocardiográficos de arritmogênese aumentada mostram resultados promissores como ferramenta na estratificação de risco para morte súbita em indivíduos com arritmias ventriculares. Por esse motivo, o objetivo desta pesquisa foi investigar o valor prognóstico de tais marcadores em cães da raça Boxer acometidos pela CAVD. Para isso, foi conduzido um estudo retrospectivo com Boxers acima de 4 anos de idade no qual os traçados eletrocardiográficos convencionais e ambulatorial de 24 horas foram revisados para a obtenção dos seguintes marcadores: dispersão e duração do QRS e do intervalo QT corrigido, Tpico-Tfinal, Tpico-Tfinal/QTc, JTpico/JT, Tpico-Tfinal/JTpico, intervalo de acoplamento e índice de prematuridade. Ademais, no Holter foram analisadas as características das arritmias ventriculares e a variabilidade da frequência cardíaca (VFC). Os proprietários dos cães foram contactados a respeito de sinais clínicos, morte súbita e histórico familiar da CAVD. Curvas *Receiver operating characteristic* (ROC) foram construídas para investigar a sensibilidade e especificidade dos marcados em diferenciar cães com CAVD dos saudáveis. Curvas Kaplan-Meier e o teste Log-rank foram utilizados para análise do valor prognósticos das variáveis e da VFC.

Palavras-chave: arritmia, complexos cardíacos prematuros, eletrocardiografia ambulatorial, morte súbita, prognóstico

ELECTROCARDIOGRAPHIC SURROGATES OF AUGMENTED ARRHYTHMOGENESIS IN BOXER DOGS

ABSTRACT – The arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited myocardial disease observed in the adult Boxer, characterized by the replacement of right ventricle (RV) cardiomyocytes by fibrofatty tissue, generating areas prone to ventricular arrhythmias, which may culminate in sudden death. Due to advances in genetic mapping in domestic dogs, it can be concluded that Boxers are affected by ARVC in a similar way to what occurs in humans, therefore being considered a natural animal model for the study of human ARVC. In humans, several studies with electrocardiographic markers of increased arrhythmogenesis show promising results to stratify risk for sudden death in individuals with ventricular arrhythmias. For this reason, the objective of this research was to investigate the value of such markers in Boxers. A retrospective study with Boxers older than 4 years was conducted, in which conventional and 24-hour ambulatory electrocardiographic records were reviewed to obtain the following markers: QRS dispersion and duration and corrected QT interval, Tpeak-Tend, Tpeak-Tend/QTc, JTpeak/JT, Tpeak-Tend/JT, coupling interval and prematurity index. In addition, Holter was analyzed to the characteristics of ventricular arrhythmias and heart rate variability (HRV). Owners were contacted regarding clinical signs, sudden death and family history of ARVC. Receiver operating characteristic curves (ROC) were constructed to investigate the sensitivity and specificity of discriminate individuals affected by ARVC from healthy subjects. Kaplan-Meier curves and the Log-rank test analyzed the prognostic value of such parameters and HRV.

Keywords: arrhythmia, premature cardiac complexes, ambulatory electrocardiography, sudden death, prognosis

CAPÍTULO 1 – Considerações gerais

1. INTRODUÇÃO

A cardiomiopatia arritmogênica do ventrículo direito (CAVD) é uma doença miocárdica familiar observada no Boxer adulto, caracterizada pela substituição dos cardiomiócitos do ventrículo direito (VD) por tecido fibroadiposo, gerando áreas propensas à formação de arritmias ventriculares (Meurs et al., 2014). Indivíduos humanos e caninos afetados pela CAVD são predispostos à morte súbita, em alguns casos, como a primeira manifestação da doença (Meurs et al., 2014; Corrado et al., 2017). Pesquisas demonstram semelhanças clínicas e patológicas da doença em seres humanos e em Boxers, o que torna a raça um modelo animal natural para o estudo da CAVD (Basso et al., 2004; Meurs, 2004).

A prevalência de CAVD em seres humanos é estimada em 1 caso a cada 5000 indivíduos na população em geral, e de 1 caso a cada 2000 pessoas em alguns países europeus, como a Itália e Alemanha (Rampazzo et al., 1994; Peters et al., 2004). Pode ser considerada a causa de até 10% das mortes súbitas cardíacas em pessoas com menos de 65 anos e 22% em atletas jovens (Kiès et al., 2006; Azaough et al., 2011). Embora a prevalência em Boxers não tenha sido estudada, clinicamente observa-se a CAVD como frequente causa *mortis* nessa raça, seja por insuficiência cardíaca congestiva, eutanásia ou morte súbita.

A infiltração fibroadiposa no miocárdio apresenta instabilidade eletrofisiológica, predispondo ao desenvolvimento de circuitos elétricos reentrantes. Em seres humanos, diversos marcadores eletrocardiográficos têm sido estudados como ferramenta diagnóstica na estratificação de risco em indivíduos com arritmias ventriculares, tais como a dispersão do QRS (Peters et al., 1999), a dispersão do intervalo QT (Tse e Yan, 2016), o JTpico/JT (Moise et al., 1990; Coronel et al., 2009), o intervalo de acoplamento (Kim et al., 2014) e o índice de prematuridade (Igarashi et al., 2012). Assim, a identificação do valor prognóstico dos marcadores

eletrocardiográficos de arritmogênese poderia auxiliar na monitoração e estratificação de risco de pessoas e cães acometidos pela CAVD.

2. REVISÃO DE LITERATURA

A CAVD, também conhecida como displasia arritmogênica do VD, é uma doença miocárdica hereditária que afeta principalmente o VD (Corrado et al., 2017). A perda do miocárdio ventricular direito com substituição dos cardiomiócitos por tecido fibroadiposo é a principal característica histopatológica desta doença (Thiene et al., 1988). Infiltrados inflamatórios irregulares (principalmente linfócitos T) são frequentemente observados em associação com os cardiomiócitos degenerados, sugerindo que o processo patológico pode ser imunologicamente mediado (Basso et al., 1996; Corrado et al., 1997).

A cicatriz fibroadiposa resultante estende-se do epicárdio até o endocárdio, e envolve predominantemente a parede livre do VD (Corrado et al., 1997). Em seres humanos, as lesões estão tipicamente localizadas na via de entrada do VD (região subtricuspídea), via de saída do VD (região infundibular), e ápice (“triângulo da displasia”) (Marcus et al., 1982).

A infiltração fibroadiposa no miocárdio apresenta instabilidade eletrofisiológica, predispondo ao desenvolvimento de circuitos elétricos reentrantes (Basso et al., 2009; Corrado et al., 2017). Assim, a estimulação adrenérgica pode aumentar a propensão às arritmias ventriculares ou até mesmo induzi-las diretamente (Corrado et al., 2017). A atividade esportiva aumenta o risco de morte súbita cardíaca em adolescentes e adultos jovens com CAVD. Sabe-se que em humanos afetados pela CAVD o exercício físico pode agravar o desacoplamento mecânico dos cardiomiócitos, desencadeando arritmias ventriculares malignas, e por esse motivo é considerado um fator ambiental crítico para o desenvolvimento e progressão da doença (Corrado et al., 2017).

Etiologicamente, trata-se de uma doença genética, de caráter autossômico dominante, com padrão de transmissão de penetrância incompleta (Oyama et al., 2008; Meurs et al., 2014). Mutações nos genes que codificam proteínas

desmossômicas, importantes estruturas na adesão célula a célula, desempenham um papel fundamental na patogênese da CAVD (Corrado et al., 2017).

Seis loci independentes já foram identificados como possíveis causadores de mutações em quatro proteínas de ligação dos desmossomos: a desmoplaquina, placoglobina e placofilina, e estriatina (Indik e Marcus 2003; Macrae et al., 2006; Meurs et al., 2014). Além disso, ocorre mutação nos genes dos receptores cardíacos rianodina, o principal canal intracelular cardíaco de liberação de cálcio, tanto em cães da raça Boxer, quanto em seres humanos (Macrae et al., 2006; Oyama et al., 2008).

A CAVD é uma causa importante de morte súbita cardíaca em humanos jovens e atletas (Corrado et al., 2017). Durante anos notou-se que os Boxers eram predispostos ao desenvolvimento de arritmias ventriculares e morte súbita (Basso et al., 2004). Devido aos avanços no mapeamento genético em cães domésticos, pode-se concluir que os Boxer são acometidos pela CAVD de maneira similar ao que ocorre em pessoas, e, portanto, são considerados um modelo animal natural para o estudo da CAVD humana (Basso et al., 2004).

Nos Boxers, existem três formas clínicas da doença, propostas por Harpster (1991). Na categoria I ou forma oculta, os cães são assintomáticos e identifica-se pequeno número de arritmias ventriculares. Na categoria II ou forma evidente, os cães apresentam síncopes e cansaço fácil, principalmente durante exercícios ou momentos de excitação, associados à presença de extrassístoles monomórficas. Na categoria III ou forma de disfunção do miocárdio, que é a menos comum, os animais apresentam sinais de insuficiência cardíaca congestiva, como tosse, intolerância ao exercício, efusões e síncopes. Nesta fase, há disfunção sistólica biventricular, com presença de taquiarritmias ventriculares e ocasionalmente, taquiarritmias supraventriculares, como a fibrilação atrial (Harpster, 1991).

Ainda não está definido se as formas de manifestação da doença representam uma continuidade entre elas ou se são diferentes expressões de manifestações genéticas distintas (Harpster, 1991). Alguns animais apresentam um grande número de eventos arrítmicos e não progridem para a forma sintomática da doença, enquanto outros, com o mesmo grau de ectopia ventricular progridem gradualmente e podem

desenvolver sinais clínicos secundários às arritmias (Harpster, 1991; Meurs et al., 2014).

Em cães da raça Boxer a presença de extrassístoles ventriculares (EV) e supraventriculares na eletrocardiografia convencional de três minutos, apresentou 83,33% de sensibilidade, 100% de especificidade, valor preditivo positivo (VPP) de 100% e valor preditivo negativo (VPN) de 86% no diagnóstico da CAVD (Pereira, 2011). Os parâmetros de variabilidade da frequência cardíaca (VFC), obtidos no domínio do tempo no Holter de 24 horas, SDNNidx e pNN>50, foram significativamente menores nos Boxers com mais de 1000 EV/24h, em comparação aos cães com menos que 1000 EV/24h (Pereira, 2011). Assim, os resultados sugerem que a eletrocardiografia convencional e a VFC são técnicas que podem contribuir substancialmente no diagnóstico da CAVD em cães Boxers (Pereira, 2011). No que diz respeito à contribuição do exame ecocardiográfico no estudo da CAVD do Boxer, resultados recentes obtidos pelo Laboratório de Cardiologia Veterinária da Faculdade de Ciências Agrárias e Veterinárias da Unesp Jaboticabal demonstraram que, tanto na avaliação do VD por índices sistólicos convencionais, quanto por aqueles derivados da técnica de *speckle tracking* não foram capazes de detectar disfunção miocárdica do VD em Boxers considerados acometidos pela CAVD (Fenerich, 2018).

3. OBJETIVOS

Os objetivos do presente trabalho são: (1) avaliar as características das arritmias ventriculares em cães da raça Boxer monitorizados por Holter de 24 horas, no que diz respeito à morfologia, ocorrência, número ao longo do dia, intervalo de acoplamento, índice de prematuridade, e variabilidade da frequência cardíaca no domínio do tempo; (2) investigar o impacto de tais variáveis na sobrevida global dos animais; (3) avaliar os marcadores eletrocardiográficos de condução, despolarização e repolarização miocárdica em Boxers saudáveis, acometidos pelo CAVD, e grupo controle (não Boxer).

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CAPÍTULO 2 - Cardiomiopatia arritmogênica do ventrículo direito em cães da raça boxer: atualidades no diagnóstico e tratamento[1]

(Arrhythmogenic right ventricular cardiomyopathy in the Boxer dog: updates on diagnosis and treatment)

RESUMO

A cardiomiopatia arritmogênica do ventrículo direito (CAVD) é uma doença miocárdica hereditária comumente observada no Boxer adulto, caracterizada pela substituição dos cardiomiócitos do ventrículo direito (VD) por tecido fibroadiposo, gerando áreas propensas à formação de arritmias ventriculares, que podem culminar em morte súbita. Devido aos avanços no mapeamento genético em cães domésticos, pode-se concluir que os Boxers são acometidos pela CAVD de maneira similar ao que ocorre em seres humanos, e, portanto, são considerados um modelo animal natural para o estudo da CAVD na espécie humana. Não há um teste de diagnóstico único e específico para CAVD, portanto, em Medicina Veterinária o diagnóstico baseia-se na presença de uma combinação de achados, que podem incluir presença de taquiarritmia ventricular sem outras causas documentáveis para a arritmia, síncope e histórico familiar de CAVD. O tratamento é direcionado à redução da frequência e complexidade da arritmia ventricular, e cloridrato de sotalol e/ou mexiletine são os antiarrítmicos mais comumente prescritos, bem como o ômega 3 advindo do óleo de peixe. Entretanto, não é sabido se o tratamento em cães assintomáticos está associado à melhores prognósticos. Embora alguns cães acometidos apresentem morte súbita ou desenvolvam insuficiência cardíaca congestiva, muitos deles têm arritmias ventriculares controláveis por antiarrítmicos e vivem uma vida normal. Algumas características dos complexos ventriculares prematuros, tais como polimorfismo e taquicardia ventricular, além da presença de sinais clínicos, e

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disfunção sistólica ventricular esquerda e direita estão associados à piores prognósticos.

Palavras-chave: arritmia; arritmogênese; eletrocardiografia; Holter; prognóstico,

ABSTRACT

The arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited myocardial disease commonly observed in the adult Boxer, characterized by the replacement of right ventricle (RV) cardiomyocytes by fibrofatty tissue, what generate areas prone to the formation of ventricular arrhythmias, which may culminate in sudden death. Due to advances in genetic mapping in domestic dogs, it could be concluded that Boxers are affected by ARVC in a closed related way to human beings, and therefore are considered a natural animal model for the study of human ARVC. There is not a single and specific diagnostic test for ARVC, thus, in veterinary practice the diagnosis is best based on a combination of findings, which may include the presence of ventricular tachyarrhythmia with no other documented causes for the arrhythmia, as well as syncope, and family history to ARCV. Treatment is directed to decrease the occurrence and the complexity of the ventricular arrhythmias, and sotalol and/or mexiletine are the antiarrhythmics most commonly prescribed, as well as omega 3 from fish oil. However, it is unknown if the treatment on an asymptomatic dog prolongs survival. Although some affected dogs show sudden death or develop congestive heart failure, many of them have ventricular arrhythmias that are controllable by antiarrhythmics and live a normal life. Some characteristics of ventricular premature complexes, as polymorphism and ventricular tachycardia, the presence of clinical signs, and systolic dysfunction of left and right ventricles are associated with worst prognosis.

Keywords: arrhythmia; arrhythmogenesis; electrocardiography; Holter; prognosis

INTRODUÇÃO

A cardiomiopatia arritmogênica do ventrículo direito (CAVD) no Boxer é uma doença miocárdica familiar observada em adultos, caracterizada pela substituição dos cardiomiócitos do ventrículo direito (VD) por tecido fibroadiposo, gerando áreas propensas à formação de arritmias ventriculares (Meurs et al., 2014). Embora a CAVD tenha sido mais bem caracterizada no Boxer, casos isolados, suspeitos e confirmados, têm sido descritos no Dachshund (Simpson et al., 1994), Bullmastiff (Bright et al., 1995), Husky Siberiano (Palacio et al., 2001), Buldogue Inglês (Santilli et al., 2009; Nakao et al., 2011; Cunningham et al., 2018), Labrador Retriever (Nakao et al., 2011), Dálmata (Nakao et al., 2011), Pastor de Shetland (Nakao et al.,2011), e Weimaraner (Eason et al., 2015), além de ser descrita em felinos domésticos (Fox et al., 2000).

Indivíduos afetados pela CAVD são altamente predispostos à morte súbita, em alguns casos, como a primeira manifestação da doença (Corrado et al. 2017; Meurs et al., 2014). Pesquisas demonstram fortes semelhanças clínicas e patológicas da enfermidade em seres humanos e cães da raça Boxer, o que torna essa raça um modelo animal natural para o estudo da CAVD (Basso et al., 2004; Meurs et al., 2004).

A prevalência de CAVD em seres humanos é estimada em 1 caso a cada 5000 indivíduos na população em geral, e de 1 caso a cada 2000 pessoas em alguns países europeus, como a Itália e Alemanha (Rampazzo et al. 1994; Peters et al. 2004). A CAVD pode ser considerada a causa de até 10% das mortes súbitas cardíacas em pessoas com menos de 65 anos, e 22% em atletas jovens (Kiès et al. 2006; Azaouagh et al. 2011). Embora a prevalência em Boxers não tenha sido estudada, clinicamente observa-se a CAVD como frequente *causa mortis* nessa raça, seja por insuficiência cardíaca congestiva, eutanásia ou morte súbita.

ETIOLOGIA

A CAVD, também conhecida como displasia arritmogênica do ventrículo direito, é uma doença miocárdica hereditária que afeta principalmente o ventrículo direito (VD) (Corrado et al. 2017). A perda do miocárdio ventricular direito com substituição dos

cardiomiócitos por tecido fibroadiposo é a principal característica histopatológica desta doença (Thiene et al. 1988). Em seres humanos acometidos, infiltrados inflamatórios irregulares (principalmente linfócitos T) são frequentemente observados em associação com cardiomiócitos degenerados, sugerindo que o processo patológico pode ser imunologicamente mediado (Basso et al. 1996; Corrado et al. 1997).

A cicatriz fibroadiposa resultante estende-se do epicárdio até o endocárdio, e envolve predominantemente a parede livre do VD (Corrado et al. 1997). Em seres humanos, as lesões estão tipicamente localizadas na via de entrada do VD (região subtricuspídea), via de saída do VD (região infundibular), e ápice ventricular ("triângulo da displasia") (Marcus et al. 1982; Corrado et al. 1997).

Nos Boxers, etiologicamente, trata-se de uma doença genética, de caráter autossômico dominante, com padrão de transmissão de penetrância incompleta (Oyama et al. 2008; Meurs et al. 2014). Mutações nos genes que codificam proteínas desmossômicas, especialmente no gene estriatina, localizado no cromossomo 17 (Meurs et al., 2010), importantes estruturas na adesão célula a célula, desempenham um papel fundamental na patogênese da CAVD (Corrado et al. 2017).

Seis loci independentes já foram identificados como possíveis causadores de mutações em quatro proteínas de ligação dos desmossomos: a desmoplaquina, placoglobina e placofilina, e estriatina (Indik et al., 2003; Macrae et al. 2006; Meurs et al. 2010). Além disso, ocorre mutação nos genes dos receptores cardíacos rianodina, o principal canal intracelular cardíaco de liberação de cálcio, tanto em cães da raça Boxer, quanto em seres humanos (Macrae et al. 2006; Oyama et al. 2008).

INSTABILIDADE ELETROFISIOLÓGICA

A infiltração fibroadiposa no miocárdio apresenta instabilidade eletrofisiológica, predispondo ao desenvolvimento de circuitos elétricos reentrantes (Basso et al. 2009; Corrado et al. 2017). Assim, sabe-se que em seres humanos a estimulação adrenérgica pode aumentar a propensão às arritmias ventriculares ou até mesmo induzi-las diretamente (Corrado et al. 2017). A atividade esportiva aumenta o risco de morte súbita cardíaca em adolescentes e adultos jovens com CAVD (Thiene et al.

1988; Corrado et al. 2017). Sabe-se que em humanos afetados pela CAVD o exercício físico pode agravar o desacoplamento mecânico dos cardiomiócitos, desencadeando arritmias ventriculares malignas, e por esse motivo é considerado um fator ambiental crítico para o desenvolvimento e progressão da doença (Corrado et al. 2017).

A CAVD é uma causa importante de morte súbita cardíaca em humanos jovens e atletas (Corrado et al. 2017). Durante anos notou-se que os Boxers eram predispostos ao desenvolvimento de arritmias ventriculares e morte súbita (Basso et al. 2004). Devido aos avanços no mapeamento genético em cães domésticos, pode-se concluir que os Boxer são acometidos pela CAVD de maneira similar ao que ocorre em pessoas, e portanto, são considerados um modelo animal natural para o estudo da CAVD humana (Basso et al. 2004).

Em seres humanos, diversos marcadores eletrocardiográficos têm sido estudados como ferramenta diagnóstica e na estratificação de risco em indivíduos com doenças cardíacas. Dentre tais marcadores, destacam-se a dispersão do QRS, definida como a diferença máxima entre a duração do complexo QRS entre derivação precordial direita (rV2 nos animais) e esquerda (V4 nos animais) (Peters et al., 1999), a dispersão do intervalo QT, definida como a máxima diferença na duração do intervalo QT na eletrocardiografia de 12 derivações (Tse et al., 2016), o JTpico/JT, razão entre o JTpico (intervalo entre ponto J, ou seja, término do complexo QRS, e pico da onda T) e JT (ponto J até o final da onda T), avaliada em pequenos animais na derivação bipolar D II, e pré-cordiais rV2 e V4 (Moise et al., 1990; Coronel et al., 2009), ilustrados na Figura 1.

Enquanto a dispersão do QRS é um indicador da diferença na velocidade de condução do impulso elétrico entre duas regiões miocárdicas distintas, e, portanto, reflete a despolarização cardíaca, a dispersão do intervalo QT reflete a diferença no potencial de ação entre duas regiões do miocárdio, ou seja, é um marcador eletrocardiográfico de repolarização cardíaca, da mesma forma que o índice JTpico/JT. O intervalo de acoplamento (IA), definido como o intervalo R-R entre o batimento ectópico e o sinusal precedente, e o índice de prematuridade, razão entre o IA e o intervalo R-R dos dois batimentos sinusais precedentes, são parâmetros eletrocardiográficos utilizados na classificação e estratificação de risco das arritmias ventriculares em seres humanos e animais (Igarashi et al., 2012; Kim et al., 2014;

Carvalho et al., 2018). Em cães da raça Boxer, estudos que avaliem a utilidade de tais marcadores de arritmogênese aumentada são escassos.

APRESENTAÇÃO CLÍNICA

A CAVD no Boxer é mais comumente diagnosticada entre 5 e 7 anos de idade, embora alguns animais possam ser identificados precocemente, com 1 a 3 anos (Meurs, 2004; Meurs, 2017).

Nos Boxers, existem três formas clínicas da doença, propostas por Harpster et al (1991). Na categoria I ou forma oculta, os cães são assintomáticos e identifica-se pequeno número de arritmias ventriculares. Na categoria II ou forma evidente, os cães apresentam síncopes e cansaço fácil, principalmente durante exercícios ou momentos de excitação, associados à presença de extra-sístoles monomórficas. Na categoria III ou forma de disfunção do miocárdio, que é a menos comum, os animais apresentam sinais de insuficiência cardíaca congestiva, como tosse, intolerância ao exercício, efusões e síncope. Nesta fase, há presença de taquiarritmias ventriculares e ocasionalmente, taquiarritmias supraventriculares, como a fibrilação atrial (Harpster et al., 1991), além de disfunção sistólica biventricular, diagnosticada por meio de parâmetros ecocardiográficos de função sistólica radial (fração de encurtamento, *strain* e *strain rate* radiais e circunferenciais do ventrículo esquerdo, *fractional area change* (FAC) do ventrículo direito), e/ou função sistólica longitudinal (excursão sistólica do plano anular mitral ou tricúspide, MAPSE e TAPSE, respectivamente, *strain* e *strain rate* longitudinais) .

Ainda não está definido se as formas de manifestação da doença representam uma continuidade entre elas ou se são diferentes expressões de manifestações genéticas distintas (Harpster et al., 1991), embora saiba-se que os Boxers homozigotos para a mutação de deleção do gene estriatina são mais propensos a manifestação da categoria III da CAVD (Meurs et al., 2010). Alguns animais apresentam um grande número de eventos arrítmicos e não progridem para a forma sintomática da doença, enquanto outros, com o mesmo grau de ectopia ventricular

progridem gradualmente e podem desenvolver sinais clínicos secundários às arritmias (Harpster et al., 1991; Meurs et al., 2014).

DIAGNÓSTICO

Com o intuito de padronizar o diagnóstico da CAVD em humanos, em 1994 uma força-tarefa internacional propôs diretrizes sob a forma de um sistema de pontuação qualitativa com critérios maiores e menores (McKenna et al. 1994). Em 2010, as diretrizes foram revisadas para melhorar a sensibilidade diagnóstica, incluindo principalmente o rastreio de membros familiares dos acometidos, critérios quantitativos para o diagnóstico de anormalidades do ventrículo direito e adição de critérios genéticos moleculares (Marcus et al. 2010).

Em pessoas com suspeita de CAVD são avaliados: a função global e regional do VD, por meio da ecocardiografia bidimensional, ressonância magnética e angiografia; caracterização tecidual do VD na biópsia miocárdica; anormalidades eletrocardiográficas de repolarização, como a presença de onda T negativa nas derivações pré-cordiais direitas; anormalidades eletrocardiográficas na condução e despolarização, como a presença de onda épsilon, episódios confirmados de taquicardia ventricular sustentada ou paroxística com morfologia de bloqueio de ramo esquerdo; e histórico familiar de CAVD em parentes de primeiro ou segundo grau. Para cada um desses tópicos, critérios maiores e menores são atribuídos.

Embora diversos aspectos sejam ponderados no diagnóstico da CAVD humana, existem situações nas quais podem haver equívocos no diagnóstico definitivo (Corrado et al. 2017). Taquicardia ventricular direita idiopática, sarcoidose cardíaca, doenças cardíacas congênitas que cursem em sobrecarga ventricular direita, e cardiomiopatia dilatada biventricular são alguns exemplos (Morin et al. 2010; Steckman et al. 2012; Corrado et al. 2017).

Em Boxers, o diagnóstico é melhor baseado na presença de uma combinação de fatores, incluindo histórico familiar da CAVD, presença de taquiarritmia ventricular, histórico de síncope ou intolerância ao exercício, e confirmação histopatológica *post mortem* de infiltração fibroadiposa no miocárdio do VD (Meurs, 2004).

Exame físico

Muitos cães acometidos pela CAVD não possuem anormalidades cardiovasculares detectáveis ao exame físico (Meurs, 2004; Meurs, 2017). A auscultação cardíaca deverá ser cuidadosa, uma vez que sopro geralmente não é auscultado em Boxers afetados pela CAVD, à exceção dos casos severos e menos comuns, nos quais há disfunção miocárdica (categoria III), e o sopro sistólico grau I a IV em ápice cardíaco esquerdo pode estar presente em 42% dos casos, consistente com regurgitação mitral secundária à dilatação ventricular (Meurs, 2004; Baumwart et al., 2005b; Meurs, 2017). Outras doenças cardíacas podem estar associadas ao mesmo padrão de sopro no Boxer, tais como estenose aórtica valvar ou subvalvar (Kvart et al., 1998; Koplitz et al., 2003), e devem portanto, serem excluídas. Ocasionalmente, pode ser detectada extrassístole ventricular prematura à auscultação, com déficit de pulso periférico (Meurs, 2004; Meurs, 2017). Pacientes na categoria III podem apresentar pulso jugular e ascite, atribuíveis à insuficiência cardíaca congestiva direita (Meurs, 2017).

Eletrocardiografia

Comumente, Boxers considerados afetados pela CAVD possuem registro eletrocardiográfico de 2-5 minutos sem arritmias ventriculares detectáveis, pois estas podem ser intermitentes e variáveis ao longo do tempo (Spier et al., 2004). Porém, quando detectados, complexos ventriculares prematuros (CVP) com morfologia de bloqueio de ramo esquerdo, avaliados em derivações planas frontais, como a aVF e D II (Kraus et al. 2002), conforme ilustrado pela Figura 2, podem estar presentes de forma isolada, em pares, trios, taquicardia ventricular (> 3 CVP em sequência) paroxística, ou ainda sustentada (> 30 segundos) (Spier et al., 2004; Meurs, 2004; Meurs, 2017).

Eletrocardiografia ambulatorial (Holter)

A eletrocardiografia ambulatorial (Holter) é parte importante na triagem, diagnóstico e monitoramento da CAVD canina, na qual são investigados CVP (Meurs,

2004). Os CVP nos Boxers com CAVD são originados no VD e possuem morfologia de bloqueio de ramo esquerdo, quando avaliados no canal I (polo positivo em direção ao ápice do ventrículo esquerdo, similar à derivação D II).

Uma vez que a CAVD nos Boxers pode ser assintomática (categoria I ou forma oculta) em muitos animais acometidos, além do histórico familiar da doença nem sempre ser conhecido em cães, o diagnóstico clínico da CAVD muitas vezes passa a ser substanciado na quantidade e complexidade dos CVP no Holter de 24 horas, a despeito das inúmeras limitações dessa abordagem. As arritmias ventriculares podem ser originadas por outras doenças cardíacas que não a CAVD, como a degeneração mixomatosa da valva mitral (Crosara et al., 2010; Carvalho et al., 2018) e/ou distúrbios sistêmicos como a erliquiose (Ferreira et al., 2017) e neoplasias (Marino et al., 1994), e que, portanto, deverão ser excluídos com o auxílio de métodos diagnósticos complementares (p. ex. exames de sangue, ultrassonografia abdominal e ecocardiografia) antes de se estabelecer o diagnóstico de CAVD.

Atualmente não há consenso a despeito do número de CVP em 24 horas como ponto de corte para o diagnóstico da CAVD em Boxers. Alguns autores baseiam-se em *guidelines* não publicados do *American College of Veterinary Internal Medicine Forum* em 2009, e consideram que Boxers com mais de 300 CVP/24 horas são fortemente suspeitos para a CAVD, enquanto entre 50 e 300 CVP/24 horas a doença não pode ser descartada (Meurs et al., 2014; Meurs, 2017). Outros trabalhos sugerem que mais de 100 CVP/24 horas no Boxer adulto pode classifica-lo como provavelmente afetado, sobretudo se forem identificados padrões repetidos, como bigeminismo, trigeminismo ou taquicardia ventricular (Meurs, 2004). Cães adultos e saudáveis possuem em média 2 extrassístoles ventriculares prematuras em 24 horas (Meurs et al., 2001). Stern et al. (2010) realizaram um estudo com 301 Boxers, no qual os animais considerados clinicamente saudáveis apresentaram menos de 91 CVP isolados e monomórficos no Holter de 24 horas (Stern et al. 2010).

Ademais, pode haver variabilidade diária em relação ao número de CVP obtidos no Holter de 24 horas, e mudanças de cerca de 80% na frequência de arritmias ventriculares podem estar dentro do limite de variabilidade espontânea nos Boxers acometidos pela CAVD (Spier et al., 2004). Esta magnitude de variabilidade deverá

ser considerada ao monitorar a resposta ao tratamento com antiarrítmicos, e também ao utilizar o Holter como ferramenta diagnóstica da CAVD no Boxer.

Eletrocardiografia de alta resolução (ECGAR)

O ECGAR é um método não invasivo que detecta a presença dos potenciais tardios (PT), ou seja, sinais de alta frequência e baixa amplitude que ocorrem ao final do complexo QRS (Calvert et al., 1998). Tais sinais não são detectados pela eletrocardiografia convencional, devido à baixa amplitude e interferência que os movimentos respiratórios e tremores musculares têm sob esta técnica (Calvert et al., 1998). Os PT são considerados marcadores da condução lenta, que ocorrem entre células lesadas do miocárdio após sofreram fibrose, fato que propicia o aparecimento de taquicardia ventricular por reentrada (Spier et al., 2004b). Portanto, a identificação dos PT pode ser utilizada como fator preditivo de risco para futuros eventos arrítmicos e morte súbita (Calvert et al. 1998; Moffa et al., 2001; Ferreira 2003; Barbosa et al., 2004; Bernardic et al., 2005; Spier et al., 2004b).

No Boxer com CAVD, a presença de PT correlaciona-se positivamente com morte súbita de origem cardíaca (Spier et al., 2001; Spier et al., 2004b). Porém, demonstrou-se a presença de PT em apenas 51,3% de cães Boxer com CAVD, e em 83,3% dos cães com CAVD na categoria III (Chamas, 2011), sugerindo que estes PT aparecem mais frequentemente nos estágios mais avançados da doença, à semelhança do que ocorre em seres humanos (Nava et al., 2000; Steriotis et al., 2009) e, portanto, não é considerado preditor precoce de mortalidade na CAVD do Boxer.

Ecocardiografia

Apesar da CAVD ser considerada uma doença miocárdica, o exame ecocardiográfico da maioria dos Boxers acometidos está dentro dos padrões de normalidade em relação à dimensão e função ventricular esquerda (Meurs, 2017). Como dito anteriormente, apenas uma minoria dos casos exibe disfunção miocárdica e dilatação ventricular. A sobrevida média nestes cães com dilatação ventricular esquerda é significativamente menor do que naqueles cujo o tamanho ventricular é considerado normal (Motskula et al., 2013). Um estudo avaliou o TAPSE como indicador da função sistólica ventricular direita e demonstrou que esta variável é

menor nos Boxers com mais de 50 CVP/24 h, e que o TAPSE reduzido é associado à doença mais severa e à piores prognósticos (Kaye et al., 2015).

Exames de imagem

A radiografia torácica geralmente encontra-se normal no Boxer com CAVD, exceto nos casos em que há dilatação ventricular ou biventricular, tornando-se passível de visibilizar cardiomegalia. Nos casos severos, evidências radiográficas de insuficiência cardíaca, tais como edema pulmonar e/ou efusão pleural, podem ser constatadas (Meurs, 2017).

A ressonância magnética cardíaca é um exame de imagem não invasivo que combina tanto a avaliação morfofuncional, quanto a caracterização tecidual, uma vez que a utilização de contraste possibilita a identificação de áreas miocárdicas fibrosadas (Perazzolo et al., 2015). Ainda pouco explorada em Medicina Veterinária para o diagnóstico da CAVD no Boxer, um estudo comparou a avaliação morfológica e funcional do ventrículo direito e esquerdo em Boxers afetados e controles saudáveis por meio da ressonância magnética cardíaca, e demonstrou prejuízos na função sistólica ventricular direita nos animais doentes (Baumwart et al., 2007).

Biomarcadores

A troponina cardíaca I mostrou-se significativamente elevada em alguns Boxers afetados pela CAVD, nos quais correlacionou-se com o número e complexidade da arritmia ventricular (Baumwart et al*.,* 2007). Entretanto, há grande variabilidade nos níveis da troponina cardíaca I de caso para caso, e, portanto, a elevação nos seus níveis é um achado inconsistente nos cães acometidos pela CAVD (Meurs, 2017). A mensuração dos peptídeos natriuréticos cerebrais não mostrou ser um indicador útil da doença, a menos que ocorra disfunção miocárdica (Baumwart et al*.,* 2005).

Biopsia endomiocárdica

Apesar de pouco difundida na rotina clínica veterinária, a biópsia endomiocárdica do VD por via transvenosa é o procedimento mais confiável para o diagnóstico preciso da CAVD em seres humanos, apesar da possibilidade de fornecer resultados falso

negativos devido à natureza segmentar da doença (Basso et al., 1996). Além do caráter invasivo do procedimento, com risco de perfuração ventricular e tamponamento cardíaco, outra limitação da biópsia endomiocárdica é que, como ela é realizada por acesso intravenoso, a amostra obtida é de tecido subendocárdico, que pode não refletir o padrão transmural da infiltração fibroadiposa do miocárdio na CAVD (Turrini et al., 1999). Ainda, a biópsia endomiocárdica pode fornecer resultados duvidosos, uma vez que podem ser frequentemente encontradas, em indivíduos normais, ilhas de tecido adiposo entre os cardiomiócitos do VD (Mckenna et al., 1994; Thiene et al., 2001).

Triagem

Uma mutação genética de deleção no gene da estriatina (STRN), localizada no cromossomo 17, está associada ao desenvolvimento de CAVD em diversos cães da raça Boxer (Meurs et al., 2010), e, portanto, um kit comercialmente disponível (http://www.laboklin.co.uk/laboklin/GeneticDiseases.jsp) tem sido utilizado para a triagem da doença em alguns países. A estriatina está localizada na região do disco intercalado nos cardiomiócitos, juntamente com outras três proteínas desmossômicas (placofilina-2, placoglobina, e desmoplaquina), todas envolvidas na patogênese da CAVD em seres humanos (Al-Jassar et al., 2013). A deleção no STRN foi identificada em 94% dos Boxers acometidos pela CAVD em um estudo, entretanto, também foi encontrada em 24% dos controles saudáveis (Meurs et al., 2010). Embora a CAVD no Boxer seja conhecida como uma doença hereditária associada à deleção no STRN em muitos casos, é também uma doença familiar herdada com penetrância incompleta e expressão fenotípica variável (Meurs, 2017). Cães que são homozigotos para a deleção no STRN parecem apresentar uma forma mais grave de CAVD, demonstrada por um número maior de arritmias ventriculares, ocorrência de morte súbita e, em alguns casos, o desenvolvimento da categoria III da enfermidade (Meurs et al., 2010; Meurs et al., 2013).

TRATAMENTO

O tratamento da CAVD no Boxer geralmente é voltado para o controle das arritmias ventriculares, uma vez que a maior parte dos animais não desenvolve disfunção sistólica e não progride para insuficiência cardíaca (Meurs, 2017). Evidentemente, alguns cães assintomáticos afetados podem apresentar morte súbita como a primeira manifestação. Ainda não há um consenso a respeito do melhor momento para se iniciar a terapia antiarrítmica nos Boxers assintomáticos (Meurs, 2017).

No Boxer, algumas características das arritmias ventriculares parecem estar mais associadas ao desenvolvimento de sinais clínicos, como por exemplo: taquicardia ventricular sustentada, complexos ventriculares prematuros em padrões repetidos (ex. pares, trios, bigeminismo, trigeminismo), e fenômeno R em T (Meurs, 2004; Meurs, 2017) Porém, vale ressaltar que, no melhor do nosso conhecimento, não há estudos referentes ao impacto do tratamento com antiarrítmicos na sobrevida e prognóstico em cães da raça Boxer assintomáticos e acometidos pela CAVD . Com base nisso, alguns clínicos optam por iniciar a terapia uma vez que um determinado número de CVP no Holter de 24 horas tenha sido identificado (p. ex. quando houver mais de 1000 CVP/24 h, ou se houver taquicardia ventricular ou quaisquer padrões repetidos de CVP) mesmo em um cão assintomático.

Foi demonstrado que nos Boxers, o tratamento antiarrítmico diminui tanto o número de CVP em 24 horas, quanto a complexidade da arritmia (MEURS *et al.,* 2002), entretanto, a variabilidade diária no número de CVP registrados pelo Holter de 24 horas pode ser um fator de confundimento. Logo, ao optar pelo tratamento, os tutores deverão ser informados de que o uso de antiarrítmicos pode exercer um efeito contrário, pró-arrítmico, como exacerbação das extra-sístoles com a amiodarona, e bloqueio atrioventricular de primeiro e/ou segunda grau com o cloridrato de sotalol (Meurs, 2017).

Cães acometidos pela CAVD e que apresentem síncope devem iniciar o tratamento antiarrítmico (Meurs, 2004; Meurs, 2017). Um estudo mostrou redução significativa dos episódios de síncope após a instituição da terapia (Meurs et al*.,* 2002). As opções de antiarrítmicos que demonstraram reduzir o número e complexidade das arritmias ventriculares, bem como as síncopes, são o cloridrato de sotalol, na dose de 1,5 a 2 mg/kg via oral a cada 12 horas, e o mexiletine, na dose de 5 a 6 mg/kg via oral

a cada 8 horas, sendo que este último não se encontra comercialmente disponível no Brasil. Ótimos resultados foram referidos com a associação de ambos (Meurs, 2017). A bradicardia neurocardiogênica é uma causa de síncope em cães da raça Boxer, embora menos comum que a CAVD, e não esteja obrigatoriamente relacionada à cardiomiopatia, embora possam coexistir (Thomason et al., 2008). Quando o Holter é realizado próximo ao evento da síncope no Boxer, e nenhum ou raros CVP são identificados, a síncope secundária à bradicardia deverá ser investigada (Thomason et al., 2008). O uso de betabloqueadores, ou de sotalol, pode agravar a bradicardia neurocardiogênica no Boxer (Thomason et al., 2008).

Idealmente, um controle pré e pós tratamento com Holter de 24 horas deve ser preconizado, para avaliar a resposta à terapia antiarrítmica, porém, animais sintomáticos e com arritmia ventricular observadas no ECG convencional deverão iniciar o tratamento o quanto antes (Meurs, 2017). Devido à variação diária no número de CVP documentados pelo Holter de 24 horas, preconiza-se que numa resposta positiva ao tratamento haja redução de cerca de 80% no número de CVP e na complexidade das arritmias (Meurs, 2017).

Diversos ensaios clínicos em seres humanos demonstram efeitos cardioprotetores e antiarrítmicos dos ácidos graxos ômega 3 (Marchioli et al., 1997; GISSI-Prevenzione Investigators, 1999; Albert et al., 2002). Em Boxers, o ômega 3 oriundo do óleo de peixe (ácido eicosapentanóico 78 mg + ácido docosahexenóico 497 mg) administrado por 6 semanas consecutivas foi capaz de reduzir significativamente o número de CVP (Smith et al., 2007). Estudos clínicos mais recentes, que avaliaram os efeitos dos ácidos graxos poliinsaturados ômega-3 na morte súbita cardíaca produziram resultados conflitantes. Em cães submetidos ao infarto miocárdico experimental, a suplementação com 1-4 gramas/dia de óleo de peixe (ácido eicosapentanóico + ácido docosahexenóico) exibiu efeito pró-arrítmico na população estudada (Billman et al., 2012).

PROGNÓSTICO

Há sempre o risco de morte súbita em cães da raça Boxer acometidos pela CAVD. Entretanto, grande parte dos animais assintomáticos vivem por anos, mesmo sem tratamento (Meurs, 2017). Outra parcela, dos cães sintomáticos, podem ser manejados satisfatóriamente com antiarrítmicos durante anos (Meurs et al., 2014). Uma pequena parte destes, podem desenvolver disfunção sistólica e dilatação ventricular, nos quais a sobrevida é consideravelmente menor (Meurs et al., 2005; Meurs et al., 2014). Um estudo realizado com 122 Boxers, identificou que a presença de taquicardia ventricular, polimorfismo, presença de >50 CVP/24 h, idade acima de 4,6 anos, animais do sexo masculino e presença de dilatação ventricular esquerda na ecocardiografia são fatores preditores independentes de mortalidade cardíaca, tendo sido associados à piores prognósticos, a despeito da presença de sinais clínicos (Motskula et al., 2013). O TAPSE <15,1 mm está associado à menor sobrevida, mesmo nos Boxers sem disfunção sistólica (Kaye et al., 2015). Ademais, recentemente em uma investigação que incluiu 69 Boxers, foi demonstrado que a presença de síncope e episódios de taquicardia ventricular resultam em piores prognósticos (Chamas et al., 2016).

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Figura 1 - Esquema representando a mensuração de marcadores eletrocardiográficos. A) Intervalo QT e subintervalos (QRS, JTpico, Tpico, Tfinal e JT). B) Marcadores eletrocardiográficos utilizados na avaliação das arritmias ventriculares.

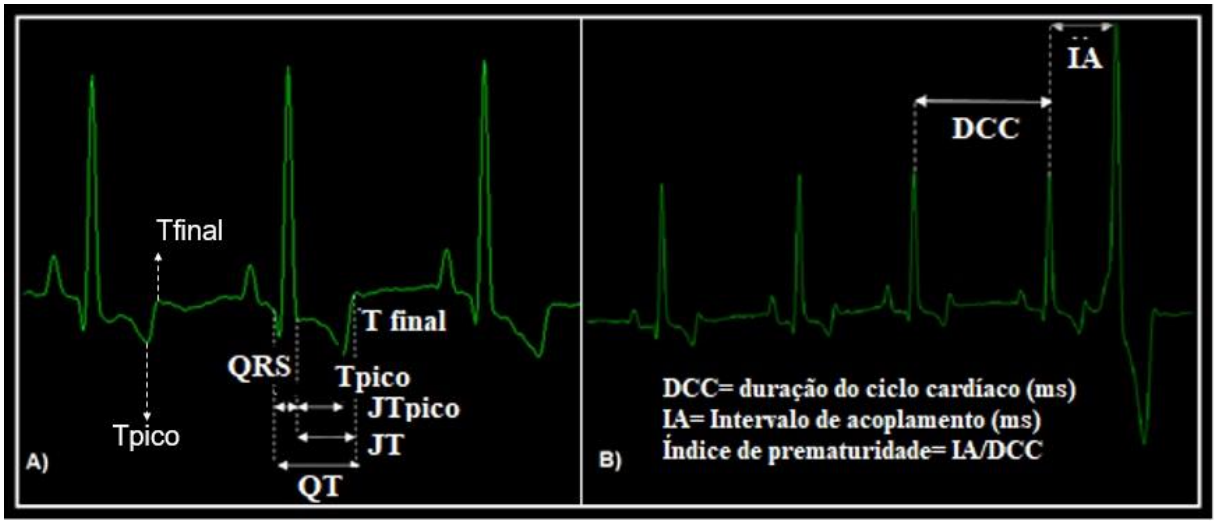
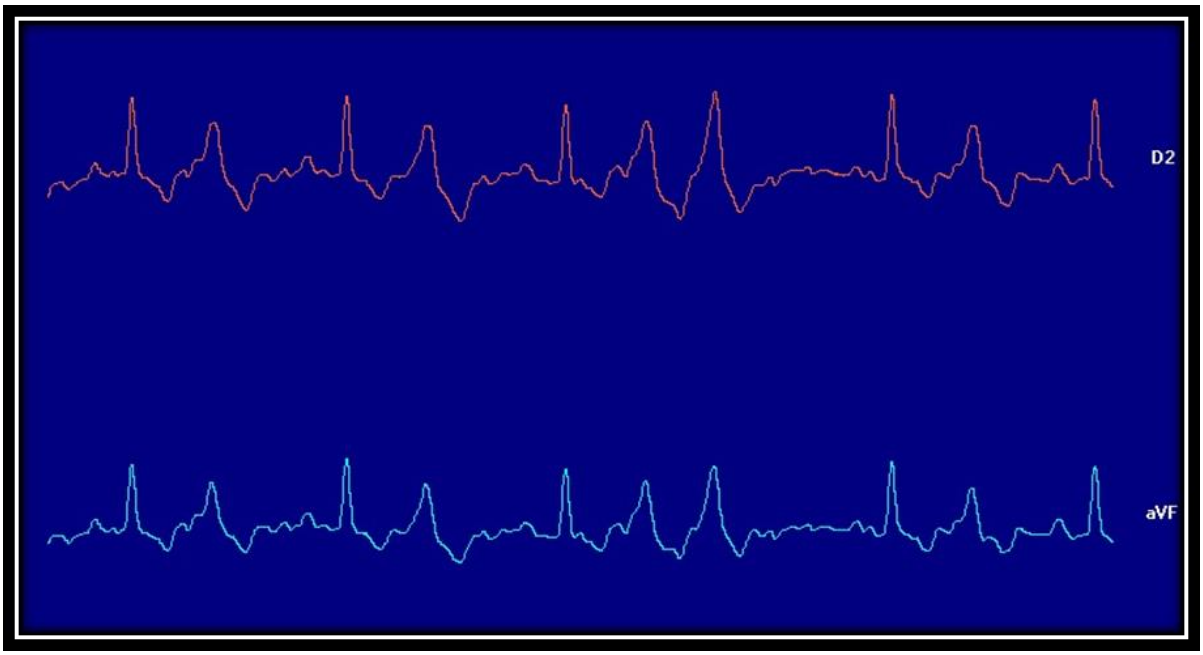


Figura 2 - Traçado eletrocardiográfico de um cão da raça Boxer considerado acometido pela cardiomiopatia arritmogênica do ventrículo direito. Nota-se bigeminismo ventricular, e dois complexos ventriculares prematuros pareados após o terceiro batimento sinusal, seguido de pausa compensatória. A morfologia de bloqueio de ramo esquerdo nas derivações DII e aVF sugerem a origem ventricular direita da arritmia.



CAPÍTULO TRÊS - Prognostic value of coupling interval, prematurity index and heart rate variability in Boxer dogs[2]

Abstract

The coupling interval (CI) and prematurity index (PI) of ventricular arrhythmias (VA), and the heart rate variability (HRV) was investigated in 36 client-owned Boxers. The first 24-hour Holter from animals meeting inclusion/exclusion criteria was evaluated for the number, morphology, site of origin, complexity, CI and PI, of ventricular premature complexes (VPCs), and time domain HRV. The effect on survival was assessed, considering the presence/absence of ventricular tachycardia (VT), and syncope. All-cause mortality was considered as the end-point, with median survival times being obtained by Kaplan-Meier analyses and compared by log-rank test. The CI and PI were not demonstrated to be prognostic surrogates in Boxer dogs with VA. HRme≥ 112 bpm is 100% sensitive but only 46% specific for detecting VT in Boxers on the 24-hour Holter. Presence of VT, SDNN≤245 ms, or SDANN≤134 ms at the time of the first 24-hour Holter was associated with a shorter survival.

Abbreviation list

ARVC: arrhythmogenic right ventricular cardiomyopathy

AUC: areas under the curve

CHF: congestive heart failure

CI: coupling interval

ECG: electrocardiography

HR: heart rate

[2] Este capítulo corresponde ao artigo científico submetido à revista Canadian Veterinary Journal e encontra-se em avaliação para publicação. Ademais, está formatado de acordo com as normas deste periódico.

HRmax: maximum heart rate over 24-hour recording

HRme: mean heart rate over 24-hour recording

HRmin: minimum heart rate over 24-hour recording

HRV: heart rate variability

PI: prematurity index

pNN>50: percentage of adjacent R-R intervals differing in duration by more than 50 milliseconds

rMSSD: square root of the mean squared differences of successive R-R intervals

SCD: sudden cardiac death

SCL: sinus cycle length

SDANN: standard deviation of the mean R-R intervals obtained at 5 minutes intervals

SDNN: standard deviation of the R-R intervals

SDNNidx: mean of the standard deviation of the R-R intervals obtained at 5 minutes intervals

VA: ventricular arrhythmias

VT: ventricular tachycardia

VPCs: ventricular premature complexes

Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC) was first described in Boxer dogs in the early 1980's [1] as a myocardial disease in which cardiomyocytes are replaced by fibrofatty tissue, making affected dogs prone to developing ventricular arrhythmias (VA). Boxer ARVC is a familial disease apparently inherited as an autosomal dominant trait [2]. Three clinical presentations have been proposed: (1) asymptomatic dogs with occasional ventricular premature complexes (VPC), (2) Boxer

dogs with tachyarrhythmias and syncope or exercise intolerance, and (3) least commonly diagnosed, animals with myocardial systolic dysfunction and ventricular dilation, sometimes with evidence of congestive heart failure (CHF) [3].

There is no single specific test to diagnose Boxer ARVC [4], and 24-hour Holter monitoring plays a fundamental role as part of screening, diagnosis, and monitoring of treatment in this condition. The severity of VA is usually described by the number, pattern, morphology and occurrence. Although many affected animals live a normal life, Boxer dogs with ARVC are at risk of sudden cardiac death (SCD), thus, the study of additional electrocardiographic characteristics may help stratify risk. The prognosis of Boxers with ARVC without CHF is widely variable and predicting outcome is usually challenging [1].

The coupling interval (CI), defined as the R-R interval between the VPC and the preceding sinus complex, and the prematurity index (PI), defined as the ratio of CI to the R-R interval of the sinus cycle just before the VPC, have been extensively studied in VA of human beings in order to evaluate the prematurity of VPCs, although the results are still controversial [5-7]. In dogs with degenerative mitral valve disease, our group recently demonstrated that PI differs between symptomatic and pre-clinical stages, but the prognostic value of such indices has not been evaluated [8]. However, heart rate variability (HRV), a non-invasive way to assess the interaction between the sympathetic and parasympathetic autonomic nervous system, is a well-established method to identify people at risk of developing CHF or SCD [9-12]. Curiously, in Boxer dogs the prognostic value of HRV has not yet been completely characterized.

Therefore, the aims of this study were twofold: (1) to investigate the characteristics of VA, including the CI and PI, and the HRV variables in Boxer dogs with respect to clinical status and the presence/absence of ventricular tachycardia - previously established as an independent predictor of mortality in Boxer ARVC [13]; and (2) to evaluate the impact of such characteristics on the overall survival time.

Materials and Methods

800 ***Animals***

801 The study was approved by the institutional Animal Care and Use Committee
802 (protocol 012277/17). Boxer dogs investigated at the Cardiology section of a referral
803 teaching practice were identified by searching the medical record database. The
804 database was searched to identify all boxers which met the following inclusion criteria:
805 ≥ 4 years old, examined between 2012 and 2017, with an available conventional
806 electrocardiography (ECG) [a], in addition to VA recorded at a three-channel 24-hour
807 Holter monitoring [b], and echocardiography [c], performed by the attending cardiologist
808 within seven days of one another. For animals in which more than one Holter recording
809 was available, only the first examination was used for analysis. Dogs found to have
810 any heart disease other than ARVC diagnosed by echocardiography, CHF, a systemic
811 disease process that could explain the observed arrhythmias (*e.g*., ehrlichiosis [14],
812 detected neoplasia [15]), as well as those with syncope due to neurocardiogenic
813 bradycardia, and those receiving antiarrhythmics were excluded. Baseline clinical
814 details obtained from medical records included age, gender, body weight, and history
815 of syncope at the time of the first 24-hour Holter Recording.

816

817 ***Electrocardiographic evaluation***

818 One investigator (E.R.C), who was blinded to the clinical status of dogs,
819 manually reviewed Holter records checking the QRS templates, including possible
820 misinterpretation for horizontal and vertical resolution, and checked the digital 24-h
821 editing program [d]. Measurements, as well as analyses, were made on channel 1 of the
822 Holter recording (positive pole toward the apex of the left ventricle).

823 Ventricular arrhythmias over a 24 hour period were characterized according to
824 [2]: (1) presence or absence of ventricular premature complexes (VPC); (2) number of
825 VPCs; (3) site of origin of VPCs: right ventricle (left bundle branch block morphology -
826 LBBB), left ventricle (right bundle branch block morphology - RBBB), or both; (4) VPC
827 morphology: monomorphic (unifocal – same QRS morphology and polarity throughout
828 the exam) or polymorphic (multifocal – QRS morphology changing with regard to
829 amplitude and/or polarity); and (5) VPC complexity: isolated VPC, repeating patterns

(*e.g.,* couplets, triplets, bigeminy, trigeminy) or ventricular tachycardia (VT). VT was defined as a sequence of more than three consecutive large QRS complexes, with heart rate (HR) > 180 beats/min and atrioventricular dissociation. Boxers were divided according to presence or absence of syncope (syncope *versus* non-syncopal), as well as according to presence or absence of VT (VT *versus* non-VT).

Boxers were further classified according to the degree of ventricular ectopy, based on the following criteria: 0 to 20 VPCs per 24 hours were interpreted as normal; 21 to 300 VPCs per 24 hours were interpreted as indeterminate; and animals with more than 300 VPCs/24h were considered likely to be affected by ARVC [1].

In addition, the CI of VPC, defined as the R-R interval between the VPC and the preceding sinus complex, and the sinus cycle length (SCL), defined as the R-R interval of the sinus cycle just before the VPC, were determined as described elsewhere (Figure 1) [6,8]. The PI of VPCs was calculated as the ratio of CI to SCL. Five VPCs per dog were manually chosen at random, all measurements were made five times, the highest and lowest values were excluded and a mean of three measurements was used for statistical analysis. When the randomization included a run of VT, the first VPC of the run and the SCL from the sinus beats before the episode were used. Boxers with fewer than 5 VPCs recorded had all ventricular premature beats measured, and a mean was obtained.

The 24-h HRV was assessed at the time domain of sinus beats, and included the following parameters: (1) the standard deviation of the R-R intervals (SDNN); (2) the standard deviation of the mean R-R intervals obtained at 5 minute intervals (SDANN); (3) the mean of the standard deviation of the R-R intervals obtained at 5 minute intervals (SDNNidx); (4) the square root of the mean squared differences of successive R-R intervals (rMSSD); (5) percentage of adjacent R-R intervals differing in duration by more than 50 milliseconds (pNN>50); (6) minimum HR over 24-hour recording (HRmin); (7) mean HR (HRme); and (8) maximum HR (HRmax).

Areas under the curve (AUC) above 0.5 were obtained for PI and HRme parameters to try to differentiate dogs with VT from others at the time of the first 24-h Holter. For this reason, two proposed cut-off values to PI and HRme, based on the better match between specificity and sensitivity, were proposed.

Survival analyses

Survival analyses was performed using an end-point of all-causes of death. Follow-up information was documented from medical records, or a telephone call with owners, in up to six attempts over a number of days. If survival data were still incomplete, referring veterinarians were contacted. Outcome data included patient status (alive or dead), date of death or euthanasia, and the probable cause of death. Additionally, owners were asked whether there was a family history of ARVC. SCD was defined as an unexpected death without apparent clinical signs during the preceding 24 hours [16]. For dogs that were still alive at the end of the study, or those lost to follow-up, the date that were last seen alive was recorded and then they were censored of the analysis.

Statistical analyses

The Shapiro–Wilk test was used to investigate the normal distribution of data. Syncopal and non-syncopal animals, and dogs that presented with VT and those without were compared using either Mann–Whitney test or Student's t-test according to distribution. Parametric data are represented as mean ± standard deviation, while non-parametric variables are shown as median [interquartile range]. The contingency was evaluated using either Fisher's exact test or the Chi-square when three or more categories were compared. Receiver operating characteristic (ROC) curves were constructed to investigate sensitivity and specificity of CI, PI and HRV parameters to distinguish dogs with VT from those without, as VT has previously been established as an independent predictor of mortality in Boxer ARVC [13]. Kaplan-Meier survival curves were constructed and compared using both Log-rank and Gehan-Breslow-Wilcoxon tests to identify factors that significantly influenced mortality, and to estimate median survival time. Statistical processing was performed using commercially available statistical software [e], and significance was defined as $P<0.05$.

Results

Between 70 evaluated dogs, a total of 36 dogs met the inclusion/exclusion requirements and were included in this study. The sample comprised 17 females and 19 males, with a mean of 8 [6-10.8 y] years old, weighing 27.9 kg [24.3-32 kg]. Fourteen animals (38.9%) had ≤ than 20 VPCs/24-h and were considered normal, seven (19.4%) had between 21 and 300 VPCs/24-h being considered as indeterminate to ARVC, and fifteen (41.7%) had more than 300 VPCs/24-h and were considered likely to be affected by ARVC. Ten dogs (27.8%) had only VPCs with first deflection positive, suggesting the right ventricle as site of origin, five (13.9%) animals had only VPCs with the first deflection negative, suggesting the left ventricle as site of origin, and twenty-one (58.3%) dogs showed both.

Seven dogs (18.9 %) experienced syncope at the time of first 24-h Holter recording, and no animal had clinical signs of CHF (ascites, pulmonary edema, pleural effusion). Characteristics evaluated in syncopal and non-syncopal Boxer dogs are summarized in Table 1. Age and weight did not differ between groups; however, male dogs were overrepresented in the syncopal group (P=0.001). In addition, symptomatic patients were found to have more polymorphic VPCs (P=0.0002), as well as a higher SDNN (P=0.02) and SDNNidx (P=0.009).

After the first 24-h Holter recording, Boxer dogs that had syncope, and/or VPCs in couplets, triplets, bigeminy or VT, regardless of the number of VPCs, were given antiarrhythmic therapy, a total of 18 animals (48.6%). The most commonly prescribed drug was sotalol (77.8%) at a dose of 30-60 mg per dog orally every 12 hours. Amiodarone was prescribed to four animals (22.2%), at a dose of 150-200 mg per dog orally every 12 hours. Twelve dogs (66.7%) received omega-3 fish oil (500 mg orally daily) in addition to sotalol or amiodarone.

Boxers were categorized according to the presence of VT, and the CI, SCL, PI, and HRV features were compared, as shown in Table 2. The CI was considered similar between dogs with or without VT (P=0.09), however, the SCL was shorter in the VT group (280 *vs* 410 ms; P=0.008), therefore, the PI was higher (less premature) in these patients (0.85 *vs* 0.68; P=0.02). Furthermore, among HRV parameters, only the HRme

differed between non-VT and VT dogs, being higher in the latter (89 *vs* 104 bpm; P=0.01).

Areas under the curve (AUC) above 0.5 were obtained for CI, PI and HRV parameters to try to differentiate dogs with VT from others at the time of the first 24-h Holter (Table 3), but only PI (AUC=0.73; P=0.02) and HRme (AUC=0.72; P=0.03) were considered relevant and cut-off values were presented in Table 4.

Median follow-up period for the whole population was 700 days [354-934 days]. Sixteen dogs (44.4%) reached the end-point. Among them, three animals were euthanized due to end-stage cancer (18.75%), SCD occurred in two (12.5%), and eleven died of unknown causes (68.75%). Among dogs that died suddenly, one had 8 isolated monomorphic VPCs over 24-h, and another 2 isolated monomorphic VPCs and a run of VT of 8 seconds over 24-h at the time of first Holter.

A family history was known in five animals (13.9%) (7-11 years old), with three dogs having ARVC parents that died suddenly, and two with healthy parents. The three dogs with family history of ARVC showed more than 300 polymorphic VPCs/24-h. Also, two of these dogs had VT and syncope, and the third had isolated VPCs and was asymptomatic at the time of the first Holter monitoring.

Median survival time was significantly shorter for dogs with VT compared to those with isolated or repeated patterns of VPC (463 *vs* 1173 days; HR= 4.25; 95% CI= 1.23-14.64; P=0.02). The presence of couplets, triplets and bigeminy were not statistically significant (P=0.057). There was no significant difference in survival time between the dogs in the monomorphic or polymorphic VPCs group (P=0.26). The presence of syncope (P=0.79), number of VPCs over 24-h (P=0.18), as well as CI (P=0.29) and PI (P=0.57) was not associated with overall survival at the proposed cut-off values, as shown in Figure 2.

Of the HRV features, SDNN was shown to be associated with prognosis (SDNN>245 ms= 1645 days *vs* SDNN≤245 ms= 271 days; HR=19.97; 95% of CI= 3.91-101.8; P=0.0003), also SDANN (SDANN>134 ms= 1645 days *vs* SDANN≤134 ms= 463 days; HR=3.95; 95% CI=2.13–38.79; P=0.003) (Figure 3). Survival analyses according to HRV features are summarized in Table 5.

Discussion

In this study, the impact on overall survival of some characteristics of ventricular arrhythmias and heart rate variability on the first 24-h Holter monitoring in thirty-six Boxer dogs, was assessed. In these analyses, both the dogs with syncope and those examined for routine breed screening were included. The mean age in the whole population was similar, approximately 8 years old, although only animals ≥ 4 years old were actually accepted into the study. Boxer ARVC is usually of adult-onset, more frequently seen in animals above 5 years of age [2,17]. Among symptomatic dogs, a male predominance was observed, similarly to previously reported data [17,18].

The diagnosis of ARVC is best based on a combination of findings, that may include a middle age Boxer dog with syncope, ventricular arrhythmias with left bundle branch block morphology in caudoventral leads (II, III and aVF) [19] without other documentable causes for the arrhythmia, and a family history of disease [2,4]. Although in man the familial history of ARVC is considered a major criterion for diagnosis [20], in this study the family history was known in only three dogs, 13.9% of cases.

The number of VPCs over 24-h was not statistically different between asymptomatic and symptomatic dogs. In addition, the complexity of VA did not differ between symptomatic and asymptomatic dogs. The small number of symptomatic dogs enrolled in our study may have affected these findings. Palermo *et al.* (2011) found that symptomatic Boxers with ventricular dilation (form 3 of ARVC) usually had a higher number of ventricular ectopic beats, in addition to more complex arrhythmias (couplets, triplets, VT) in comparison to those without ventricular dilation (forms 1 and 2). However, only a small proportion of dogs in Palermo's study had a Holter recording available, making it difficult to draw conclusions regarding the association between the different ARVC forms and either severity or frequency of arrhythmias.

Although 24-h Holter monitoring provides a better assessment of presence, overall frequency and complexity of VA and should be used as part of the diagnosis of Boxer ARVC [18,21], there is no established cut-off for the number of VPCs over 24-hour that would classify a Boxer as ARVC affected. Ventricular arrhythmias have a

spontaneous daily variability, which can be as high as 80% in Boxer dogs with ARVC [22]. The proposed cut-off value of 300 VPCs over 24-h was not associated with a worse prognosis in this study, contrasting with another investigation in which more than 50 VPCs/24-h was associated with a shorter survival [13]. In addition, the two dogs that died suddenly would have been considered normal at the first 24-h Holter based solely on the number of VPCs over the day.

Symptomatic Boxer dogs are more likely to have polymorphic VPCs, in concordance with what has been previously reported in Boxer dogs with CHF due to ARVC [13], and symptomatic dogs with degenerative mitral valve disease [8]. Although survival was considered similar between Boxer dogs exhibiting either monomorphic or polymorphic VA (P=0.26), the difference in median survival between these groups has clinical significance (1993 *vs* 910 days). Other authors have previously reported that polymorphism is associated with cardiac mortality in Boxer dogs [13]. The impact of polymorphic ventricular arrhythmias on mortality has not been clearly elucidated in veterinary studies. In the human medical literature, however, it is recognized as a negative prognostic indicator, especially polymorphic VT, which is considered a rhythm with a potential to progress to ventricular fibrillation, a major cause of sudden cardiac death [23]. None of animals on this study had polymorphic VT.

The presence of VT was associated with an increased all-cause mortality in this study, similar to previously studies, in which Boxer dogs with VT were about 3 times more likely to suffer cardiac death [13,24]. In people, many studies have reported that more frequent VPCs are closely associated with ventricular dilation, systolic dysfunction, malignant forms of VT, as well as a poor prognosis [5-6, 25-30]. Although the PI and HRme were the two evaluated variables that better distinguished dogs with VT from the remaining population, the specificity and sensitivity obtained for PI were low, and none of them were associated with prognosis. However, the HRme=112 bpm was 100% sensitive but only 46% specific for VT, meaning that all dogs without VT had an HRme < 112 bpm. This finding suggests that if VT is not observed in a 24-hour Holter record in an adult Boxer but HRme is above 112 bpm, it could be reasonable to pursue further Holter monitoring.

Boxer dogs with ARVC can experience SCD regardless of presence/absence of clinical signs [4]. Our findings suggested that syncope at the time of the first 24-h Holter

monitoring was not associated with a worse prognosis, however our population was heterogenous and only 41.7% was considered likely to be affected by ARVC according to the degree of ventricular ectopy. Palermo *et al*. 2011 found that the presence of syncope was associated with shorter survival times in Boxers affected with ARVC. Drawing conclusions about the significance of syncope and survival in Boxer dogs is difficult, as the majority of symptomatic animals received antiarrhythmic therapy after the first Holter, which may skew the survival analyses.

There has been interest in the study of VPC CI and PI as an aid to better stratification of risk in patients with VA, although these variables have been poorly investigated in veterinary patients. In human patients, results are conflicting, likely reflecting heterogeneous study populations [6, 31-33]. The CI and PI were not statistically significant different between symptomatic and asymptomatic Boxer dogs, in contrast with our own study in dogs with mitral endocardiosis, in which the VPCs in symptomatic animals were less premature [8]. However, the VPCs of dogs with clinical mitral endocardiosis could be considered relatively less premature, mainly because the influence of heart rate, or sinus cycle length, at PI. In addition, these two variables were not associated with overall mortality, and, at least to the best of our knowledge, this was the first time that the prognostic value of CI and PI has been evaluated in Boxer dogs. The VPCs in dogs with VT were found to be less premature (higher PI), due to a shorter sinus cycle length, despite CI being comparable to those without VT. The authors believe these findings could be explained by the effect of heart rate on the prematurity index, since dogs with VT had higher mean heart rate and thus higher PI. Moreover, PI at the proposed cut-off values does not adequately distinguish patients with and without VT.

The HRV is determined by autonomic nervous system activity. The propensity for lethal arrhythmias associated with either increased sympathetic or reduced vagal activity, reflected as decreased HRV, have been extensively studied in people [3–5].From our data, some HRV variables, such as SDNN and SDNNidx, in symptomatic Boxer dogs were higher than in the asymptomatic group. When looking at animals with VT, only HRme was different from the other parameters, suggesting that elevated HRme (≥112 bpm) could be associated with an increased risk of developing VT, although with a poor specificity. Previous studies of HRV in Boxer dogs did not identify a lower HRV

in patients with complex and frequent VA [22,37]. Although HRV was not evaluated in relation to syncope in such investigations, Boxer dogs with CHF had a lower HRV [22,24]. Our findings suggest that symptomatic Boxer dogs, as well as those with VT and no CHF, did not have a persistently high sympathetic tone, or HRV changes have reflected subclinical diseases processes at the time that the first 24-hour Holter was made.

Nevertheless, the SDNN and SDANN, estimates of long-term components of HRV, which are thought to be more sympathetically mediated [38-40] of HRV, were found to have prognostic value in Boxer dogs, as a SDNN ≤ 245 ms, or SDANN ≤ 134 ms were associated with worse outcome. Conversely, a reduced HRV has already been associated with CHF and risk of SCD in people with VA [10-12]. Chamas *et al*. 2016 found no association between HRV variables and outcome in Boxer dogs, even though the lowest SDANN (58 ± 13 ms) was documented in animals with CHF, as previously reported by Spier & Meurs 2004b (82 ± 21 ms).

The results of this study should be interpreted in the context of its limitations. This was a retrospective investigation and accepts the inherent criticisms of the methodology, as the animals could not be genetically tested for the striatin deletion. The unequal groups and small sample size with regard to syncope, limits the stratification of VA characteristics and HRV between such individuals. The total sample size was small, including Boxer dogs considered normal, equivocal and likely affected by ARVC. In addition, at the end of follow-up period fewer than 50% of population had reached the end-point. Although any cause of mortality is of clinical relevance, a study to asses cardiac mortality would be preferable. Finally, the influence of antiarrhythmic therapy after the first 24-h Holter potentially played a major role in overall survival, and this was not standardized due to the retrospective methodology. Despite all the limitations, these preliminary results add new information about the risk stratification in Boxer dogs with VA, and may contribute to prospective studies.

In conclusion, our major findings are as follows. (1) Symptomatic Boxer dogs are more likely to have polymorphic VPCs than asymptomatic ones; (2) The VPCs of dogs with ventricular tachycardia are relatively less premature, probably due to the heart rate influence over the prematurity index in spite of a comparable coupling interval; (3) The PI and HRme were significant different between VT and non-VT dogs, whilst these parameters alone did not discriminate adequately between groups, HRme

≥ 112 bpm may provide additional information regarding the presence of VT, in a population known to have significant day-to-day variation of VA; (4) The presence of VT, SDNN ≤ 245 ms, or SDANN ≤134 ms in an adult Boxer dog at the time of the first 24-h Holter recording is associated with a shorter overall survival time.

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Conflict of interest

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

[a] ECGPCVet, Tecnologia Eletrônica Brasileira, São Paulo, Brazil.

[b] CardioLight, Cardios Sistemas, São Paulo, Brazil.

[c] My Lab 30, Esaote, Italy.

[d] CardioManenger S540, Cardios Sistemas, São Paulo, Brazil.

[e] Prism Windows 6.0, GraphPad Software, California, USA.

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premature ventricular complexes from right ventricular outflow tract improves left ventricular dilation and clinical status in patients without structural heart disease. J Am Coll Cardiol 2005;45:1259–1265.

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Figure 1. Schematic representation of the measurement of coupling interval (CI), sinus cycle length (SCL) and calculation of the prematurity index (PI).

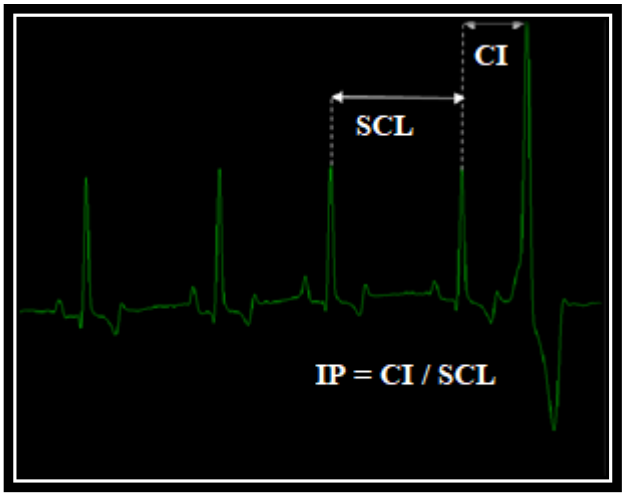
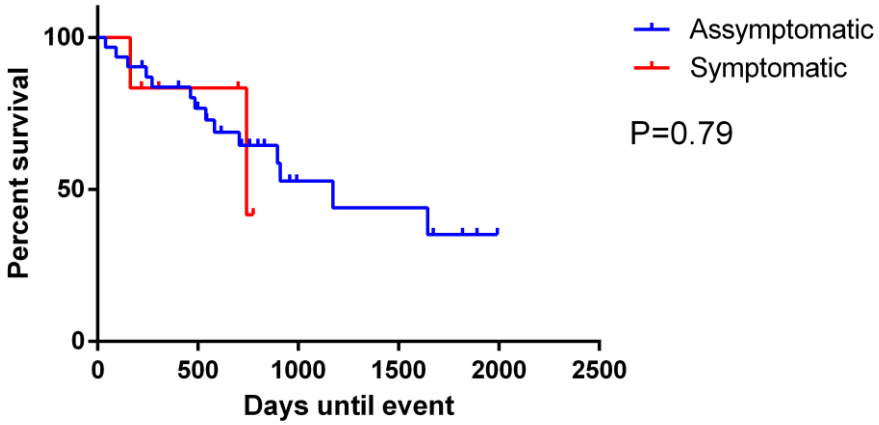
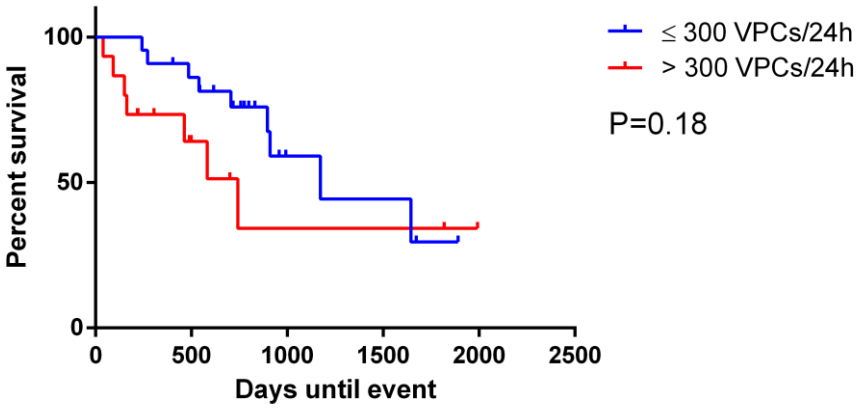
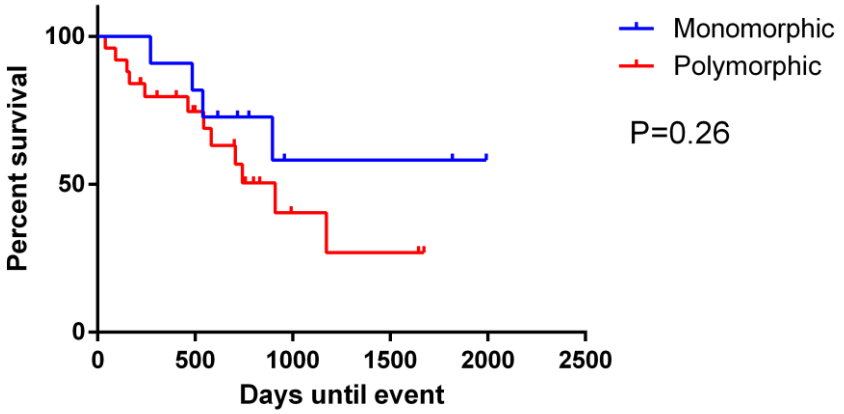
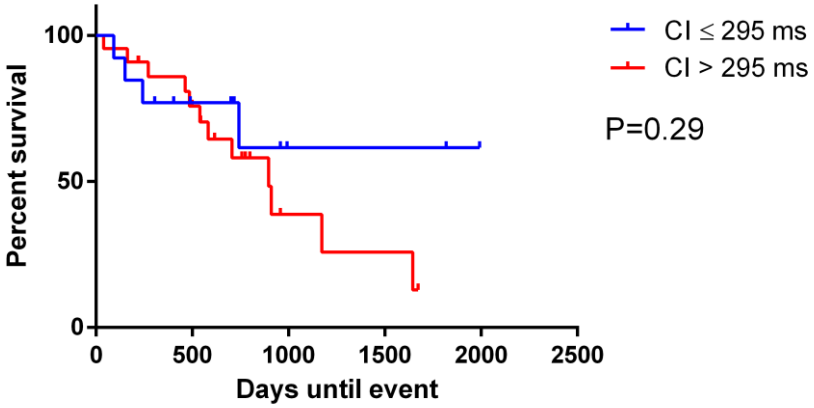


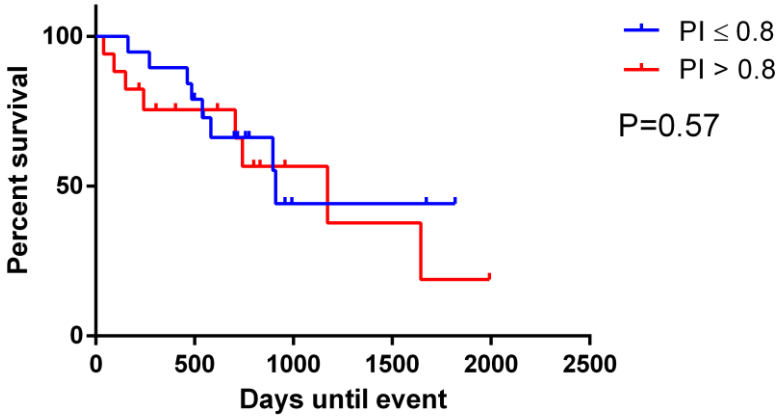
Figure 2. Kaplan-Meier analyses used to assess the prognostic value of several characteristics of ventricular arrhythmias and syncope in 36 adult Boxer dogs. The end-point was all-cause of death.





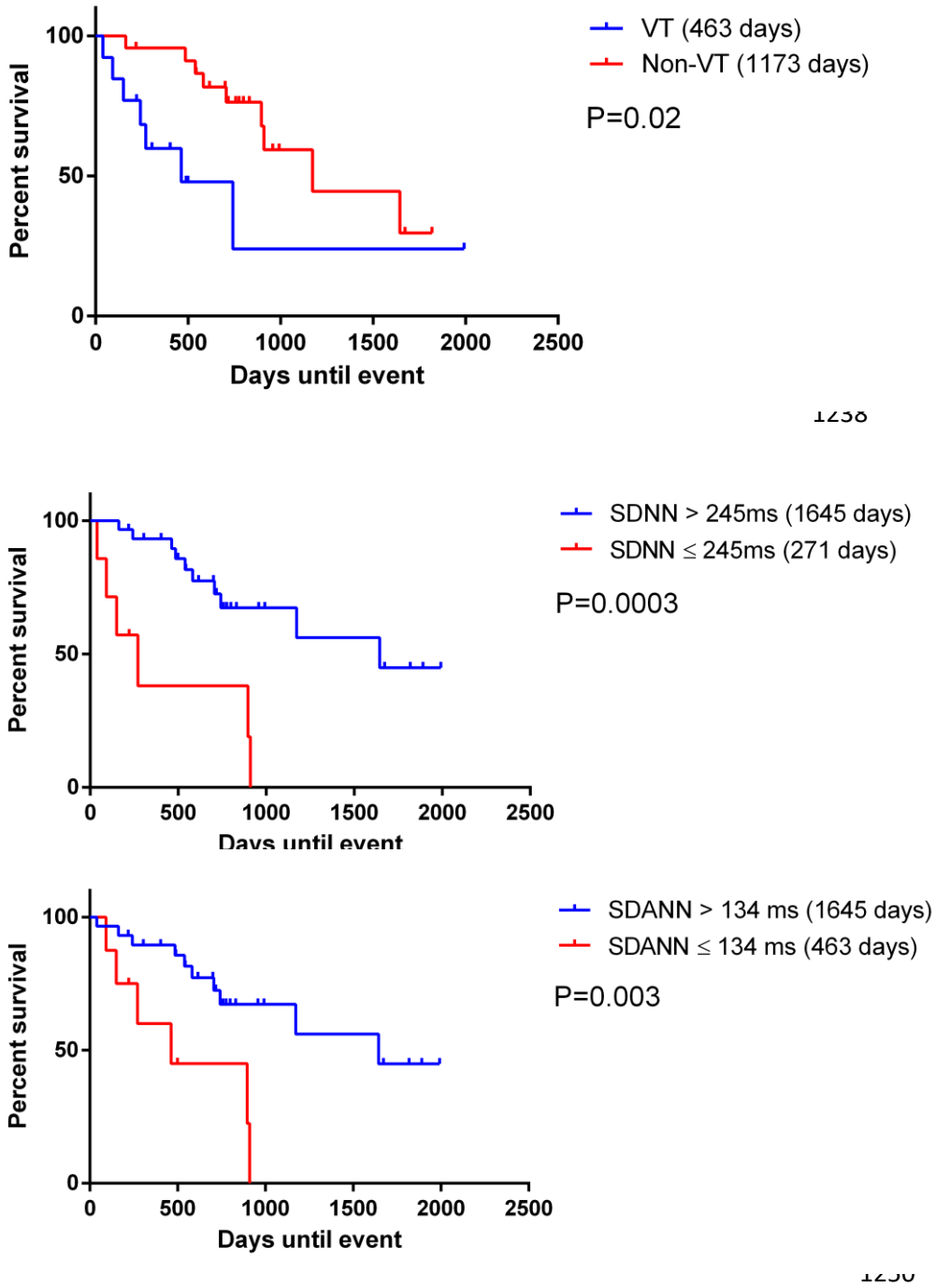






VPCs: ventricular premature complexes; CI: coupling interval; PI: prematurity index.

Figure 3. Kaplan-Meier analyses with prognostic value at the first 24-Hour Holter monitoring in 36 adult Boxer dogs. The end-point was all-cause of death.



VT: ventricular tachycardia; SDNN: the standard deviation of the RR intervals; SDANN: the standard deviation of the mean RR intervals obtained at 5 minutes intervals, over 24-hours.

Table 1. Sample characterization, characteristics of ventricular arrhythmias and time domain heart rate variability in 36 adult Boxer dogs according to the presence of syncope. Parametric data are represented as mean ± standard deviation, while non-parametric variables are shown as median [interquartile range].

		Non-Syncopal (n=30)	Syncopal (n=6)	P
Age (years)		8.1±2.8	7.7±2.8	0.77
Body weight (kg)		26.5 [24-30.4]	32.8 [22.4-40]	0.42
Gender (%)				
	Female	53	28	0.001
	Male	47	72	
Number of VPC		25 [3-2538]	1023 [47-3517]	0.22
Morphology (%)				
	Monomorphic	38	14	0.0002
	Polymorphic	62	86	
Occurrence				
	Isolated	45	42	0.11
	Repeated patterns	17	29	
	VT	38	29	
Coupling interval (ms)		320 [275-370]	300 [270-360]	0.49
Prematurity index		0.75±0.19	0.70±0.24	0.52
HRmin (bpm)		39 [36-45]	34 [31-38]	0.06
HRme (bpm)		93 [85-105]	90 [83-92]	0.30
HRmax (bpm)		235 [226-250]	250 [223-250]	0.38
SDNN (ms)		300 [245-352]	369 [327-429]	0.02
SDANN (ms)		170±55	210±43	0.09
SDNNidx		236 [178-282]	297 [269-353]	0.009
rMSSD (ms)		135.5±62.4	151.4±70	0.56
pNN>50 (%)		45.8±14.7	57.5±8.9	0.05

Repeated patterns: couplets, triplets, bigeminy, trigeminy; VT: ventricular tachycardia; HRmin: minimum heart rate over 24 hours; HRme: mean heart rate over 24h; HRmax: maximum heart rate over 24h; SDNN: the standard deviation of the RR intervals; SDANN: the standard deviation of the mean RR intervals obtained at 5 minute intervals; SDNNidx: mean of the standard deviation of the RR intervals obtained at 5 minute intervals; rMSSD: the square root of the mean squared differences of successive RR intervals; pNN>50: percentage of adjacent RR intervals differing by more than 50 milliseconds in duration.

Table 2. Electrocardiographic features of ventricular arrhythmias and time domain heart rate variability in 36 adult Boxer dogs according to the presence of ventricular tachycardia. Parametric data are represented as mean ± standard deviation, while non-parametric variables are shown as median [interquartile range].

	CI (ms)	SCL (ms)	PI	HRmin (bpm)	HRme (bpm)	HRmax (bpm)	SDNN (ms)	SDANN (ms)	SDNNidx	rMSSD (ms)	pNN>50 (%)
VT (n=13)	280 [260-340]	360 [280-475]	0.85±0.12	43±12	104±21	231 [229-248]	292 [140-386]	158±68	223 [103-333]	114±69	45±17
Non-VT (n=23)	320 [300-380]	490 [410-600]	0.68±0.20	38±6	89±10	245 [224-250]	317 [278-374]	186±42	252 [201-300]	150±56	50±13
P	0.09	0.008	0.02	0.11	0.01	0.29	0.37	0.14	0.38	0.09	0.34

VT: ventricular tachycardia; CI: coupling interval; SCL: sinus cycle length; PI: prematurity index; HRmin: minimum heart rate over 24 hours; HRme: mean heart rate over 24h; HRmax: maximum heart rate over 24h; SDNN: the standard deviation of the RR intervals; SDANN: the standard deviation of the mean RR intervals obtained at 5 minute intervals; SDNNidx: mean of the standard deviation of the RR intervals obtained at 5 minute intervals; rMSSD: the square root of the mean squared differences of successive RR intervals; pNN>50: percentage of adjacent RR intervals differing by more than 50 milliseconds in duration.

Table 3. Receiver operating characteristic of coupling interval, prematurity index and time domain heart rate variability parameters to distinguish adult Boxer dogs with ventricular tachycardia from those without VT.

	CI	PI	HRmin	HRme	HRmax	SDNN	SDANN	SDNNidx	rMSSD	pNN>50
AUC	0.67	0.73	0.60	0.72	0.60	0.59	0.62	0.59	0.68	0.58
95% CI	0.46- 0.87	0.56-0.89	0.39-0.81	0.53-0.90	0.41-0.80	0.38-0.80	0.40-0.83	0.37-0.80	0.48-0.87	0.36-0.78
P	0.09	0.02	0.31	0.03	0.30	0.36	0.23	0.37	0.08	0.45

AUC: area under the curve; CI: coupling interval; PI: prematurity index; HRmin: minimum heart rate over 24 hours; HRme: mean heart rate over 24h; HRmax: maximum heart rate over 24h; SDNN: the standard deviation of the RR intervals; SDANN: the standard deviation of the mean RR intervals obtained each 5 minutes; SDNNidx: mean of the standard deviation of the RR intervals obtained each 5 minutes; rMSSD: the square root of the mean squared differences of successive RR intervals; pNN>50: percentage of adjacent RR intervals differing by more than 50 milliseconds in duration.

Table 4. Cut-off values to prematurity index (PI) and mean heart rate (HRme) that better distinguished Boxer dogs with VT from others at the time of the first 24-h Holter recording.

	Cut-off	Sensitivity (%)	Specificity (%)	Likelihood ratio
PI	0.79	65.22	69.23	2.12
	0.83	65.22	53.85	1.41
HRme (bpm)	94	76.16	53.85	1.65
	112	100	46.15	1.85

Table 5. Log-rank comparison of Kaplan-Meier survival curves analyses of heart rate variability features to the proposed cut-off values (in parentheses), in 36 Boxer dogs monitored by 24-h Holter.

Parameter	P
SDNN (245 ms)	0.0003
SDANN (134 ms)	0.003
SDNNidx (192 ms)	0.16
rMSSD (173 ms)	0.14
pNN>50% (52.1%)	0.26
HRmin (37 bpm)	0.34
HRme (95 bpm)	0.07
HRmax (247 bpm)	0.16

SDNN: the standard deviation of the RR intervals; SDANN: the standard deviation of the mean RR intervals obtained at 5 minutes intervals, over 24-hours; SDNNidx: mean of the standard deviation of the RR intervals obtained each 5 minutes; rMSSD: the square root of the mean squared differences of successive RR intervals; pNN>50%: percentage of adjacent RR intervals differing by more than 50 milliseconds in duration; HRmin: minimum heart rate over 24 hours; HRme: mean heart rate over 24h; HRmax: maximum heart rate over 24h.

CAPÍTULO 4 - Electrocardiographic markers of myocardial conduction and repolarization in Boxer dogs[3]

ABSTRACT. - Carvalho E.R, Zacché E., Fenerich M., Camacho A.A., Santos J.P. dos, Sousa M.G. 2019. **Electrocardiographic markers of myocardial conduction and repolarization in Boxer dogs.** College of Veterinary Medicine and Agricultural Sciences, São Paulo State University (UNESP) Jaboticabal. Via de Acesso Prof. Paulo Donato Castellane s/n. 14884-900 - Jaboticabal, SP. E-mail: beth_rcarvalho@hotmail.com

Electrocardiographic markers have been used in people to classify arrhythmogenic risk. The aims of this study were twofold: (1) to investigate electrocardiographic markers of conduction and repolarization in Boxers and non-Boxer dogs; (2) to compare such findings between groups. Ten-lead standard electrocardiograms of Boxer dogs and non-Boxers recorded between 2015 and 2018 were retrospectively reviewed. Dogs ≥ 4 years of age and weighing >20 kg were included. Animals with valvular insufficiencies, congenital cardiopathies, cardiac dilation, suspected systolic dysfunction, biphasic T-wave, bundle branch blocks, and those receiving antiarrhythmics were excluded. Electrocardiographic markers of conduction, QRS duration (QRSd) and dispersion (QRSD), and repolarization (corrected QT interval, Tpeak-Tend, JT and JTpeak), as well as derived indices, were measured. Two hundred dogs met the inclusion/exclusion requirements, including 97 Boxers (8.1 ± 2.5 years old; 30 ± 7 kg) and 103 non-Boxer (8.8 ± 2.5 years old; 30 ± 8 kg). QRSd and QRSD, and repolarization markers in lead II and left precordial lead V4 were considered similar between groups. Dispersion of late repolarization on lead rV2, Tpeak-Tend interval, was considered longer in Boxers (45 ± 8 ms vs 38 ± 10 ms; P= 0.01). The Tpeak-Tend / JTpeak and the JTpeak/JT also differed between groups. Our results indicate that the dispersion of myocardial late repolarization in lead rV2 is slower in Boxers than other dog breeds.

[3] Este capítulo corresponde ao artigo científico submetido à revista Pesquisa Veterinária Brasileira e encontra-se em avaliação para publicação. Ademais, está formatado de acordo com as normas deste periódico.

INDEX TERMS: arrhythmias, canine, electrocardiography, premature cardiac complexes.

RESUMO.- [Marcadores eletrocardiográficos de condução e repolarização miocárdica em cães da raça Boxer]

Marcadores eletrocardiográficos têm sido estudados em seres humanos para estratificação do risco arritmogênico. Os objetivos deste estudo foram duplos: (1) investigar os marcadores eletrocardiográficos de condução e repolarização miocárdica em Boxers e em cães de outras raças; (2) comparar tais resultados entre os grupos. Para tal, a eletrocardiografia convencional de 10 derivações registradas entre 2015 e 2018 foram avaliadas de maneira retrospectiva. Cães com idade igual ou superior a 4 anos e pesando > 20 quilos foram incluídos. Animais com insuficiência valvar, cardiopatias congênitas, dilatação cardíaca, suspeita de disfunção sistólica, onda T bifásica, bloqueio(s) de ramo(s), ou aqueles que recebiam antiarrítmicos foram excluídos. Variáveis eletrocardiográficas de condução, como a duração e dispersão do complexo QRS (QRS_d e QRSD, respectivamente), e repolarização (intervalo QT corrigido, Tpico-Tfinal, JT e JTpico), bem como índices derivados, foram mensurados. Duzentos cães que se adequaram aos critérios de inclusão/exclusão foram incluídos, 97 Boxers (8,1 ± 2,5 anos; 30 ± 7 kg) e 103 não Boxers (8,8 ± 2,5 anos; 30 ± 8 kg). O QRSd e o QRSD, e os marcadores de repolarização nas derivações II e V4 foram similares entre os grupos. O marcador de dispersão da repolarização tardia na derivação rV2, Tpico-Tfinal, foi considerado mais longo no Boxers (45 ± 8 ms *versus* 38 ± 10 ms; P= 0.01). O Tpico-Tfinal/JTpico e o JTpico/JT também diferiram entre os grupos. Nossos resultados indicam que a dispersão da repolarização miocárdica tardia na derivação precordial direita, rV2, é mais lenta no Boxer do que nas outras raças.

TERMOS DE INDEXAÇÃO: arritmias, canino, eletrocardiografia, complexos cardíacos prematuros.

INTRODUCTION

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited myocardial disease commonly seen in adult Boxer dogs. The disease is characterized by the replacement of right ventricle (RV) cardiomyocytes by a fibrofatty tissue, a condition that eventually generates areas prone to the formation of ventricular arrhythmias, which may culminate in sudden death (Harpster 1983, 1991). There is no single and specific diagnostic test for ARVC, thus, in veterinary practice, the diagnosis is best based on a combination of findings which may include: the presence of ventricular tachyarrhythmia with no other documented causes for the arrhythmia, syncope, and family history of ARVC (Meurs 2004, Meurs 2017). Although many Boxers with ARVC live for years even without treatment, they are always at an increased risk of sudden cardiac death (Meurs 2017).

In people many electrocardiographic markers have been studied to identify individuals at risk for malignant arrhythmias and sudden death (Kurl et al. 2012, Kurosaki et al. 2013, Tse & Yan 2016, Zumhagen et al. 2016). These can be based on myocardial conduction, such as QRS duration (QRS_d), which is a surrogate marker of conduction velocity (CV), and QRS dispersion (QRS_D) reflecting CV dispersion. Conduction abnormalities associated with reduced CV, along with a functional or structural obstacle, are conditions for re-entry (Tse & Yan 2016).

Abnormal repolarization properties can increase the risk of triggered activity and re-entrant arrhythmias, the latter known to be involved in the pathogenesis of ARVC (Basso et al. 2015). Electrocardiographic markers based on repolarization include: corrected QT (QT_c), QT dispersion (QT_D), interval from the peak to the end of the T-wave (T_{peak}-T_{end}), and interval from J point to the peak and/or end of the T-wave (JT_{peak} and JT, respectively).

Although studied in many conditions known to be associated with increased arrhythmogenicity in man, such as ARVC (Zorzi et al. 2013), diabetes (Stettler et al. 2007), sepsis (Ozdemir et al. 2016), and myocardial infarction (Zhao et al. 2012), studies investigating electrocardiographic markers of conduction and repolarization in dogs are lacking. Therefore, the aims of this study were twofold: (1) to investigate

electrocardiographic markers of conduction and repolarization in Boxers and non-Boxer dogs over 20 kg; and (2) to compare such findings between the general population and a breed widely known to be predisposed to developing ventricular arrhythmias.

MATERIALS AND METHODS

Study Design and Ethics Statement. This retrospective study was approved by the institutional Animal Care and Use Committee (protocol 012277/17).

Animals. Boxer dogs (BG) evaluated between 2015 and 2018 at the Cardiology sections of two referral teaching practices were identified by searching the medical record databases. Only dogs that met the following inclusion criteria were admitted into the study: ≥ 4 years old, body weight > 20 kg, with a standard high-quality 10-lead electrocardiogram and an echocardiogram. The electrocardiograms (ECG) (ECGPCVet, Tecnologia Eletrônica Brasileira) were recorded for a minimum of 3 minutes with the animal positioned in right lateral recumbency. The electrodes for frontal and precordial leads were placed as described elsewhere (Tilley & Burtinick 1999). The echocardiogram (My Lab 30, Esaote) was obtained within a maximum of seven days from the evaluated ECG. Dogs found to have valvular insufficiencies, suspected systolic dysfunction (defined as shortening fraction ≤ 25%), left atrial dilation (defined as left atrial-to-aortic root ratio ≥ 1.6 (Hansson et al. 2002), ventricular dilation (defined as body weight normalized left ventricular internal diameter in diastole ≥ 1.7 (Cornell et al. 2004), congenital cardiopathies, biphasic T-wave, QRS complexes with bundle branch block morphology, and those receiving antiarrhythmics were excluded. In addition, matched non-Boxer dogs (NBG) were selected based on the same inclusion/exclusion requirements.

In BG 37 dogs had an available 24-Hour Holter monitoring recorded within seven days of the evaluated ECG and echocardiogram. Boxers were further classified according to the degree of ventricular ectopy, based on the following criteria: 0 to 20 ventricular premature complexes (VPCs) per 24 hours were interpreted as normal; 21 to 300 VPCs per 24 hours were interpreted as indeterminate; and animals with more

than 300 VPCs/24h were considered likely to be affected by ARVC (Meurs 2017). Only the subgroup of Boxers considered normal (n=12) and the subgroup considered likely to be affected by ARVC (n=15) utilizing the Holter criteria were compared for all electrocardiographic parameters listed in Figure 3. The subgroup considered indeterminate (n=8) was excluded from such comparisons.

The clinical history of syncope at the time of ECG and echocardiogram was searched in medical records of all dogs in BG and NBG. In NBG no animal had a recorded syncope, 88 Boxers in BG were unaffected but nine had suffered at least one syncopal episode. All electrocardiographic parameters listed in Figure 3 were also compared for syncopal and non-syncopal Boxers.

Electrocardiographic evaluation. One investigator, who was blinded to the breed and clinical status of dogs, measured all the parameters listed in Figure 3 and illustrated in Figure 1. The measurements were made in a run of five sequential sinus beats. The highest and lowest values obtained for each parameter were excluded and the final result was considered as the average of three measurements. Parameters obtained from precordial leads were measured using leads rV2 as right and V4 as left.

Statistical analyses. The Shapiro–Wilk test was used to investigate the normal distribution of data. Groups were compared using either Mann–Whitney test or Student's t-test according to distribution. Parametric data are represented as mean ± standard deviation, while non-parametric variables are shown as median [interquartile range]. The contingency was evaluated using the Fisher's exact test. Statistical processing was performed using commercially available statistical software (Prism Windows 6.0, GraphPad Software), and significance was defined as $P<0.05$.

RESULTS

A total of 200 dogs met the inclusion/exclusion requirements and were recruited for this study. The sample comprised 97 Boxers and 103 non-Boxer dogs. No differences in gender were documented within or between groups. The mean body weight did not differ between groups (Boxers 30 ± 7 kg, non-Boxers 30 ± 8 kg, P=0.82).

The mean age was also considered similar between groups (Boxers 8.1 ± 2.5 years old, non-Boxers 8.8 ± 2.5 years old, P=0.16). Among NBG, mixed breed dogs were most represented (n=67, 65%), followed by Labrador Retrievers (n=11, 11%), Pitbulls (n=9, 9%), and Golden Retrievers (n=8, 8%). No animal from NBG had a history of syncope. In BG, nine dogs (9%) had a clinical history of syncope, and 88 (91%) had no previous history.

The electrocardiographic markers of conduction, QRS_d and QRS_D, were considered similar between BG and NBG. Also, no differences existed between BG and NBG regarding the myocardial repolarization markers obtained from leads II and V4. When looking at lead rV2, the dispersion of late repolarization evaluated as T_{peak}-T_{end} was longer in Boxers (45 ± 8 ms) than it was in non-Boxer dogs (38 ± 10 ms) (P=0.01). Similarly, the dispersion of repolarization normalized to JT_{peak} interval obtained from lead rV2, i.e. T_{peak}-T_{end} / JT_{peak}, was found to be higher in BG (0.41 [0.35-0.51]) than in NBG (0.37 [0.27-0.40]) (P=0.03). Curiously, the dispersion of repolarization normalized to JT interval, the JT_{peak}/JT, was found to be slightly lower in Boxers (0.72 [0.67-0.75] *vs* 0.73 [0.71-0.79], P=0.04). Our results are summarized in Table 1, and box-plots of markers found to be different between groups are represented in Figure 2.

Interestingly, no differences were documented in the electrocardiographic markers between syncopal and non-syncopal Boxers (Table 2), or between Boxers considered normal (n=12) and those likely affected by ARVC (n=15) according to the 24-Hour Holter criteria for the degree of ventricular ectopy (Table 3).

DISCUSSION

In this study, traditional and novel electrocardiographic conduction and repolarization markers of augmented arrhythmogenesis were evaluated and compared between Boxers and dogs of other breeds. In these retrospective analyses, both Boxers who had history of syncope (n=9) and those without (n=88) were included, as well as Boxers considered normal (n=12) or likely affected by ARVC (n=15) according to Holter criteria. None of the dogs had received antiarrhythmic therapy at the time the

electrocardiogram was recorded. The body weight and age were considered similar between BG and NBG, although only animals ≥ 4 years old and > 20 kg were included in the study to minimize the impact of the dog's size and age on electrocardiographic parameters.

The pathological features of ARVC in both people and Boxer dogs, which are considered as a spontaneous animal model for the study of the human ARVC, include cardiomyocyte atrophy and fibrofatty replacement (Marcus et al. 1982, Basso et al. 2015). Depolarization delay in right precordial leads is a common feature in people with ARVC (Nasir et al. 2004, Cox et al. 2008). Thus, the QRS prolongation (>110 ms) in right precordial leads is considered a major criteria for diagnosis of ARVC in people (Marcus et al. 2010), and an independent predictor of risk of sudden cardiac death in the general population (Kurl et al. 2012). However, our findings suggest that the electrocardiographic surrogates of conduction velocity of myocardial depolarization impulse behave similarly in Boxers and in medium to large non-Boxer dogs, and Boxers with a high degree of ventricular ectopy (> 300 VPCs/24h) compared to those with a low number of VPCs (0-20 VPCs/24h).

The QT interval reflects the duration of the action potential at the cellular level, being associated with depolarization and triggered activity. The QT_c interval in people with type 1 diabetes is considered an independent predictor of cardiovascular mortality (Stettler et al. 2007). However, although it is a poor indicator of heterogeneity in repolarization across the heart, it is known that increased heterogeneities in repolarization increase the risk of arrhythmias (Tse & Yan 2016). No differences were noticed either in QT_c or QT_D between groups in this investigation.

The fibrofatty tissue that replaces healthy myocardium in ARVC is thought to contribute to the development of ventricular arrhythmias by slowing intra-ventricular conduction, and acting as a substrate for arrhythmias through a scar-related macro-reentry mechanism (Fontaine et al. 1984). The T_{peak}-T_{end} interval is important as a lead-dependent marker of global dispersion of late repolarization (Xia et al. 2005a, b), since repolarization dispersion varies in different cardiac regions (Bieganowska et al. 2013). Therefore, it was proposed that T_{peak}-T_{end} interval should be determined from right precordial leads for right ventricular disorders (Tse & Yan 2016). When the right

precordial lead rV2 is considered, our results for the electrocardiographic markers T_{peak}-T_{end} and T_{peak}-T_{end}/JT_{peak} suggest that the late dispersion of RV repolarization occurs more slowly in Boxer dogs than in other breeds. In people T_{peak}-T_{end} is increased in short QT syndrome (Watanabe et al. 2010), Brugada syndrome (Zumhagen et al. 2016), and patients with *torsade des pointes* (Yamaguchi et al. 2003).

While T_{peak}-T_{end} represents late repolarization, JT_{peak} represents early repolarization (Johannesen et al. 2014a, b), and both are potential markers of abnormal repolarization (Clemente et al. 2012). In people, the JT_{peak}/JT and the T_{peak}-T_{end}/JT_{peak} showed a higher sensitivity and specificity than T_{peak}-T_{end} for identifying patients who suffered myocardial infarction (Alvarado-Serrano et al. 2006). The dispersion of repolarization normalized to JT or JT_{peak} interval at rV2 differs in Boxers form non-Boxers in our study. Unexpectedly, the marker JT_{peak}/JT was slightly lower in BG, mainly due to the interquartile range difference between groups, since median values were similar in both groups.

The small and unequal sample size between syncopal and non-syncopal Boxers may have contributed significantly to our findings in such a comparison. According to our results, myocardial conduction and repolarization is similar in supposedly healthy and syncopal Boxers. As previously mentioned, a diagnosis of ARVC in veterinary patients is based on a combination of findings, including ventricular arrhythmias, syncope, and familial history of ARVC. Many disorders, such as neurocardiogenic bradycardia (Thomason et al. 2008), can ultimately lead to syncopal events. Since most syncopal Boxers admitted to a referring cardiology service are already receiving antiarrhythmic therapy, it is challenging to study electrocardiographic markers in such individuals. Antiarrhythmic drugs such as sotalol and mexiletine act by blocking ion channels, potassium and sodium respectively, in the cellular membrane, thus, interfering in electrocardiographic measurements, especially those related to conduction and repolarization.

The results of this study should be interpreted in the context of its limitations. This was a retrospective investigation and accepts the inherent criticisms of the methodology. Moreover, it is difficult to determine the end of the T-wave on conventional electrocardiography, as previously reported by many researchers and

this may introduce inaccuracies (Bieganowska et al. 2013, Kaplan et al. 2015, Tse & Yan 2016, Zumhagen et al. 2016). Also, the low number of syncopal Boxers, and those with a 24-Hour Holter recording may have compromised the accuracy of our analysis.

CONCLUSIONS

We conclude that (1) myocardial electrical conduction occurs similarly in BG and NBG; (2) global dispersion of myocardial late repolarization in the right precordial lead rV2 occurs more slowly in Boxers than dogs from other breeds; (3) traditional and novel electrocardiographic markers of myocardial conduction and repolarization were obtained in Boxers and non-Boxers dogs > 20 kg.

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Figure 1. Schematic representation of J point, QT interval, JT interval and JT_{peak} interval measurements used to obtain electrocardiographic markers in lead II.

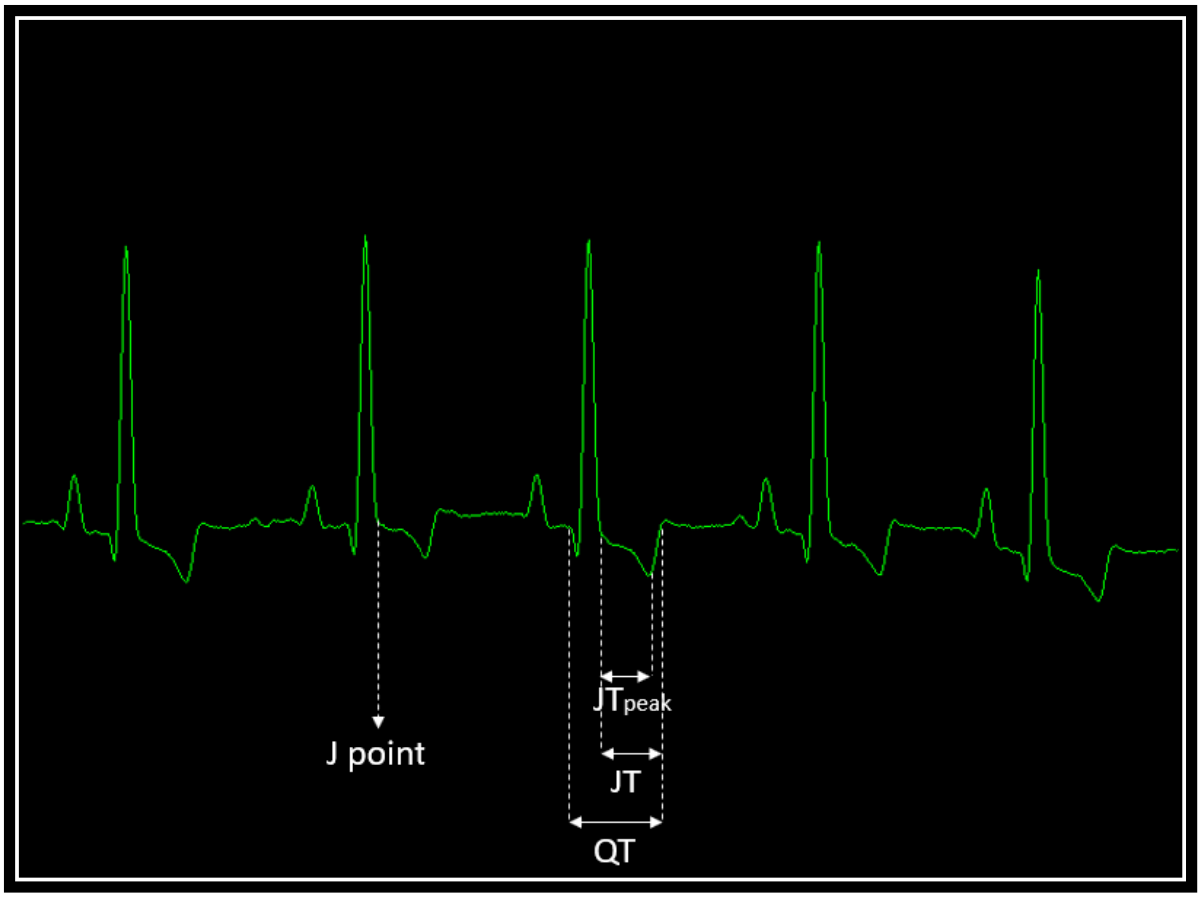

Figure 2. Box-plots of data distribution for electrocardiographic markers of repolarization found to differ between Boxer (n=97) and non-Boxer (n=103) dogs.

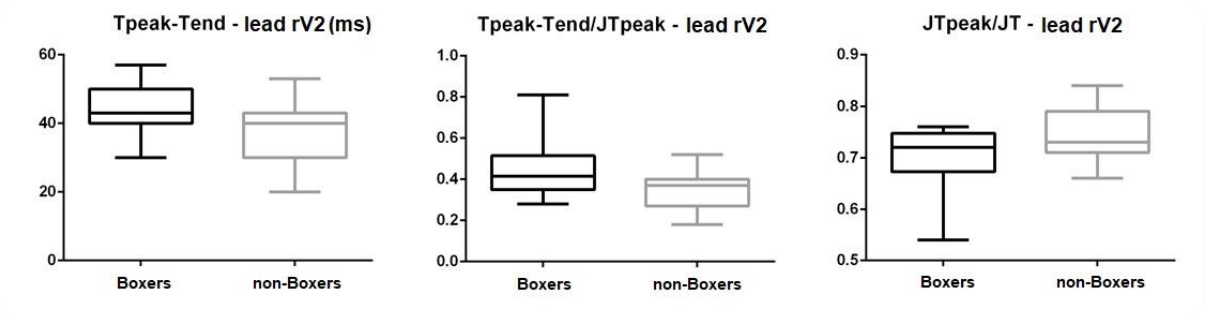

Figure 3. Summary of electrocardiographic markers evaluated in this study, which are based on either myocardial conduction or repolarization.

Classification of risk marker	Electrocardiographic marker	Definition	Pre-clinical marker correlate	Reference
Conduction	QRS_d	QRS duration, interval between the beginning and the end of QRS complex measured in leads II, rV2 and V4	Conduction velocity (CV)	(Kurl et al. 2012)
	QRS_D	QRS dispersion, maximum difference between QRS durations measured in the right (rV2) and left (V4) precordial leads	CV difference between two regions	(Peters et al. 1999; Tse et al. 2015)
Repolarization	QT_c	QT interval at DII corrected for heart rate using the Van de Water' formulae	Action potencial duration (APD)	(Surawicz 1987; Oliveira et al. 2014)
	QT_D	Maximum difference between QT intervals in two leads of the 10-lead ECG (applying the correction proposed by Malik et al., 2000)	Difference in APD values between two regions	(Linker et al. 1992; Elming et al. 1998; Malik and Batchvarov 2000)
	T_{peak}-T_{end}	Interval from the peak to the end of the T-wave, measured in leads II, rV2 and V4	Dispersion of late repolarization	(Xia et al. 2005)
	T_{peak}-T_{end}/QT_c	Interval from the peak to the end of the T-wave divided by QT_c	Dispersion of repolarization divided by APD	(Castro Hevia et al. 2006)
	JT_{peak}/JT	JT_{peak}: interval from J-point to peak of the T-wave. JT: interval from J-point to end of T-wave. Measured in leads II, rV2 and V4	Dispersion of repolarization normalized to JT interval	(Alvarado-Serrano et al. 2003; Brisinda et al. 2004; Johannesen et al. 2014)
	T_{peak}-T_{end} / JT_{peak}	Measured in leads II, rV2 and V4	Dispersion of repolarization normalized to JT_{peak} interval	(Alvarado-Serrano et al. 2003; Brisinda et al. 2004)

Table 1. Comparison of electrocardiographic surrogates of myocardial conduction and repolarization in Boxer dogs (n=97) and non-Boxer dogs (n=103) obtained from the 10-lead standard electrocardiography. *Statistical significance (P<0.05)

Electrocardiographic marker	Boxer	non-Boxer	P	Pre-clinical marker correlate
QRS_d lead II	67 [61-76] ms	60 [57-67] ms	0.06	
QRS_d lead rV2	70 [67-77] ms	67 [60-73] ms	0.11	Conduction velocity (CV)
QRS_d lead V4	64 ± 9 ms	64 ± 10 ms	0.89	
QRS_D	10 [3-16] ms	7 [3-13] ms	0.50	CV difference between two regions
QT_c	257 [246-272] ms	254 [242-258] ms	0.22	Action potencial duration (APD)
QT_D	7 [5-11] ms	7 [5-8] ms	0.54	Difference in APD values between two regions
T_{peak}-T_{end} lead II	34 ± 9 ms	31 ± 7 ms	0.22	
T_{peak}-T_{end} lead rV2	45 ± 8 ms	38 ± 10 ms	0.01*	Dispersion of late repolarization
T_{peak}-T_{end} lead V4	35 ± 9 ms	34 ± 12 ms	0.51	
T_{peak}-T_{end} /QT_c lead II	0.14 ± 0.04	0.12 ± 0.03	0.34	
T_{peak}-T_{end} /QT_c lead rV2	0.17 [0.15-0.19]	0.15 [0.13-0.18]	0.07	Dispersion of repolarization divided by APD
T_{peak}-T_{end} /QT_c lead V4	0.13 [0.11-0.16]	0.12 [0.09-0.17]	0.67	
JT DII	146 ± 22 ms	150 ± 10 ms	0.47	
JT rV2	150 ± 19 ms	148 ± 8 ms	0.77	Global dispersion of repolarization
JT V4	153 ± 20 ms	151 ± 12 ms	0.74	
JT_{peak} DII	112 ± 25 ms	121 ± 11 ms	0.21	
JT_{peak} rV2	105 ± 18 ms	110 ± 7 ms	0.29	Dispersion of early repolarization
JT_{peak} V4	119 ± 20 ms	117 ± 14 ms	0.78	
JT_{peak}/JT lead II	0.76 [0.71-0.83]	0.82 [0.76-0.86]	0.08	
JT_{peak}/JT lead rV2	0.72 [0.67-0.75]	0.73 [0.71-0.79]	0.04*	Dispersion of repolarization normalized to JT interval
JT_{peak}/JT lead V4	0.78 [0.73-0.81]	0.77 [0.73-0.83]	0.64	
T_{peak}-T_{end} / JT_{peak} lead II	0.33 [0.23-0.42]	0.26 [0.19-0.29]	0.08	
T_{peak}-T_{end} / JT_{peak} lead rV2	0.41 [0.35-0.51]	0.37 [0.27-0.40]	0.03*	Dispersion of repolarization normalized to JT_{peak} interval
T_{peak}-T_{end} / JT_{peak} lead V4	0.29 [0.25-0.37]	0.27 [0.18-0.40]	0.50	

Table 2. Comparison of electrocardiographic surrogates of myocardial conduction and repolarization in syncopal (n=9) and non-syncopal Boxer dogs (n=88) obtained from 10-lead standard electrocardiography.

Electrocardiographic marker	Non-syncopal (n=88)	Syncopal (n=9)	*p*
QRS_d lead II	63 [60-70] ms	73 [63-80] ms	0.11
QRS_d lead rV2	67 [63-73] ms	73 [67-80] ms	0.14
QRS_d lead V4	64 ± 8 ms	63 ± 8 ms	0.60
QRS_D	6 [3-13] ms	10 [6-17] ms	0.21
QT_c	257 ± 16 ms	267 ± 28 ms	0.19
QT_D	5 [4-10] ms	7 [5-11] ms	0.26
T_{peak}-T_{end} lead II	31 ± 10 ms	35 ± 9 ms	0.26
T_{peak}-T_{end} lead rV2	43 [38-53] ms	50 [37-50] ms	0.77
T_{peak}-T_{end} lead V4	37 ± 11 ms	33 ± 9 ms	0.34
T_{peak}-T_{end} /QT_c lead II	0.12 ± 0.04	0.13 ± 0.04	0.38
T_{peak}-T_{end} /QT_c lead rV2	0.16 [0.15-0.20]	0.18 [0.16-0.19]	0.83
T_{peak}-T_{end} /QT_c lead V4	0.14 ± 0.05	0.12 ± 0.05	0.13
JT_{peak}/JT lead II	0.79 [0.73-0.85]	0.75 [0.73-0.82]	0.24
JT_{peak}/JT lead rV2	0.72 [0.70-0.75]	0.71 [0.65-0.73]	0.14
JT_{peak}/JT lead V4	0.77 ± 0.06	0.78 ± 0.07	0.52
T_{peak}-T_{end} / JT_{peak} lead II	0.26 [0.20-0.36]	0.34 [0.24-0.40]	0.21
T_{peak}-T_{end} / JT_{peak} lead rV2	0.38 [0.34-0.47]	0.44 [0.38-0.52]	0.30
T_{peak}-T_{end} / JT_{peak} lead V4	0.29 [0.25-0.37]	0.28 [0.20-0.34]	0.57

Table 3. Comparison of electrocardiographic surrogates of myocardial conduction and repolarization obtained from 10-lead standard electrocardiography in Boxer dogs considered normal (n=12) and affected by ARVC (n=15) according to the degree of ventricular ectopy on 24-Hour Holter monitoring.

Electrocardiographic marker	Normal Boxers (n=12)	ARVC Boxers (n=15)	P
QRS_d lead II	63 [60-67] ms	73 [63-80] ms	0.05
QRS_d lead rV2	69 ± 7 ms	72 ± 7 ms	0.15
QRS_d lead V4	67 [60-70] ms	63 [57-70] ms	0.48
QRS_D	8 ± 9 ms	9 ± 6 ms	0.37
QT_c	260 ± 15 ms	267 ± 28 ms	0.40
QT_D	5 [4-11] ms	7 [5-11] ms	0.25
T_{peak}-T_{end} lead II	32 ± 10 ms	35 ± 9 ms	0.33
T_{peak}-T_{end} lead rV2	43 [37-54] ms	50 [37-50] ms	0.99
T_{peak}-T_{end} lead V4	39 ± 12 ms	33 ± 9 ms	0.14
T_{peak}-T_{end} /QT_c lead II	0.13 ± 0.04	0.13 ± 0.04	0.45
T_{peak}-T_{end} /QT_c lead rV2	0.16 [0.15-0.19]	0.18 [0.16-0.19]	0.79
T_{peak}-T_{end} /QT_c lead V4	0.15 ± 0.05	0.12 ± 0.05	0.07
JT_{peak}/JT lead II	0.78 [0.72-0.87]	0.75 [0.73-0.82]	0.38
JT_{peak}/JT lead rV2	0.73 [0.69-0.75]	0.71 [0.65-0.73]	0.21
JT_{peak}/JT lead V4	0.76 ± 0.07	0.79 ± 0.06	0.36
T_{peak}-T_{end} / JT_{peak} lead II	0.24 [0.19-0.37]	0.34 [0.24-0.40]	0.23
T_{peak}-T_{end} / JT_{peak} lead rV2	0.43 ± 0.15	0.45 ± 0.10	0.30
T_{peak}-T_{end} / JT_{peak} lead V4	0.34 ± 0.15	0.29 ± 0.11	0.27