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Cardiac diagnostic test results and outcomes in 44 dogs naturally infected with *Trypanosoma cruzi*

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Abstract

Background: The protozoal parasite *Trypanosoma cruzi* causes myocarditis in dogs.

Objectives: To describe the cardiac diagnostic test results and outcomes of dogs naturally infected with *T. cruzi*.

Animals: Forty-four client-owned dogs.

Methods: Medical records were retrospectively reviewed to identify dogs with an indirect fluorescent antibody test result for *T. cruzi* ≥1 : 80. Data collected included signalment, cardiac diagnostic test results (ECG, echocardiography, cardiac troponin I) and outcome. Outcomes were categorized as alive, dead (cardiac or noncardiac) or lost to follow up.

Results: ECG abnormalities were present in 41 dogs with ventricular arrhythmias (n = 28) and atrioventricular block (AVB) (n = 15) most commonly identified. Echocardiographic chamber enlargement was present in 28 dogs and most often included the right ventricle (RV) (n = 15) and left atrium (n = 12). Troponin was ≥2 times the reference range in 20/36 (56%) dogs. In univariate analysis using nonparametric Kaplan-Meier, ventricular arrhythmias with a modified Lown score ≥2 ($P = .02$), presence of AVB ($P = .04$), and RV enlargement ($P = .006$) were associated with decreased survival times. Right ventricular enlargement (HR 3.6; 95% confidence interval [CI] 1.4-9.3; $P = .007$) and higher body weight at presentation (HR 1.0; 95% CI 1.0-1.1; $P = .04$) were associated with decreased time to death in the final explanatory multivariable model.

Conclusions and Clinical Importance: Cardiac abnormalities were common and variable, and RV enlargement was associated with shorter survival time. A diagnostic evaluation that includes screening for arrhythmias, echocardiography, and cTnI can provide useful information related to the characterization of heart disease in dogs seropositive for *T. cruzi*.

Abbreviations: AVB, atrioventricular block; CI, confidence interval; cTnI, cardiac troponin I; DMVD, degenerative mitral valve disease; IFA, indirect fluorescent antibody; LA : Ao, ratio of the left atrial diameter to the aortic valve diameter; LVIDdN, left ventricular internal dimension at end-diastole normalized to body weight; LVIDsN, left ventricular internal dimension at end-systole normalized to body weight; RA : LA, ratio of the right atrial diameter to the left atrial diameter; RV, right ventricle.

KEYWORDS
canine, Chagas disease, echocardiography, heart, myocarditis, troponin

1 | INTRODUCTION

Chagas disease, caused by the protozoal parasite *Trypanosoma cruzi* and transmitted by triatomine insect vectors, causes myocarditis in dogs in the United States and Latin America.[1] The parasite invades cardiac myocytes where the amastigote form replicates causing cell destruction and an inflammatory response that can damage cardiac tissue.[2,3] Chagas disease is characterized by 3 phases that include acute, indeterminate, and chronic. The acute phase occurs in the first 21 days after *T. cruzi* infection and, while it is often associated with nonspecific illness, acute myocarditis and sudden death can occur.[4-6] Dogs in the indeterminate phase are characterized as antibody positive while clinically asymptomatic. A subset of dogs progress into a chronic phase characterized by arrhythmias, myocardial dysfunction, heart failure and sudden death.[1,3-6]

Infection status is typically established using the indirect fluorescent antibody (IFA) test in dogs.[7] An association between titer results and clinical disease has not been reported. Other methods of antemortem testing include the detection of parasite DNA in the blood using polymerase chain reaction, however, after initial infection and intracellular localization of the parasite, parasitemia is often low and difficult to detect.[8,9] Rapid serology tests have also been used off label for research but are not currently a standard for veterinary diagnosis in dogs.[10]

Studies in experimentally infected dogs document cardiac abnormalities after inoculation with *T. cruzi* organisms.[5,11] In an experimental model of Chagas disease, 25/78 (32%) dogs inoculated with *T. cruzi* organisms died 1 to 6 months after infection, and 13 of the 46 (28%) infected dogs that remained alive and had echocardiographic imaging had a variable reduction in left ventricular ejection fraction that occurred 6 to 9 months after infection.[11] Beagles monitored approximately every 10 days after inoculation developed evidence of acute and chronic Chagas disease that included atrioventricular block (AVB), ventricular arrhythmias, segmental thinning and wall motion abnormalities, global systolic dysfunction and biventricular failure over nearly a 10 month period.[6] However normal left ventricular function was also present in dogs with severe myocarditis documented at necropsy.[6]

A limited number of studies have described cardiac abnormalities and clinical outcomes in naturally infected dogs seropositive for *T. cruzi*.[10,12-15] Thirty percent (9/30) of naturally infected, seropositive dogs in Mexico had ECG and echocardiographic abnormalities detected.[15] The clinical outcomes of Chagas disease and indicators of prognosis in naturally-infected dogs are variable and not well understood. Additional clinical description and outcome reporting would be useful particularly in the absence of well-established antiparasitic treatments or preventatives for *T. cruzi* infection in dogs.

The objective of this study was to evaluate electrocardiographic and echocardiographic studies and report cardiac troponin I (cTnI) concentrations and clinical outcomes in dogs seropositive for *T. cruzi* based on IFA testing.

2 | MATERIALS AND METHODS

2.1 | Case selection criteria

Electronic medical records of all dogs that presented to the Texas A&M University Small Animal Veterinary Medical Teaching Hospital in College Station, Texas, between January 1, 2010 and January 1, 2018 were reviewed to identify dogs with a *T. cruzi* IFA titer result ≥1 : 80.

2.2 | Medical records review

Patient data collected from the medical records for the purpose of this study included signalment, body weight, diet, IFA test titer result, cardiac troponin I (cTnI) concentration, ECG (standard and ambulatory) and echocardiographic findings, and clinical outcome when available. Diet was classified as either traditional or as nontraditional (boutique, exotic-ingredient, or grain free) based on a potential association with diet and dilated cardiomyopathy in dogs.[16] For clinical outcome data, each dog was determined to be alive, dead (cardiac-related or noncardiac), or lost to follow up at study end date January 1, 2018. An attempt was made to contact owners or referring veterinarians to determine last known date alive or dead and reason for death, and the information was used for assigning outcome. All diagnostic test data collected were from the time of serologic testing.

2.3 | Procedures

2.3.1 | Laboratory analysis

Serological testing for anti-*T. cruzi* IgG antibodies was performed using an IFA by the Texas A&M Veterinary Medical Diagnostic Laboratory (College Station, Texas). For the purposes of this study, samples with IgG titers of ≥1 : 80 were considered positive to decrease the potential for interpretive error that can occur with the color dilution assay utilized. Dogs with titers of 1 : 20 were not included in this study due to the variable results encountered with repeated testing in the authors' experience and the lack of availability of other methods of testing to confirm positive or negative status during the majority of the duration of this study. Additionally, end titer results were not

reported throughout the duration of this study until 2014 when titer results >1 : 160 were reported when requested.

Cardiac troponin I concentrations were measured using 1 of 2 commercially available assays. For dogs admitted from 2010 to 2013, cTnI analysis was performed using a 2-site chemiluminescent immunometric assay (Immulite 2000, Siemens Healthcare Diagnostics, Los Angeles, California). The Immulite assay has a sensitivity of 0.2 ng/mL and has been validated in dogs.[17,18] Since 2013, cTnI analysis was performed using the ADVIA Centaur CP immunoassay (Ultra-TnI, Siemens Medical Solutions USA, Inc, Malvern, Pennsylvania) with a reported range for cTnI of 0.006 to 50.0 ng/mL that has been validated in dogs.[19] For the purposes of this study, results were divided into 3 categories: within the reference range of healthy dogs (low); 1-2 times the upper reference limit (medium); and ≥2 times the upper reference limit as previous described (high).[20] For the Immulite assay, this represents ≤0.5 ng/mL (low), 0.51-1.0 ng/mL (medium) and ≥1.01 ng/mL (high), and for the ADVIA assay, this represents ≤0.128 ng/mL (low), 0.129-0.255 ng/mL (medium) and ≥0.256 ng/mL (high).

2.3.2 | Echocardiography

All transthoracic echocardiographic studies were performed by a board-certified cardiologist or cardiology resident under direct supervision of a boarded cardiologist. The echocardiographic studies were reviewed on a digital workstation (GE EchoPAC v203; GE Medical Systems, Horten, Norway), and offline analysis was performed. Measurements included left ventricular internal dimension at end diastole and end systole from M-mode images in a right parasternal short axis view that were normalized to body weight (LVIDdN, LVIDsN). Values outside of the 95% confidence interval [CI] of a healthy dog reference cohort were considered abnormal.[21,22] Left ventricular fractional shortening was calculated from M-mode measurements. Left ventricular ejection fraction was calculated using the single plane Simpson's method of discs from images obtained in a left parasternal apical 4 chamber view. For the purposes of this study, fractional shortening <20% and ejection fraction <40% were considered low.[23] Left atrium to aorta ratio (LA : Ao) was calculated from measurements obtained in both right parasternal short and long axis views.[24,25] Left atrial enlargement was defined as an LA : Ao in short axis >1.57 or in long axis >2.4.[24,25] The diameter of the right atrium was measured in a right parasternal, long axis 4 chamber view across the midsection of the chamber parallel to the tricuspid annulus in the last frame before tricuspid valve opening.[26] A right atrium to left atrium ratio (RA : LA) was calculated as the long axis diameter of the right atrium to the long axis diameter of the left atrium. Right atrial enlargement was defined as RA : LA > 1. Enlargement of the right ventricle (RV) was subjectively characterized from a right parasternal long axis view as none, mild, moderate, or severe as previously described, with severe enlargement indicating the RV chamber was larger than the left.[27] The pattern of enlargement was subjectively characterized as concentric hypertrophy, eccentric hypertrophy, or both. The presence of ascites, pleural, or pericardial effusion was recorded. The presence of acquired and congenital heart disease was recorded. Degenerative mitral valve disease (DMVD) was classified based on the 2019 American College of Veterinary Internal Medicine Consensus Statement guidelines.[21]

2.3.3 | Electrocardiography

Paper or digital ECG recordings obtained at the time of diagnosis were reviewed and complexes measured in Lead II. Measurements made on sinus beats included durations of the P wave, PR interval, and QRS complex, as well as the amplitudes of the P and R waves. ECG variables were evaluated based on published reference ranges.[28] A description of the QRS complex morphology in Lead II was recorded. The presence of supraventricular and ventricular arrhythmias and conduction abnormalities, characterized as AVB, bundle branch block, and atrial standstill was recorded.[29] For the purposes of this study, an ECG abnormality included any of the following 3 categories: conduction abnormality, abnormal interval duration or amplitude measurement, or a rhythm diagnosis other than sinus rhythm, sinus arrhythmia, sinus tachycardia, and sinus bradycardia. Ventricular arrhythmias were assigned a modified Lown score as follows: 1 = single ventricular premature complexes, 2 = ventricular bigeminy or trigeminy, 3 = accelerated idioventricular rhythm, 4 = ventricular couplets or triplets, 5 = ventricular tachycardia or R-on-T phenomenon.[30-32] The highest grade observed was the grade assigned. Supraventricular arrhythmias were categorized as supraventricular premature complexes, supraventricular tachycardia, or atrial fibrillation.

Results from a contemporaneous ambulatory ECG (Holter) recording were recorded if performed. Data recorded included duration of analysis, mean heart rate, number of pauses >2.5 seconds, longest pause in seconds, the presence of supraventricular arrhythmias, ventricular arrhythmias and AVB. Ventricular and supraventricular arrhythmias were classified as described for the standard ECG.

2.3.4 | Outcome information

Survival time was calculated by determining the duration of time, in days, from the time of diagnosis to outcome (death) or censoring. Dogs that were alive at study end on January 1, 2018 were right censored. Follow-up data to document survival time and cause of death were obtained from the medical record or telephone interviews with owners and referring veterinarians. If death was confirmed, the cause of death was determined to be cardiac or noncardiac related. Dogs that died from a cardiac cause were subdivided into sudden cardiac death versus euthanasia. If no outcome could be determined, these dogs were determined to be lost to follow up and censored at the last known point of contact.

2.3.5 | Statistical analysis

Descriptive statistics were calculated. The Shapiro-Wilk test was used to verify normal distribution of variables. Age and weight were not normally distributed and thus were reported as median and range.

Variables investigated as potential risk factors for cardiac related death included weight (kg) and age at presentation, sex, LA : Ao in short axis, LVIDdN, LVIDsN, fractional shortening, ejection fraction, cTnI concentration, modified Lown score, QRS duration, AVB (absence or presence), RA : LA (none or enlarged ≥1), and RV enlargement (absence or presence). To ensure adequate observations in each group for analysis, the following variables were dichotomized: cTnI to <1.0 and ≥1.0, modified Lown score to <2 and ≥2, and QRS duration <0.07 seconds and ≥0.07 seconds. The cutoff for cTnI is clinically relevant as common acquired canine heart diseases rarely exceed this threshold and cases of severe confirmed myocarditis often exceed this threshold.[18,33] A modified Lown score ≥2 was selected to represent the clinical definition of complex ventricular arrhythmias.

A cardiac related death was considered the "event" and all other unrelated causes of death were right censored. To evaluate potential risk factors for death, data were imported into RStudio 1.0.136 software for survival analysis. Categorical variables were analyzed using the nonparametric Kaplan-Meier method and log-rank test with right censoring to calculate the median time to event and plot time to event curves. With some variables, survival was greater than 50% at the longest time point and median survival could not be calculated and was reported as undefined.

Univariate Cox regression analysis with right censoring was used to screen variables to determine whether there was an association with the event (cardiac related death). Variables with a $P \leq .10$ from the initial screening were included in a multivariable Cox proportional-hazards model. Variables were evaluated to determine if the proportional hazards assumption was met and multicollinearity was assessed. Backward stepwise elimination was performed. The variable with the highest P value was eliminated at each step until the final remaining variables in the model had P values <.05. During backwards selection the Cox models were compared by using the Bayesian Information Criteria and the Cox model with the lowest Bayesian Information Criteria was selected as the final exploratory model. After evaluating the results of the first multivariable model, a second multivariable Cox proportional-hazards model was generated to analyze results if the echocardiographic variables with a $P \leq .10$ at initial screening (LA : Ao short axis, RV enlargement) were not included in analysis. Similar to before, backward stepwise elimination was performed and variables removed until the final remaining variable(s) in the model had P values <.05. This was performed to further explore ECG abnormalities and elevated cTnI which are identified in Chagas disease and could be overshadowed by the echocardiographic parameters.

3 | RESULTS

A search of the Texas A&M University Veterinary Medical Teaching Hospital medical records between January 2010 and January 2018 resulted in 466 dogs with an IFA test. In total, 422 dogs were excluded because IFA results were negative (n = 411) or the titer was reported as 1 : 20 (n = 11). Forty-four of 466 dogs (9%) had an antibody titer ≥1 : 80 to *T. cruzi* and were included in analysis. Median IFA titer result

TABLE 1 Clinical characteristics and cardiac diagnostic test results in 44 dogs seropositive for *Trypanosoma cruzi*

Variable	N	Median [IQR] (range)
Age (years)	44	4.9 [1.8-8.5] (0.3-12.7)
Weight (kg)	44	22.5 [13.2-31.5] (4.9-83.4)
Sex	27 M (61%)	
	17 F (39%)	
cTnI (low/medium/high)	9/7/20	
Echocardiography	43	
LVIDdN	43/43 (100%)	1.61 [1.45-1.82] (1.04-2.63)
LVIDdN >1.85	8/43 (19%)	
LVIDsN	43/43 (100%)	1.09 [0.82-1.25] (0.58-2.05)
LVIDsN >1.26	3/43 (7%)	
FS (%)	43/43 (100%)	28.89 [21.28-39.02] (10.61-54.25)
FS <20%	8/43 (19%)	
EF (%)	42/43 (98%)	53.5 [45.0-65.0] (25.0-86.0)
EF <40%	5/42 (12%)	
LA:Ao short axis	43/43 (100%)	1.38 [1.20-1.67] (1.10-2.42)
LA:Ao short axis >1.57	12/43 (29%)	
LA:Ao long axis	37/43 (86%)	2.37 [2.01-2.79] (1.41-4.49)
LA:Ao long axis >2.4	18/37 (49%)	
RA:LA	41/43 (95%)	0.81 [0.70-0.99] (0.47-2.12)
RA:LA >1	10/41 (24%)	
RV enlargement	15/43 (35%)	
(none/mild/moderate/severe)	27/11/4/0	
Effusion	4/43 (9%)	
(ascites/pericardial/pleural)	3/1/0	
ECG	43	
P wave amplitude (mV)	38/43 (88%)[a]	0.20 [0.15-0.30] (0.10-0.40)
P wave amplitude >0.4 mV	0/38 (0%)	
P wave duration (seconds)	38/43 (88%)[a]	0.04 [0.04-0.04] (0.02-0.08)
P wave duration >0.04 seconds	4/38 (11%)	
PR duration (seconds)	25/43 (58%)[a,b]	0.10 [0.10-0.12] (0.06-0.16)
PR duration >0.13 seconds	3/25 (12%)	
QRS duration (seconds)	30/43 (70%)[b]	0.06 [0.05-0.08] (0.04-0.14)
QRS duration >0.07 seconds	8/30 (27%)	
R wave amplitude (mV)	27/43 (63%)[b,c]	1.0 [0.50-1.28] (0.1-1.9)
R wave amplitude <0.5 mV	7/27 (26%)	

(Continues)

TABLE 1 (Continued)

Variable	N	Median [IQR] (range)
Supraventricular arrhythmias	7/43 (16%)	
SVPC	2/7 (3%)	
SVT	2/7 (3%)	
AF	3/7 (4%)	
Conduction abnormalities	21/43 (49%)	
Atrial standstill	1/21 (5%)	
1AVB	3/21 (14%)	
BBB	8/21 (38%)	
3AVB	12/21 (57%)	
Ventricular arrhythmias	28/43 (65%)	
Modified Lown score 1/2/3/4/5	19/1/0/2/6	

Abbreviations: AF, atrial fibrillation; 1AVB, first degree atrioventricular block; 3AVB, third degree atrioventricular block; BBB, bundle branch block; EF, ejection fraction; FS, fractional shortening; LA, left atrium; LA : Ao, left atrium to aorta ratio; LVIDdN, left ventricular internal dimension at end-diastole normalized to body weight; LVIDsN, left ventricular internal dimension at end-systole normalized to body weight; RA:LA, right atrium to left atrium diameter in long axis ratio; RV, right ventricle; SVT, supraventricular tachycardia; SVPC, supraventricular premature complex.
[a]Unable to be measured in 4 dogs with atrial standstill or AF.
[b]Unable to be measured in 13 dogs with atrial standstill or 3AVB.
[c]Unable to be measured in 1 dog with a QS morphology in lead II.

was 1 : 160 (range, 1 : 80-1 : 1280). Clinical characteristics for the 44 dogs are reported in Table 1. Fifty percent of dogs in the study population were less than 5 years old (n = 22), and 27% were less than 2 years old (n = 12). Of the more than 30 breeds represented, Labrador retrievers were the most common breed (n = 5, 11%). No other breed was represented by more than 2 dogs. All of the American Kennel Club breed groups were represented, with the sporting group most common (12 of 44, 27%). Diet was recorded in 35 of 44 (80%) dogs.

Serum cTnI concentration was obtained in 36 of 44 dogs (82%) (Table 1). Eleven of 36 dogs (31%) had samples analyzed on the Immulite assay, 5 of which were below the lower detection limit. The median concentration for the remaining 6 dogs was 0.48 ng/mL (range, 0.31-28.20). Twenty-five of 36 dogs (69%) had samples analyzed on the ADVIA Centaur TnI-Ultra assay, 1 of which was below the lower detection limit. The median concentration in 24 dogs was 0.429 ng/mL (range, 0.006-13.3 ng/mL). Overall in the 36 dogs, 20 (56%) had a high cTnI concentration as defined in the methods and 10 (28%) had a cTnI concentration ≥1.0 ng/mL.

3.1 | Echocardiography

An echocardiogram was available for review in 43 of 44 dogs (98%) and select variables are reported in Table 1. Concurrent congenital (n = 4) or acquired (n = 9) heart disease was documented in 13 of 43 (30%). Congenital heart disease included ventricular septal defect (n = 2) and mild pulmonic stenosis (n = 3); 1 dog had both. The most common acquired heart disease was DMVD (n = 8) characterized as stage B1 (n = 7) and stage B2 (n = 1).[21]

TABLE 2 Nonparametric Kaplan-Meier method and log-rank test to evaluate associations between variables and survival in 42 dogs that were seropositive for *Trypanosoma cruzi*

	Total sampled	Nonsurvivors	Median survival (days)	*P*-value (log rank test)
Sex				.67
Female	17	9	850	
Male	25	10	Undefined	
Troponin I[a]				.75
<1 ng/mL	24	10	850	
≥1 ng/mL	10	4	Undefined	
Modified Lown score				.02
0-2	34	13	Undefined	
≥2	8	6	179	
AV block				.04
Present	14	9	541	
Absent	28	10	Undefined	
QRS[b]				.09
≥0.07 ms	8	6	298	
<0.07 ms	20	9	918	
RV[c]				.006
Enlargement	14	10	298	
No enlargement	26	9	Undefined	

Abbreviations: AV, atrioventricular; RV, right ventricle.
[a]Thirty-four dogs had troponin I results.
[b]Twenty-eight dogs had QRS width measurements.
[c]Forty dogs had echocardiographic images to evaluate RV enlargement.

Other acquired heart disease included heartworm disease (n = 1) in a dog with DMVD stage B1, a right auricular mass (n = 1), and a tricuspid valve thrombus or mass (n = 1). Twenty-eight of 43 (65%) had enlargement of at least 1 chamber. Chamber enlargement included the RV (36%), left atrium (29-49% depending on measurement), right atrium (24%), and left ventricle in diastole (19%) and systole (7%). Ten dogs had enlargement of both left and right chambers while only left or only right sided enlargement was present in 10 and 8 dogs, respectively. Left ventricular ejection fraction and fractional shortening were low in 12 to 19% of dogs, respectively. Six dogs with LVIDdN and LVIDsN greater than the 95% CI of a healthy dog reference cohort did not have diet history available while the remaining were fed traditional diets. Two of the 10 dogs with right atrial enlargement had concurrent right sided heart disease (heartworm disease in 1 dog, pulmonic stenosis in 1 dog). When present, RV enlargement was categorized as mild to moderate, and the pattern of enlargement was eccentric hypertrophy in all but 1 dog with concentric hypertrophy and pulmonic stenosis. Effusion (n = 4) was not identified in the dogs with concurrent heart disease associated with the right side of the heart (eg, right auricular mass, heartworm disease, pulmonic stenosis).

3.2 | Electrocardiography

An ECG was available for interpretation in 43 of 44 dogs (98%) consisting of a 6-lead ECG performed by the cardiology service (38 of 43) or for the remainder, a rhythm strip performed at admission. Measurements

made on sinus beats are reported in Table 1. One of the 4 dogs with a P wave duration >0.04 seconds had left atrial enlargement. The 3 dogs with a prolonged PR interval >0.13 seconds also had a prolonged QRS complex duration of 0.08 to 0.09 seconds. The 8 dogs with a QRS duration >0.07 seconds (range, 0.08-0.14 seconds) were large breeds (>20 kg), and the QRS complex morphology was characterized as predominately negative consistent with a right bundle branch block (n = 5), predominately positive consistent with a left bundle branch block (n = 1), isoelectric (n = 1), or splintered (n = 1). An additional dog had a splintered QRS complex of normal width. The R wave amplitude did not exceed the upper limits of normal for any dog. Five of the 7 dogs with R wave amplitude <0.5 mV had abnormal QRS complex morphology (wide and predominately negative or splintered) and none had pleural or pericardial effusion.

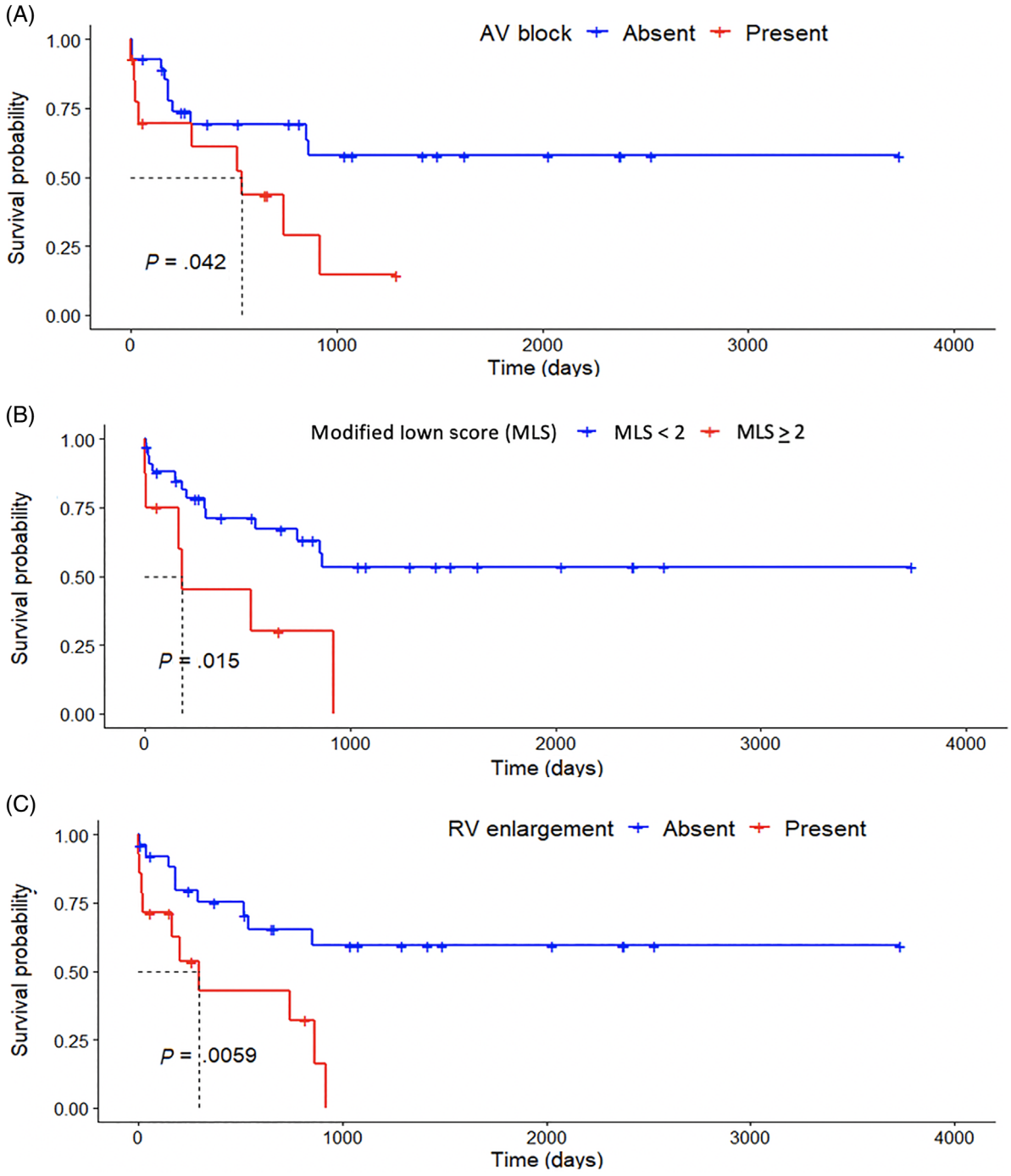

FIGURE 1 Kaplan-Meier survival curves for dogs seropositive for *Trypanosoma cruzi*. Each cross represents when a dog was censored and days are measured from first exam at a veterinary medical teaching hospital. Variables measured include, A, presence or absence of AV block, B, ventricular arrhythmias with modified Lown score (MLS) categorized as <2 or ≥2, and C, presence or absence of right ventricular (RV) enlargement. Only significant variables are shown

An ECG abnormality (eg, arrhythmia, conduction abnormality or abnormal complex measurement) was identified in 41 of 43 dogs (95%), and 14 of 43 dogs (33%) had more than 1 abnormality. The most common combination of abnormalities included conduction abnormalities, specifically AVB, and ventricular arrhythmias in 8 of 14 dogs (57%), supraventricular and ventricular arrhythmias in 5 of 14 dogs (36%), and supraventricular arrhythmias and conduction abnormalities in 1 of 14 dogs (7%).

An ambulatory ECG (Holter) was available for review in 12 of 44 dogs (28%), all of which had a concurrent ECG performed. The median length of time analyzed was 24 hours (range, 3.5-26 hours). One dog did not have any abnormalities detected with ambulatory ECG. Two of 12 dogs (2%) had numerous pauses more than 2.5 seconds (506, 2292 pauses). Eleven of 12 dogs (92%) had ventricular arrhythmias documented that were classified using a modified Lown score. Compared to the 6-lead ECG results, the modified Lown score based on ambulatory ECG results was the same (n = 4) or higher (n = 7). In 6 of the 7 dogs, the modified Lown score on the ambulatory ECG was ≥2 scores higher. Additionally, in 4 dogs ventricular (n = 2) and supraventricular (n = 2) arrhythmias were identified with ambulatory ECG that were not identified with a standard ECG.

3.3 | Outcomes

During the study period, out of 44 dogs, 19 events (cardiac-related deaths) were recorded, and 23 dogs were right censored including 11 dogs that were alive at study end, 5 dogs that were lost to follow up and 7 dogs that died for unrelated reasons (6 euthanized and 1 died for reasons unknown). Of the 5 dogs lost to follow up, 3 had been examined once by referring veterinarians after discharge from the teaching hospital, and that date was used for survival analysis. Two dogs were excluded from survival analysis because no outcome information was available beyond the first visit. The median survival time for all of the study dogs was 863 days after the first visit. Cardiac-related deaths were attributed to euthanasia (n = 14) and sudden death (n = 5).

3.3.1 | Identification of risk factors for death in seropositive dogs

When predictors of cardiac-related death were independently analyzed using nonparametric Kaplan-Meier, a modified Lown score ≥2 ($P = .02$), presence of AVB ($P = .04$), and presence of RV enlargement ($P = .006$) were significantly associated with reduced survival, while QRS duration ≥0.07 seconds ($P = .09$), cTnI concentration ≥1 ng/mL ($P = .75$) and sex ($P = .67$) were not associated with survival (Table 2, Figure 1). In the univariate Cox regression analysis, 14 variables were tested and 6 were associated with cardiac-related death ($P < .1$) including LA : Ao short axis, modified Lown score ≥2, QRS duration ≥0.07 seconds, weight (kg), presence of AVB, and RV enlargement were initially included in multivariable analysis. Backwards elimination was performed and multivariable analysis identified body weight and

TABLE 3 Results from univariate and multivariate Cox proportional hazard models on 42 dogs that were seropositive for *Trypanosoma cruzi* evaluated at a veterinary medical teaching hospital

	Univariate			Multivariate		
Variable	Hazard ratio	95% confidence interval of the hazard ratio	*P*-value (Likelihood ratio test)	Hazard ratio	95% confidence interval of the hazard ratio	*P*-value
Weight (kg)	1.02	1.00-1.05	.07	1.03	1.0-1.1	.04
Age at presentation (years)	0.99	0.88-1.12	.9			
LA : Ao (0.01 unit increment)	3.77	1.17-12.14	.04			
LVIDdN (0.01 unit increment)	2.06	0.47-9.00	.4			
LVIDsN (0.01 unit increment)	2.34	0.67-8.17	.2			
FS (%, 1 unit increment)	0.08	0.94-1.02	.3			
EF (%, 1 unit increment)	0.99	0.96-1.03	.6			
RA:LA (enlargement)	0.97	0.21-4.53	1			
Sex (male)	0.82	0.33-2.03	.7			
Troponin (>1 ng/mL)	0.83	0.26-2.64	.7			
Modified Lown score (≥2)	3.08	1.15-8.21	.04			
QRS duration (>0.07 ms)	2.45	0.85-7.01	.1			
AVB (presence)	2.50	1.00-6.24	.05			
RV (enlargement)	3.39	1.35-8.48	.01	3.6	1.4-9.3	.007

Abbreviations: AVB, atrioventricular block; EF, ejection fraction; FS, fractional shortening; LA : Ao, ratio of the left atrial diameter to the aortic valve diameter; LVIDdN, left ventricular internal dimension at end-diastole normalized to body weight; LVIDsN, left ventricular internal dimension at end-systole normalized to body weight; RA : LA, ratio of the right atrial diameter to the left atrial diameter; RV, right ventricle.

RV enlargement to be associated with survival (Table 3). The hazard of death was 3.6 times greater among dogs with RV enlargement compared to those without enlargement (HR 3.6; 95% CI 1.4-9.3; $P = .007$) and 1.0 times greater with each 1 kg increase in body weight (HR 1.0; 95% CI 1.0-1.1; $P = .04$). In the second multivariable Cox proportional-hazards model with echocardiographic variables excluded, the hazard of death was 3.2 times greater among dogs with ventricular arrhythmias with modified Lown score ≥2 (HR 3.2; 95% CI 1.2-8.5; $P = .02$).

4 | DISCUSSION

This study reports cardiac abnormalities and variables associated with an increased incidence of death in naturally infected dogs that were antibody positive for *T. cruzi* and that presented to a veterinary medical teaching hospital. Cardiac abnormalities in Chagas disease are attributed to damaged myocytes and the presence of inflammation, edema and scarring of the myocardium associated with the *T. cruzi* parasitic infection.[2,3,34] Electrocardiographic abnormalities were common in this group of dogs with 95% (41/43) of the dogs with an ECG performed having at least 1 abnormality identified. ECG abnormalities are frequently described with *T. cruzi* infection in dogs and humans, and arrhythmias are the most common reason dogs are tested for Chagas disease.[1,20,34] In this group of dogs, ventricular arrhythmias were documented most often, occurring in 65% of the dogs with 44% categorized as modified Lown score 1 (single ventricular premature beats) and 14% as a modified Lown score 5 (ventricular tachycardia). A higher Lown score is associated with an increased risk of sudden cardiac death and a greater need for antiarrhythmic therapy in Boxers with arrhythmic right ventricular cardiomyopathy.[31] This was also suggested in our study in which we found a modified Lown score ≥2 was associated with shorter survival times based on univariate Kaplan Meier analysis of our study population ($P = .02$), although it was not significant in the multivariate model unless echocardiographic variables were excluded. Though only a subset of dogs with a standard ECG had an ambulatory ECG performed (n = 12), in some dogs the modified Lown score was higher and additional arrhythmias were detected with ambulatory ECG that were not present on standard ECG. Therefore, the additional information gained from the ambulatory ECG recording could provide useful clinical information and impact recommendations for antiarrhythmic therapy and monitoring.

Changes to the ECG complex described in dogs with Chagas disease include decreased R wave amplitude, prolonged duration of the P wave and PR interval as well as axis shifts and bundle branch block characterized as a prolonged QRS duration.[5,6,12,13,35] While decreased R wave amplitude has been reported to occur at day 20 postinfection in dogs experimentally infected with *T. cruzi* and was described in 2 *T. cruzi* positive dogs with concurrent pericardial effusion,[4,12] it can also be identified with abnormal QRS morphologies including right bundle branch block and splintered complexes as observed in dogs in this study. Prolongation of the P wave, PR interval and QRS complex duration indicative of delayed conduction were documented in dogs in this study with and without cardiac enlargement. Direct damage, fibrosis, and scarring of the conduction system have been described as the cause of conduction abnormalities including AVB in *T. cruzi* infected dogs.[2-4,13] Atrioventricular block was the most common conduction abnormality found in our study population and 64% of dogs with AVB were nonsurvivors from cardiac-related death compared to 36% of dogs without AVB ($P = .04$), although this was not significant in multivariate analysis. Electrocardiographic abnormalities are consistently encountered with *T. cruzi* infection and the lack of significance in multivariate analysis in this retrospective study could be related to the number of dogs included.

Echocardiographic abnormalities described with *T. cruzi* infection include chamber enlargement and ventricular myocardial dysfunction.[5,11,12] In humans with Chagas disease, diastolic dysfunction, left atrial enlargement, left and right ventricular systolic dysfunction and ventricular tachycardia are associated with a higher risk of cardiac mortality.[36] In experimental infections in dogs, a reduction in left ventricular fractional shortening occurred over time and the degree of reduction was variable and included hypokinetic wall motion.[5,11] Both right and left ventricular enlargement were reported equally in a subset of naturally infected dogs in Mexico.[15] Additionally, RV enlargement and left ventricular systolic dysfunction were reported in a retrospective study of naturally infected dogs in Texas.[12] The variation in cardiac abnormalities between dogs could be explained in part by the biologic behavior of the *T. cruzi* organism which can display a tissue tropism to myocardial cells that can vary with host species and *T. cruzi* strain type.[37] Additionally, the role of repeated exposure to infected kissing bugs and how reinfection affects the heart in dogs has not been well established. In our study, enlargement of at least 1 cardiac chamber was present in 63% of dogs and was complicated by concurrent heart disease in a subset of dogs. Left ventricular size and indices of systolic function, while present, were not significantly associated with survival. Right ventricular enlargement was most common, and was classified as mild to moderate eccentric hypertrophy when present. The presence of RV enlargement was associated with a 3.6 times greater risk of death in multivariable analysis (HR 3.6; 95% confidence interval [CI] 1.4-9.3; $P = .007$). However, heart enlargement and arrhythmias are frequently interrelated in many diseases and arrhythmias are commonly identified in Chagas disease.[5,30,38] When echocardiographic criteria including RV enlargement were removed from the multivariable analysis in our study, the presence of ventricular arrhythmias with modified Lown score ≥2 was associated with 3.2 times greater risk of death (HR 3.2; CI 1.2-8.5; $P = .02$). This suggests arrhythmias remain an important component of Chagas disease but also suggests the presence of RV enlargement indicates a more advanced disease process and a higher risk of death.

Elevated cTnI concentration serves as an indicator of myocardial damage and elevations are reported in dogs infected with *T. cruzi*.[17,39] The highest reported cTnI concentration in a validation study of healthy dogs using the ADVIA Centaur TnI-Ultra assay was 0.128 ng/mL.[19] The median concentration in our study population was 0.40 ng/mL, indicating myocardial damage was likely present in the majority of dogs that had cTnI measured. It is important to note that while *T. cruzi*

induced myocyte damage is a potential cause of elevated cTnI concentration, additional factors including other infectious or acquired heart diseases and age have all been shown to increase cTnI.[17] Although cTnI concentration was not significantly associated with survival in this population ($P = .07$), of the dogs that had a cTnI ≥1 ng/mL, 40% were nonsurvivors from cardiac-related death.

Dogs in this study were predominately male (61%) and included many breeds with the majority representing the American Kennel Club sporting breed group similar to previous report.[40] In statistical analysis, cardiac-related death was associated with higher body weights. We attributed this to the representation of sporting breeds in this study that can be exposed to *T. cruzi* infection based on environment or lifestyle and do not believe that large dogs or specific breeds are more predisposed to death from Chagas disease. Age of infected dogs was not significantly associated with survival ($P = .9$) and was highly variable, with both young and old dogs affected. Acquired heart disease, in particular DMVD, was detected in dogs in this study and often affects dogs older than 5 years of age,[41] while 70% of deceased dogs in our study were less than 5 years of age. These findings suggest *T. cruzi* infection was a primary cause of death rather than acquired heart disease.

Infection timing was not specifically determined in this study. In the acute and chronic stage of the infection, dogs can be asymptomatic or have evidence of clinical disease.[1,4] Acute infection can be associated with severe myocardial inflammation with a shift to replacement fibrosis and less severe inflammation in the chronic stage of the disease.[2,3,34] The cardiac manifestations of *T. cruzi* infection result in arrhythmias, heart enlargement and myocardial dysfunction throughout the disease process. In experimental infections in dogs, the most severe clinical signs occurred in the youngest dogs infected (<33 days after inoculation) and affected both ventricles.[4] Dogs that survived the acute infection entered a latent, asymptomatic stage, and 2 of the 5 dogs developed clinical signs associated with heart failure in the chronic stage of the disease.

Limitations of this retrospective case series include missing data points and potential selection bias associated with being evaluated at a veterinary medical teaching hospital with a clinical indication for testing for *T. cruzi* and owners approved testing. Additionally, treatment was selected based on clinician preference and owner approval and was not standardized. However, the study provides cardiac diagnostic test information and clinical outcomes in dogs naturally infected with *T. cruzi*. Echocardiography has evolved over the years to include more specific analysis of heart size and function and assessment in this study was limited to those images that were available. Furthermore, the small sample size did not allow for complete survival analysis of categorical variables.

In summary, ECG and echocardiographic abnormalities and elevated cTnI concentration were common but variable in this group of dogs highlighting the complicated nature of this challenging disease. Ventricular arrhythmias and AVB were documented most often and ambulatory ECG (Holter) monitoring was more sensitive than a resting ECG for detection and grading of severity of arrythmias. Right ventricular enlargement, in particular, was associated with an increased risk of cardiac-related death. Therefore, a diagnostic evaluation that includes screening for arrhythmias, echocardiography, and cTnI can provide further characterization of the disease process in dogs naturally infected with *T. cruzi*.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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