JAVMA—18-09-0496—Madigan—1 fig—4 tab—LJP—RES

Investigation of a combination of amiodarone and itraconazole for treatment of American trypanosomiasis (Chagas disease) in dogs

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OBJECTIVE
To evaluate clinical, serologic, parasitological, and histologic outcomes of dogs with naturally occurring *Trypanosoma cruzi* infection treated for 12 months with amiodarone and itraconazole.

ANIMALS
121 dogs from southern Texas and southern Louisiana.

PROCEDURES
Treatment group dogs (n = 105) received a combination of amiodarone hydrochloride (approx 7.5 mg/kg [3.4 mg/lb], PO, q 24 h, with or without or a loading dosage protocol) and itraconazole (approx 10 mg/kg [4.5 mg/lb], PO, q 24 h, adjusted to maintain a plasma concentration of 1 to 2 µg/mL) for 12 months. Control group dogs (n = 16) received no antitrypanosomal medications. Serologic assays for anti–*T cruzi* antibodies, PCR assays for *T cruzi* DNA in blood, and physical evaluations were performed 1, 6, 9, 12, and 24 months after study initiation (baseline). Adverse events were recorded. Outcomes of interest were recorded and compared between groups.

RESULTS
86 of 105 treatment group dogs and 8 of 16 control group dogs survived and completed the study (5/19 and 6/7 deaths of treatment and control group dogs, respectively, were attributed to *T cruzi* infection). Mean survival time until death attributed to *T cruzi* was longer (23.19 vs 15.64 months) for the treatment group. Results of PCR assays were negative for all (n = 92) tested treatment group dogs (except for 1 dog at 1 time point) from 6 to 24 months after baseline. Clinical improvement in ≥ 1 clinical sign was observed in 53 of 54 and 0 of 10 treatment and control group dogs, respectively; adverse drug events were minor and reversible.

CONCLUSIONS AND CLINICAL RELEVANCE
Results suggested efficacy of this trypanocidal drug combination for the treatment of *T cruzi* infection in dogs. (*J Am Vet Med Assoc* 2019;255:xxx–xxx)

American trypanosomiasis, commonly known as Chagas disease, is a parasitic condition caused by the hemoflagellated protozoa, *Trypanosoma cruzi*.[1,2] Active transmission is through triatomine vectors known as kissing bugs, which are prevalent in North and South America.[3] Defecation by infected insects during or after a blood meal passes infective trypomastigotes into the bite wound.[3] Other routes of infection include transplacental transmission to offspring of infected pregnant women and bitches, oral ingestion (including consumption of infected meat), breastfeeding, and blood transfusion.[3–5] After trypomastigotes enter the circulation, there is a brief period of replication and hematogenous spread through mononuclear cells, with the final destination being various tissues in the host, including the heart, fat, and the lymphatic system, where they reside and multiply as intracellular amastigotes.[3]

A century after its discovery, *T cruzi* infection remains a serious health problem. It is a leading cause of cardiomyopathy that affects > 8 million people worldwide.[1] It is predicted that Chagas disease will cause at least 200,000 human deaths globally over the next 5 years, which is identical to the number of women living in the United States predicted to die of breast cancer in the same time period.[2,6]

ABBREVIATIONS

DTU	Discrete typing unit
IFA	Immunofluorescence antibody test
MANCOVA	Multivariate ANCOVA

Whereas the highest prevalence of cardiomyopathy due to Chagas disease in people is found in Central and South American countries, studies[7–9] show a widespread distribution of American trypanosomiasis in dogs in Texas, Louisiana, and Georgia and indicate that these southern states are the sites of important reservoirs for transmission of *Trypanosoma cruzi* in the United States. The prevalence of *T cruzi* infection in stray and shelter-housed dogs has been reported to be as high as 7.5% to 8.8% and may exceed 50% in some working dog kennels in Texas.[10,11] On the basis of a 2012 AVMA estimate[12] of the canine population in Texas, these findings suggest there could be approximately 630,000 dogs with *T cruzi* infection in that state alone.[9,10] A similar prevalence of *T cruzi* infection (ie, > 50% in hunting dogs in the region and 22% in its general canine population) has been reported in southern Louisiana.[7]

The pattern of disease progression in dogs with American trypanosomiasis is similar to that in people, which comprises acute, chronic asymptomatic, and chronic symptomatic phases.[13–15] Acute disease occurs within 3 weeks after inoculation and centers around myocarditis with severe inflammation following the death of cardiac cells.[16,17] Most affected dogs are < 1 year of age, and clinical signs commonly include tachyarrhythmias, respiratory distress, lymphadenopathy, anorexia, diarrhea, neurologic abnormalities, peripheral edema, collapse, and sudden death.[13–15] Parasitemia is profound and the mortality rate is high, especially among < 1-year-old dogs.[13,14]

A chronic phase without overt clinical signs, often described in the literature as the chronic asymptomatic phase, is most commonly encountered in dogs.[14,18] This phase is characterized by a slowly progressing, fibrosing inflammatory process in the heart and variable shedding of the organism into the circulation.[14,18] Although sudden death is a possibility at this stage, particularly as a result of fatal exercise-induced or excitement-induced arrhythmias, most signs such as mild lethargy and cardiac conduction disturbances are clinically subtle and can be overlooked by the owner.[14,18,19]

The proportion of cases of *T cruzi* infection that progress to a chronic phase with overt clinical signs in dogs is unknown; however, approximately 30% of cases progress to the chronic symptomatic stage in human patients.[3,17] Electrocardiographic abnormalities were found in 5 of 16 (31%) infected dogs in experimental studies[14,19]; these findings were mostly attributed to inflammation and fibrosis in the electrical pathways but also to selective destruction of neurons causing parasympathetic denervation in the heart. Also common in dogs during this phase are bilateral cardiac dilatation with associated pulmonary edema, pericardial effusion, and ascites; secondary mitral or tricuspid valve insufficiency; hepatosplenomegaly; and exercise intolerance and lethargy.[14,19] It is important to note that the clinical signs of cardiomyopathy in dogs that have chronic, clinically evident *T cruzi* infection are the same as those found for a number of other common heart abnormalities, including dilated cardiomyopathy and chronic valvular disease.[20] Therefore, cardiomyopathy attributable to American trypanosomiasis may be difficult to differentiate from that resulting from other causes, especially in breeds that are predisposed to these types of acquired heart dysfunction.[20]

Information on survival time in chronically infected untreated dogs is sparse. However, there is likely to be a distinct difference in prognosis according to age, in view of 1 study[21] in which investigators found dogs identified as having *T cruzi* infection at a mean age of 9 years survived for 30 to 60 months, whereas those that had the diagnosis made at a younger age (mean, 4.5 years) survived for a maximum of 5 months after diagnosis.

There are presently no drugs approved by the US FDA for the treatment of *T cruzi* infection in dogs. Currently available specific anti–*T cruzi* chemotherapeutic agents have unsatisfactory results in people and in dogs.[22,23] Much consideration has been given to nitroimidazole- and nitrofuran-derived drugs, such benznidazole and nifurtimox, which were developed in the 1960s and 1970s[24–27] and are not presently commercially available in the United States. These compounds act by generation of free radicals and damage the DNA of the parasite[28] but have several major limitations including lack of efficacy in 80% to 100% of patients in the chronic phase of the disease, inability to prevent progression of chronic cardiomyopathy in human patients with the disease,[23,27,28] and an intrinsic resistance to benznidazole in some major strains of *T cruzi*.[29,30]

Many compounds with proven trypanocidal activity are currently under development or being tested.[24,31,32] Among these are new nitroimidazole derivatives such as fexinidazole; ergosterol biosynthesis inhibitors; and experimental drugs such as cysteine proteases and inhibitors of trypanothione metabolism, kinetoplastid proteasomes, polyphosphate metabolism, and purine synthesis as well as oxaborole, fenarimol, and amidine analogs and derivatives.[32] However, very few of these drugs, such as allopurinol and the azole derivatives itraconazole, fluconazole, ketoconazole, posaconazole, and ravuconazole, have been tested in human clinical trials.[32]

Antifungal azoles work by disrupting the pathways for biosynthesis of sterols,[33] and because *T cruzi* and fungi share similar pathways for biosynthesis of sterols, antifungal azoles have been used as an alternative treatment for Chagas disease in human patients.[34–36] A cure rate of 33% was observed for itraconazole-treated human patients during long-term follow-up,[32,37] and lower cure rates of 8% to 31% have been reported in patient groups treated with newer azoles, such as posaconazole and ravuconazole.[35,38]

A human patient in Venezuela was cured of Chagas disease after receiving a combination of itraconazole and amiodarone.[36] These 2 drugs were used to

target specific metabolic pathways of the parasite related to synthesis of ergosterol and intracellular calcium modulation.[39,40] Amiodarone is an antiarrhythmic agent and the drug of choice for prevention of complex arrhythmias in people with cardiomyopathy caused by *T cruzi* infection.[36,40,41] This agent selectively disrupts calcium homeostasis in *T cruzi*,[39,42] blocks the critical protease cruzipain,[43] and inhibits production of sterols.[39] We became interested in the potential synergy that could be achieved by combining amiodarone with itraconazole to capitalize on the ability of these drugs to inhibit sterol synthesis at different steps in the ergosterol pathway and to disrupt calcium homeostasis in the parasite. The purpose of the study reported here was to evaluate the clinical, serologic, parasitological, and histopathologic outcomes of dogs naturally infected with *T cruzi* and treated with a combination of itraconazole and amiodarone for 12 months. We hypothesized that the combination of amiodarone and itraconazole would eliminate *T cruzi* parasitemia and improve clinical outcomes in these dogs.

Materials and Methods

Animals and study design

A convenience sample of client- or government-owned dogs from southern Texas and southern Louisiana that were naturally infected with *T cruzi* were enrolled in the multicenter, prospective, controlled study between October 12, 2009, and December 7, 2017. The client-owned dogs were patients at the first author's hospital and any of 11 referring hospitals. Owners were informed of their dogs' positive anti–*T cruzi* antibody status shortly after diagnosis, and the dogs were randomly assigned to treatment or control groups after owner consent was provided for study participation. Government-owned dogs were from 1 military base in Texas, and inclusion of these dogs in the study was approved by the director of the Military Working Dog program. The study protocol was approved by the Department of Defense Military Working Dog Facility animal use committee at Joint Base San Antonio-Lackland for use with government-owned dogs, and the same protocol was distributed and followed for privately owned dogs. Veterinarians at each participating facility were given the study protocol to execute. All data from referring practices and institutions were received and collated via electronic records transfer and telephone communications. The inclusion criteria were ≥ 1 positive IFA test result and 1 positive result for antibody titers (≥ 1:20) by ELISA for *T cruzi*. Pregnant bitches were excluded because of concerns that treatment could potentially be teratogenic. Dogs that received compounded itraconazole during the study were excluded from all analyses because of bioavailability concerns.[44] Demographic data (age, sex, breed, and primary housing type [indoor vs outdoor]) as well as a detailed medical history were recorded for all dogs. Dogs were assigned to the treatment and control groups by simple randomization.[a] Group allocation allowed for statistical comparison between groups while minimizing the number of dogs that did not receive treatment during the study.

Treatment group—All treated dogs received amiodarone hydrochloride[b,c] by 1 of 2 protocols. The treatment was supplied as scored 200-mg tablets to approximate the calculated dose to the nearest quarter tablet. One protocol included a loading dosage of 15 mg/kg [6.8 mg/lb], PO, every 12 hours for 7 days, followed by 15 mg/kg, PO, every 24 hours for 14 days, and then a maintenance dosage of 7.5 mg/kg [3.4 mg/lb], PO, every 24 hours for the remainder of the treatment period (total, 12 months). The other protocol was a maintenance dosage of 7.5 mg/kg, PO, every 24 hours throughout the treatment period, which was given to the military working dogs because of attending clinician concerns about potential proarrhythmic effects of a higher initial dosing regimen. The 7.5-mg/kg dose of amiodarone was halved if adverse events occurred and were thought to be related to the drug.

Itraconazole[d,e] was started on the same day as the first amiodarone treatment at a dosage of 10 mg/kg (4.5 mg/lb), PO, every 24 hours. The drug was provided as 100-mg capsules and rounded down to the nearest whole capsule, or in cases where a revised protocol was required because of low body weight, itraconazole was given at approximately 10 mg/kg, PO, every 48 hours. The dosage for each dog was adjusted to maintain a plasma itraconazole concentration of 1 to 2 µg/mL following sample collection and testing 30 to 45 days after initiation of treatment when steady state had been achieved. Plasma itraconazole concentrations were reassessed as needed for patients considered to have suboptimal circulating drug concentrations.

A complete physical examination and blood sample collection for a CBC, serum biochemical analysis, PCR assay for *T cruzi* DNA, and serologic (IFA) testing for antibodies against the organism were performed at the start of the study, immediately prior to initiation of the drug treatments (ie, baseline). An ECG was obtained and echocardiography was performed prior to treatment initiation and during follow-up when deemed appropriate by the attending clinician and authorized by the clients (for privately owned dogs). Radiographs were evaluated for patients that showed clinical signs of heart disease (if authorized by the client for privately owned dogs) prior to and on completion of the study. Follow-up visits during the treatment period took place 1, 6, 9, and 12 months after the start of drug administration. These dogs underwent complete physical examination and blood sample collection (5 mL) at all follow-up visits; a CBC, serum biochemical analysis, and measurement of plasma itraconazole concentrations were performed at the 1-month follow-up visit, and PCR assay and serologic testing (by IFA) were performed at subsequent visits. At each visit, the owners or handlers were asked whether the dogs had experienced

any clinical signs possibly associated with *T cruzi* infection, such as weakness, vomiting, diarrhea, abdominal distention, collapse, or prolonged periods of hyporexia or anorexia.[9,21] Owners or handlers were instructed to monitor their dog's appetite and behavior and to report any episodes of excessive panting or weight loss in addition to the aforementioned signs. When adverse events plausibly attributable to treatment were identified, drug doses were modified (by adjustment of itraconazole dosage on the basis of plasma drug concentrations greater than the targeted therapeutic range or, if itraconazole concentrations were deemed appropriate, adjustment of the amiodarone dosage).

Control group—Control group dogs underwent physical assessments in the same manner as treatment group dogs at the start of the study (baseline) and during treatment phase follow-up visits 1, 6, 9, and 12 months later. Follow-up information was obtained from owners and handlers, and monitoring instructions were given as for the treatment group. A CBC and serum biochemical analysis were performed at the start of the study and at the 1-month follow-up visit. Molecular assessment for parasitemia (by PCR assay) and serologic evaluation (by IFA) were performed as described for the treatment group at the time of diagnosis; PCR assays for the control group were also performed at 6, 9, and 12 months.

Posttreatment follow-up and study end points—All surviving dogs in both groups underwent complete physical examination and blood sample collection (5 mL) for PCR assay and IFA 12 months after the treatment ended (ie, 24 months after enrollment). Dogs that did not complete the study were also evaluated and had PCR assay and IFA performed at their last follow-up visit if possible. Echocardiographic and ECG evaluations were performed for dogs that had relevant physical examination abnormalities when deemed necessary by the attending veterinarian and authorized by the client (for privately owned dogs). Necropsy was performed on deceased dogs of the treatment group if possible. The study end points were clinician-reported outcomes (including subjectively assessed improvement, persistence, or worsening of clinical signs assessed from veterinary clinical observation) and indications of elimination of *T cruzi* from the peripheral blood, including negative results of PCR assay analysis, improvement in clinical signs with or without seroconversion and normalization of tissues on histologic examination, and differences in survival between treatment and control group dogs. Dogs that had clinical improvement were defined as those that had improvement in ≥ 1 assessed physical examination observation (eg, appetite, activity level, or ascites) by the time of last follow-up. Death or euthanasia attributable to *T cruzi* infection was defined as either event associated with cardiomyopathy caused by the disease, including death attributable to arrhythmia in a dog with this condition, euthanasia of a dog that failed to respond to the study treatment, or euthanasia because of quality of life concerns.

Hematologic tests for *T cruzi*

PCR assays—Blood samples (5 µL) were used for PCR assays targeting *T cruzi* kinetoplast minicircle DNA and *T cruzi* satellite DNA. The PCR assay methods (**Supplementary Appendix S1**, available at avmajournals.avma.org/doi/suppl/10.2460/javma.255.3.xxx) were performed as previously described[36,45] by study authors (CLZ and AEP-M) at VRL Laboratories, San Antonio, Tex, or Laboratorio de Enzimología de Parasitos, Universidad de Los Andes, Mérida, Venezuela. Blood samples were collected into EDTA-containing tubes and shipped and processed immediately or kept at 4°C for ≤ 1 week. For samples stored for durations > 1 week, guanidine buffer (guanidine hydrochloride; 6M; pH, 8.0; with 0.2mM EDTA) was added to blood samples (1:1 [vol:vol]), and the sample was stored in liquid nitrogen or at −80°C until analysis. Genomic DNA was extracted with a commercial kit,[f] and the PCR assays[g] were performed with commercially prepared primers[h] (for sequences proposed by Schijman et al[46]), buffer solution,[i] and a Taq polymerase product designed for enhanced amplification.[j] Detection of both targets was considered a positive result.

Serologic tests—Serum samples were obtained from whole blood with hospital-specific protocols at multiple facilities and transported (on ice in refrigerated conditions of 1°C) to a university[k] or Department of Defense[l] diagnostic laboratory to be analyzed for antibodies against *T cruzi* by IFA. Multiple dilutions were performed on all samples, and a positive result was defined as a titer ≥ 1:20. Substrate slides of whole *T cruzi*[m] and anti-dog IgG fluorescence-labeled secondary antibodies[n] were used for the IFA procedures. Indirect immunofluorescence assays were performed as previously described.[36] Confirmatory ELISAs for *T cruzi* were performed as previously described[36] at Laboratorio de Enzimología de Parasitos, Universidad de Los Andes, Mérida, Venezuela.

Other evaluations—Complete blood counts and serum biochemical analyses were performed at various commercial veterinary laboratories or in house at various hospitals. Plasma was obtained from peripheral blood samples according to hospital-specific protocols for measurement of itraconazole concentrations in dogs of the treatment group as described. Samples were stored and shipped with ice packs under refrigeration at 1°C for measurement of itraconazole concentrations at a veterinary clinical pharmacology laboratory.[o]

Echocardiography was performed in primary treatment locations by veterinarians aware of the treatment group assignment of the dogs; the equipment used varied by facility. Ventricular free wall, chamber size, and septal thickness measurements as well as fractional shortening were recorded. A standard lead II ECG tracing was recorded by veterinary staff using an ECG unit for ≥ 5 minutes. Two-view right lateral and ventrodorsal thoracic radiographic

views were also obtained in primary treatment locations when applicable. The echocardiography and ECG interpretations were read and summarized by 1 author (RM) and additionally classified as normal or abnormal for data analysis purposes.

Examination of heart tissue for dogs of the treatment group that died or were euthanized during the study was performed at 1 of 2 pathology laboratories.[p,q] Complete necropsy was performed when possible. Heart tissue was examined by light microscopic evaluation of H&E-stained slides. Areas involving the interventricular septum, apex, and both free walls of the heart were examined for inflammation, fibrosis, and presence of amastigotes.

Statistical analysis

The proportions of dogs that had clinical improvement by the end of the study and the proportion of deaths attributable to *T cruzi* infection as well as baseline and 24-month (or last available) follow-up clinical and biological results (frequencies of clinical signs attributable to *T cruzi* infection, normal vs abnormal ECG and echocardiographic findings, positive and negative IFA results, and decreased antibody titers during the study period) were compared between the treatment and control groups. Results of PCR assays (positive vs negative) were compared between groups at baseline and at each follow-up visit. Analysis of clinical improvement by the 24-month or last follow-up visit (yes vs no) included dogs that began the study with clinical signs and dogs that developed such signs during the study; findings at the last available follow-up were used for dogs that died or were euthanized before the end of the study. Given the small sample size of the control group, we used nonparametric comparisons, with Fisher exact and χ^2 tests for tabular analysis as appropriate for expected cell sizes and 2-sample Wilcoxon rank sum (Mann-Whitney) tests for continuous variables. Repeated-measures MANCOVAs were performed to determine the impact of combined amiodarone and itraconazole treatment on multiple dependent variables (ECG [abnormal vs normal result], IFA [positive vs negative results and antibody titers], and echocardiography [abnormal vs normal result]) measured at multiple time points. The analysis included between-group and within-group effects. Only the ECG, IFA, and echocardiography data were included as dependent variables in this analysis; age, sex, breed, and housing type (indoor vs outdoor) of the dogs were covariates controlled for in these analyses. Normal distribution of antibody titer data was confirmed by use of the Shapiro-Wilk test. Values of $P < 0.05$ were considered significant for all analyses.

Kaplan-Meier survival analysis was conducted to determine survival distributions of the dogs that died of *T cruzi* infection in each group. The duration of observation to event (death) was 0 to 24 months, which was the duration of the study. This analysis was performed to determine whether the incidence of death attributed to *T cruzi* infection was greater for dogs of the control group than for dogs of the treatment group. The log-rank (Mantel-Cox) test was used to determine whether there was significant difference in the overall survival distributions of dogs that died of *T cruzi* infection between groups. All analyses were performed with statistical software.[r]

Results

One hundred twenty-one dogs were enrolled in the study (105 [16 government-owned and 89 privately owned] in the treatment group and 16 [4 government-owned and 12 privately owned] in the control group). The initial enrollment included 125 dogs; however, 4 were excluded because they received compounded itraconazole. Twenty breeds and 1 mixed breed were represented, and the median age was 4.93 years (range, 5 days to 14 years). There were 63 males (27 castrated and 36 sexually intact) and 58 females (33 spayed and 25 sexually intact). In the control group, the number of dogs housed outdoors versus indoors was the same (8/16 for each housing type), whereas in the treatment group, 69 of 105 (66%) dogs were housed outdoors and 36 (34%) were housed indoors.

Most (89/105 [85%]) dogs in the treatment group had amiodarone administered according to the loading dose protocol before receiving the maintenance dose. The remaining 16 (15%) dogs in this group (all military working dogs) did not receive a loading dose prior to the maintenance dose. Three dogs received itraconazole at approximately 10 mg/kg, PO, every 48 hours (instead of every 24 hours) because of low body weight.

Eighty-six (82%) dogs in the treatment group completed the study, and 19 (18%) died before the 24-month follow-up. The mean treatment period for dogs of the treatment group that did not complete the study was 138 days (median, 145 days; range, 30 to 280 days). Eight of 16 dogs in the control group finished the study; 7 died, and 1 was lost to follow-up 60 days after starting the study. The study completion rates for the 2 groups were similar ($P = 0.47$). The proportion of dogs that died was significantly ($P < 0.001$) lower in the treatment group than in the control group (19/105 vs 7/15).

Five of 105 (5%) dogs in the treatment group had deaths attributed to *T cruzi* infection by the 24-month follow-up; 1 was euthanized, and 4 acute deaths occurred in a kennel or at home during the treatment period. In contrast, 6 of 15 dogs in the control group had deaths attributed to the disease during the same period; 2 were euthanized and 4 died acutely. The intergroup difference in rates of death attributable to the disease was significant ($P = 0.003$).

At the start of the study, the proportions of dogs with the most commonly reported clinical signs (lethargy, ascites, hyporexia or anorexia, and excessive panting or coughing) did not differ between the treatment and control groups **(Table 1)**. However, with the exception of ascites, the proportions of dogs

Table 1—Presence of common clinical signs attributed to *Trypanosoma cruzi* infection (American trypanosomiasis [commonly called Chagas disease]) for 121 naturally infected dogs in a study to evaluate the clinical, serologic, parasitological, and histopathologic outcomes following treatment with amiodarone and itraconazole.

	Baseline			Last follow-up		
Variable	Treatment (n = 105)	Control (n = 16)	*P* value	Treatment (n = 105)	Control (n = 15)	*P* value
Lethargy	28 (27)	1 (6)	0.116	2 (2)	6 (40)	< 0.001
Ascites	10 (10)	0 (0)	0.35	4 (4)	1 (7)	0.49
Hyporexia or anorexia	21 (20)	1 (6)	0.29	3 (3)	5 (33)	0.001
Excessive panting or coughing	12 (11)	0 (0)	0.36	0 (0)	5 (33)	< 0.001

Dogs of the treatment group received amiodarone hydrochloride and itraconazole for 12 months. Dogs of the control group did not receive antitrypanosomal medications. Last follow-up data represent findings for dogs 24 months after the start of the study (n = 86 in the treatment group and 8 in the control group) or at the last follow-up visit for dogs that did not survive to the 24-month time point (19 and 7 in the treatment and control groups, respectively). Data are shown as number (%) of dogs in the group; some dogs had multiple clinical signs. One dog in the control group was lost to follow-up during the study period. The *P* value reflects comparison between groups for the time point as determined by the χ^2 or Fisher exact test; values of $P < 0.05$ were considered significant.

See Figure 1 for drug administration details.

that had these clinical signs were significantly greater for the control group than for the treatment group by the end of the study.

Fifty-four of 105 (51%) dogs in the treatment group had clinical signs attributed to *T cruzi* infection at the initial evaluation, and only 1 of 54 (2%) failed to show improvement or resolution of clinical signs. For the control group, 1 of 16 dogs had clinical signs at the start of the study, and 10 had clinical signs consistent with disease progression at the end of the study. By the end of the 24-month follow-up period (or the last evaluation for dogs that did not survive to 24 months), a significantly ($P < 0.001$) greater proportion of dogs in the treatment group had improvement in ≥ 1 clinical sign (lethargy, hyporexia or anorexia, excessive panting, and ascites; 53/54 [98%]), compared with the control group (0/10 [0%]).

Adverse events

Fifteen of 105 (14%) dogs in the treatment group had adverse events attributable to the treatments or treatment combination. Recorded events included lethargy (n = 2), gastrointestinal signs (inappetence, vomiting, and diarrhea [alone or in combination [7]), weight loss (3), cutaneous eruptions (2), and serum concentrations of alanine transaminase above the reference range (10). Eleven of these 15 dogs had high plasma concentrations of itraconazole (≥ 3 µg/mL). The itraconazole dose was reduced by 33% to 50% to achieve plasma concentrations in the range of 1 to 2 µg/mL for these dogs, and no further adverse events occurred following dose adjustment. Halving the amiodarone dose ameliorated derangements in liver function tests, clinical signs of lethargy and inappetence, or both in the remaining 4 dogs. All dogs tested had measurable plasma concentrations of itraconazole that confirmed systemic exposure. Overall, amiodarone and itraconazole were well tolerated. All clinically relevant treatment-related effects detected on physical examination were mild and reversible with dose modification.

Parasitemia assessment by PCR assay

Proportions of dogs in the treatment and control groups that had positive PCR assay results for *T cruzi* in blood at the start of the study (baseline) were comparable ($P = 0.754$; **Table 2**). For the treatment group, the positive result rate was 0% for the 93 tested dogs at the 6-month visit and 0% for the 90 tested dogs at the 9-month visit (both significantly [$P < 0.001$] lower rates, compared with the baseline value); similar results were found for PCR assay results at the 12-month visit (time of final treatment) and the 24-month follow-up (1% of 87 and 0% of 86 tested dogs, respectively). The positive result rates for control dogs were 73%, 67%, and 67% for 15 tested dogs at the 6-, 9-, and 12-month visits. At the 24-month follow-up, 4 of the 8 known surviving control group dogs had positive results. The prevalence of parasitemia as determined by PCR assay was significantly ($P < 0.001$) greater for the control group than for the treatment group at every evaluation after baseline.

Serologic findings

Nineteen of 84 (23%) treatment group dogs and 0 of 8 control group dogs that initially had positive results by IFA for anti–*T cruzi* antibodies had seroconverted as assessed with this method by the time of the last follow-up examination. The proportion of dogs with a decrease in IFA-measured antibody titers over time was significantly ($P = 0.02$) greater for the treatment group (65/84 [77%]; mean titer at baseline and last follow-up, 2,282 and 893, respectively) than for the control group (3/8; mean titer at baseline and last follow-up, 1,704 and 3,349, respectively). No treated dogs had a net increase in titer; however, 3 of the 8 control dogs had a net increase in titer and 2 maintained a steady titer.

At baseline, mean anti–*T cruzi* antibody titers were not significantly ($P = 0.268$) different between the treatment and control groups **(Table 3)**. At the last follow-up, the mean antibody titer for the treatment group was significantly ($P < 0.001$) lower than that for the control group. The mean change in titers

Table 2—Results of PCR assays for *T cruzi* DNA in peripheral blood samples from the same dogs as in Table 1.

PCR assay result	Treatment (n = 105)	Control (n = 16)	Total (n = 121)	*P* value
Baseline				0.754
Negative	23/105 (22)	4/16 (25)	27/121 (22)	
Positive	82/105 (78)	12/16 (75)	94/121 (78)	
6 months				< 0.001
Negative	93/93 (100)	4/15 (27)	97/108 (90)	
Positive	0/93 (0)	11/15 (73)	11/108 (10)	
9 months				< 0.001
Negative	90/90 (100)	5/15 (33)	95/105 (90)	
Positive	0/90 (0)	10/15 (67)	10/105 (10)	
12 months				< 0.001
Negative	86/87 (99)	5/15 (33)	91/102 (89)	
Positive	1/87 (1)	10/15 (67)	11/102 (11)	
24 months				< 0.001
Negative	86/86 (100)	4/8 (50)	90/94 (96)	
Positive	0/86 (0)	4/8 (50)	4/94 (4)	

Changes in the number of dogs tested over time reflect the death of dogs (n = 26) or loss to follow-up (1) during the 24-month study period. ***See*** Table 1 for remainder of key.

Table 3—Summary statistics for (reciprocal) anti–*T cruzi* antibody titers at baseline and at the last follow-up visit for the same dogs as in Table 1.

	Treatment group		Control group		
Variable	**Mean ± SD**	**Median (range)**	**Mean ± SD**	**Median (range)**	***P* value**
Baseline titer	2,282 ± 6,336	160 (20–32,768)	1,704 ± 2,713	640 (80–8,192)	0.268
Last follow-up titer	893 ± 2,424	80 (0–16,384)	3,349 ± 5,111	1,280 (160–16,384)	< 0.001
Change in titers*	−1,389 ± 5,436	160 (15,360–28,672)	1,645 ± 2,398	0 (6,144–8,192)	0.006

At baseline, there were 105 and 16 dogs in the treatment and control groups, respectively. Last follow-up data represent the titer recorded 24 months after the start of the study (n = 86 in the treatment group and 8 in the control group) or the last titer on record for dogs that did not survive to the 24-month time point (13 and 1 in the treatment and control groups, respectively). The *P* values represent the comparison of titers between groups by Wilcoxon rank sum test.

*Change in titer values represent the mean and median results for changes in individual dogs with data at both time points.

was a substantial decrease for the treatment group (mean ± SD change for individual dogs, −1,389 ± 5,436) and a substantial increase for the control group (1,645 ± 2,398); this difference was significant ($P = 0.006$) between groups. However, results of repeated-measures MANCOVA indicated that treatment did not have significant impact on the IFA results (positive vs negative; $P = 0.66$) and only time period (last follow-up vs baseline) was significantly ($P = 0.02$) associated with this outcome. The antibody titers for all dogs (the overall study sample) at the last follow-up (mean ± SD, 893.70 ± 2,808.81) were lower than those at baseline (2,282.481 ± 5,980.02); however, the time-by-treatment interaction was not significant ($P = 0.98$).

Electrocardiographic, echocardiographic, and radiographic findings

Electrocardiographic examinations were performed for 92 of 105 (88%) dogs in the treatment group and 9 of 16 dogs in the control group at the start of the study, and all these dogs had ECG data available for the last follow-up visit **(Table 4)**. Echocardiography was performed at baseline and repeated at the last follow-up visit for 25 and 21 dogs, respectively, of the treatment group and for 6 and 4 dogs, respectively, of the control group.

The proportions of dogs in the 2 study groups with normal ECG results at baseline were comparable ($P = 0.705$; Table 4). At the last follow-up evaluation, a significantly ($P < 0.001$) lower proportion of control dogs had normal findings, compared with results for treatment group dogs. Abnormal ECG findings were detected in 25 of 92 treatment group dogs at baseline, and all these abnormalities resolved during the study period. For the control group, 3 of 9 dogs had ECG abnormalities at baseline versus 6 of 9 (including the 3 with abnormalities noted at baseline) at the last follow-up. Results of repeated-measures MANCOVA revealed that the combined amiodarone and itraconazole treatment was significantly ($P = 0.01$) associated with the outcome of normal ECG results (ie, sinus rhythm) at last follow-up (found for 92/92 [100%] dogs in the treatment group vs 3/9 in the control group) and that the time-by-treatment interaction also had a significant ($P = 0.01$) effect on this variable. A higher proportion of normal ECG results was found for dogs in the treatment group at the last follow-up than at baseline (92/92 [100%] vs 67/92 [73%], whereas the proportion of normal ECG results for dogs in the control group was lower at the last follow-up than at baseline (3/9 vs 6/9).

The proportions of dogs with echocardiographic abnormalities did not differ between groups at base-

Table 4—Results of ECG and echocardiographic evaluations at baseline and the last follow-up visit for the same dogs as in Table 1.

Variable	Treatment	Control	Total	*P* value
ECG findings at baseline				0.705
Normal	67/92 (73)	6/9 (67)	73/101 (72)	
Abnormal	25/92 (27)	3/9 (33)	28/101 (28)	
Second-degree AV block	1	0	1	
Third-degree AV block	2	0	2	
RBBB	2	0	2	
Sinus tachycardia	2	2	4	
PVC	11	1	12	
PVC with sinus tachycardia	5	0	5	
VT	2	0	2	
Electrocardiogram findings at last follow-up				< 0.001
Normal	92/92 (100)	3/9 (33)	95/101 (94)	
Abnormal	0/92 (0)	6/9 (66)	6/101 (6)	
ST segment depression	0	1	1	
Sinus tachycardia	0	3	3	
VT	0	2	2	
Echocardiographic findings at baseline				0.750
Normal	9/25 (36)	2/6 (33)	11/31 (35)	
Abnormal	16/25 (64)	4/6 (67)	20/31 (65)	
Echocardiographic findings at last follow-up				0.570
Normal	15/21 (71)	0/4 (0)	17/25 (68)	
Abnormal	6/21 (29)	4/4 (100)	8/25 (32)	

Data represent the proportion (%) or number of dogs with a given finding per group. Some dogs had > 1 cardiac abnormality. Last follow-up data represent findings 24 months after the start of the study (n = 74 treatment and 5 control group dogs for ECG and 14 treatment and 2 control group dogs for echocardiography) or at the last follow-up visit for dogs that did not survive to 24 months (all other dogs that had these evaluations). Within a time point, *P* values represent comparison of the proportions of dogs in each group that had normal (vs abnormal) results for a given variable as determined by Fisher exact or χ^2 test.

AV = Atrioventricular. PVC = Premature ventricular contractions. RBBB = Right bundle branch block. VT = Ventricular tachycardia.

line (P = 0.750) or at the last follow-up (P = 0.570; Table 4). Abnormalities found in 16 of 25 treatment group dogs included decreased fractional shortening (n = 4), septal wall hypokinesis (1), left (3) and right (7) atrial enlargement, mitral valve insufficiency (5), tricuspid valve insufficiency (2), pulmonic valve insufficiency (6), ventricular wall thickening with decreased chamber size (2) and pericardial effusion (2). All treatment group dogs that had echocardiographic abnormalities at baseline had improvement in these signs by the last follow-up, except for 1 dog that died of severe acute myocarditis and heart failure 16 days after diagnosis. Valvular regurgitation was eliminated in 11 of 13 dogs. However, 6 of 21 dogs evaluated at the last follow-up still had abnormalities present. Abnormalities in 4 of 6 control group dogs at baseline included moderate pulmonary valve insufficiency (n = 1), mild mitral valve insufficiency (1), and moderate bilateral atrial and ventricular enlargement (2). At the last follow-up, findings for the latter 2 dogs had progressed to severe bilateral atrial and ventricular enlargement, and findings for the 2 dogs with valvular insufficiency had remained the same. Results of repeated-measures MANCOVA revealed that treatment did not have significant impact on the echocardiography results (normal vs abnormal; P = 0.17); echocardiography results did not differ significantly (P = 0.76) over time, and the time-by-treatment interaction was not significant (P = 0.25) for this variable.

Forty-two dogs in the treatment group underwent radiographic examination at baseline, and 17 had signs of cardiomegaly. The signs were subtle and included right-sided heart enlargement and mild to moderate generalized cardiomegaly. One of 5 control dogs in which radiography was performed at baseline had generalized cardiomegaly with right and left atrial and ventricular dilation. Follow-up radiography was infrequently performed.

Necropsy findings and reported causes of death

Necropsy was performed for 14 of 19 dogs in the treatment group that died during the study. Gross necropsy and histopathologic findings in these 14 dogs included mild to severe myocarditis (n = 5); cardiac fibrosis, fatty infiltration, or both (6); nephritis (3); alveolar histiocytosis (2); lymphoid hyperplasia of the spleen, lymph nodes, intestines, or tonsils (alone or in combination; 3); chronic passive liver congestion (2); gastric periganglitis (1); and cardiomegaly (8). Although none of the 14 dogs that underwent necropsy completed the full 24 months of the study, 9 were free of cardiac inflammation and had no amastigotes identified at necropsy. Prior to death, 12 of these 14 dogs had clinical improvement. Five deaths (4 acute deaths and 1 euthanasia) were attributed to cardiac complications of *T cruzi* infection; causes of death or euthanasia for the remaining 14 treatment group dogs included neoplasia (n = 3), concerns about zoonotic transmission of *T cruzi* (2), intractable seizures (2), persistent ascites (1), acute renal failure (2), megaesophagus (1), chronic spinal cord degeneration (2), and limb fracture (1). One of these dogs was euthanized after premature discon-

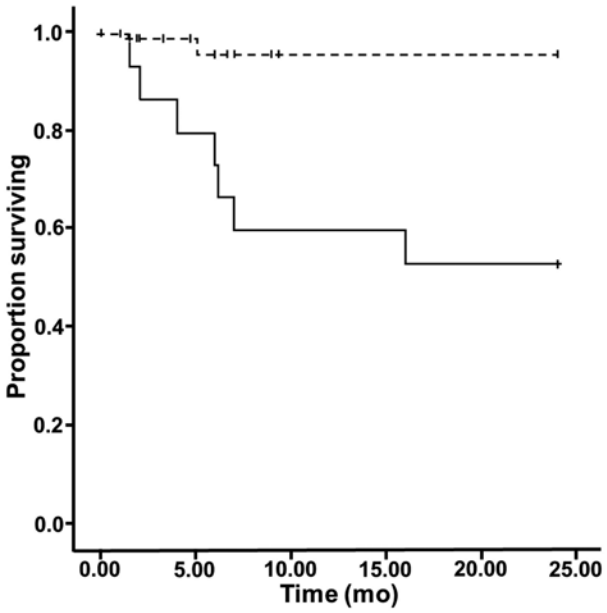

Figure 1—Kaplan-Meier plot of cumulative survival over time until death attributable to naturally acquired *Trypanosoma cruzi* infection for 121 dogs in a study to assess clinical, serologic, parasitological, and histopathologic outcomes following treatment with amiodarone and itraconazole. Dogs of the treatment group (dashed line; n = 105) were treated with a combination of amiodarone hydrochloride and itraconazole for 12 months. Amiodarone was given as a maintenance dosage of 7.5 mg/kg [3.4 mg/lb], PO, q 24 h or a loading dosage of 15 mg/kg, PO, q 12 h for 7 days followed by 15 mg/kg [6.8 mg/lb], PO, q 24 h for 14 days and then the described maintenance dosage; the treatment was supplied as scored 200-mg tablets, and the calculated dose was rounded to the nearest 50 mg. Itraconazole was given at a dosage of 10 mg/kg [4.5 mg/lb], PO, q 24 h; the treatment was supplied as 100-mg capsules, and the calculated dose was rounded down to the nearest whole capsule (for patients with low body weight, the protocol was revised to deliver approx 10 mg/kg, q 48 h). Itraconazole dosages were adjusted after measurement at steady state to maintain a plasma concentration of 1 to 2 µg/mL. Dogs of the control group (solid line; n = 16) did not receive antitrypanosomal medications. At the end of the 24-month study period, 86 dogs of the treatment group and 8 dogs of the control group were alive (and 1 dog of the control group had been lost to follow-up); 5 of 105 treatment group and 6 of 15 control group dogs had deaths attributable to *T cruzi* infection. Vertical marks indicate censored data points.

tinuation of the treatment protocol and was included in the category of other causes of death (ascites). Of the 7 control group dogs that died during the study, the only death that was not attributed to *T cruzi* infection was attributed to lymphosarcoma; these dogs did not undergo necropsy as part of the study.

Survival times for dogs with death attributed to *T cruzi* infection

There was a significant ($P < 0.001$) difference between the treatment and control groups in survival distributions of dogs with death attributed to *T cruzi* infection during the 24-month study period. Mean ± SE estimated survival time for dogs in the treatment group was 23.19 ± 0.4 months (95% confidence interval, 22.40 to 23.97 months), whereas that for dogs in the control group was 15.64 ± 2.44 months (95% confidence interval, 10.86 to 20.42 months). The difference in the survival distributions for treatment group versus control group dogs is depicted in the Kaplan-Meier survival analysis plot **(Figure 1)**.

Discussion

Preliminary evidence from this small, 2-year study that included 121 dogs with naturally occurring *T cruzi* infection (105 dogs that received a combination of amiodarone and itraconazole for 12 months [treatment group] and 16 dogs that received no antitrypanosomal treatment [control group]) suggested that the study treatment was efficacious. Results of baseline clinical and parasitological assessments were comparable between the 2 study groups, and several outcomes of interest, including the main end points of improvement in clinical signs, negative results of PCR assay for *T cruzi* DNA, and survival until death attributable to *T cruzi* infection, differed significantly between groups, with more favorable results found for dogs in the treatment group.

Clinically, cardiomyopathy is the most prominent manifestation of chronic *T cruzi* infection in dogs in North America, with very few reports[13,14,19] of gastrointestinal or neurologic involvement. Chronic cardiomyopathy can occur months or years after the initial infection but usually develops within 8 to 36 months after inoculation.[13,14,19] As in human patients, diffuse myocarditis and fibrosis lead to a number of conduction disturbances,[17,40] along with clinical signs of right-sided or left-sided heart failure, such as dyspnea (because of pulmonary edema), ascites, and hepatomegaly.[14] Dogs that enter the chronic phase of the disease without overt clinical signs (commonly termed the chronic asymptomatic phase) may not develop clinical signs; however, ventricular arrhythmias may be induced by exercise, potentially leading to death.[14,19] Our results supported these findings; the most commonly identified ECG abnormalities were premature ventricular contractions, followed by ventricular tachycardia, sinus tachycardia, atrioventricular block, and right bundle branch block.

The combination of amiodarone and itraconazole used in the present study was selected to target specific metabolic pathways of the parasite. Amiodarone is an antiarrhythmic agent that is frequently prescribed to prevent complex arrhythmias in human patients with cardiomyopathy due to *T cruzi* infection[36] and was used in the present study not only in an effort to prevent development of exercise-induced and potentially lethal ventricular arrhythmias but also because of its direct activity against *T cruzi*.[39,40] This agent disrupts calcium homeostasis in the parasite by inducing release of calcium from intracellular stores (such as the giant mitochondrion and acidocalcisomes, which are unique organelles involved in the bioenergetics of these organisms).[39] Amiodarone also blocks biosynthesis of sterols,[39,42] which is lethal in

T cruzi because the parasite uses these sterols instead of cholesterol to synthesize the ergosterol needed for proliferation.[25,47] Moreover, the spectrum of action of amiodarone extends beyond its antiparasitic effect to include direct modulation of calcium homeostasis in cardiomyocytes. The arrhythmogenic events that result from *T cruzi* infection have been shown to depend on the ability of the parasite to disrupt communication through gap junctions by decreasing concentrations of connexin 43 (an essential gap protein) to interact with actin filaments.[48] It was also confirmed experimentally that amiodarone-treated cardiomyocytes recover their normal distribution of connexin 43 and spontaneous contractility.[48] We believe that the complete reversal of arrhythmias observed in our study, with resolution of the described ECG abnormalities in 25 of 25 tested dogs of the treatment group and a significant association between treatment and the outcome of normal sinus rhythm on ECG at the last follow-up visit (as assessed by repeated-measures MANCOVA), suggested an underlying amiodarone-induced recovery of cellular events to yield clinical effects. Other possible explanations for the elimination of arrhythmias included decreased myocardial inflammation and scarring, decreased amounts of proinflammatory cytokines being released from inflamed or infected tissue, and improved diastolic filling resulting in better coronary perfusion and delivery of oxygen to the myocardium.

In addition, we observed improvement in some of the echocardiographic variables for treated dogs of our study, such as increased fractional shortening, elimination of valvular regurgitation, reduction of ventricular wall thickening, and enhanced septal wall kinesis, which suggested improved myocardial function in these dogs. This finding could be attributable to the way in which amiodarone promotes recovery of cardiac cells by recovery of the fibrillar organization of F-actin, consistent with the observations of Adesse et al[48] in regard to the finding that reduced contractility of cardiomyocytes resulting from *T cruzi* infection is corrected after treatment with amiodarone to a degree that is indistinguishable from that in noninfected healthy cardiomyocytes.[48]

Itraconazole was administered concomitantly with amiodarone in this study in an effort to take advantage of the known synergism between the 2 drugs.[36] Like other azoles, itraconazole is known to act against *T cruzi* by inhibition of cytochrome P450-dependent C14α-sterol demethylase, which is involved in several membrane-bound enzymatic pathways.[24,36] Although imidazole has previously been reported to be ineffective in the treatment of *T cruzi* infection in dogs,[13] the present study yielded some interesting results.

Although the seroconversion rate to negative results for treatment group dogs that initially tested positive for anti–*T cruzi* antibodies by IFA was 19 of 84 (23%), MANCOVA results did not reveal this as significantly associated with treatments, and the lower posttreatment antibody titers in treated dogs (a decrease in the group mean to less than half of the baseline value) versus control dogs were nonsignificant in this analysis. A possible explanation for this is the tendency for *T cruzi*–specific antibodies to persist in the bloodstream for many years, even after treatment for chronic Chagas disease in people.[49] Despite the prolonged period of treatment, only a few animals had adverse effects attributable to itraconazole or amiodarone treatment, all of which were reversible when the dosage was reduced.

Importantly, because of the intracellular nature of *T cruzi* and its well-known capacity to reside in so-called sanctuary sites such as adipose tissue,[50] mononuclear phagocytic cells,[51] and cardiomyocytes,[52] delivery of treatment poses a challenge. Nevertheless, itraconazole concentrations in cardiomyocytes, adipocytes, and splenic tissue have been found to be higher than those in plasma in people and in laboratory animals, indicating that parasites are exposed to higher concentrations of the drug in these tissues.[52,53] Amiodarone and itraconazole also accumulate in macrophages.[53–55]

Amiodarone and itraconazole concentrations are 10 and 5 times as high, respectively, in myocardium as in plasma, indicating that both drugs accumulate at the primary target organ for *T cruzi*.[54,56,57] Fat is also a reservoir from which infection can be reactivated, and therefore, penetration of the adipose tissue is critical for elimination of the chronic phase of infection.[58] Both of the drugs used in the present study are highly lipophilic, with concentrations of itraconazole in adipose tissue 25 times those in plasma.[59] Furthermore, both agents have very long half-lives in dogs (amiodarone, 3.2 days; itraconazole, 2.1 days)[60,61] and are relatively safe with dose-dependent and reversible toxic effects.

In contrast, benznidazole, which is the drug most widely used for treatment of patients infected with *T cruzi*, has poor tissue penetration[35] and does not accumulate in macrophages.[62] Moreover, benznidazole has several limitations with regard to its efficacy against this organism in the acute and chronic phases of infection in dogs. Benznidazole has an unfavorable pharmacokinetic profile that includes a short elimination half-life of only 13 hours and a maximum plasma concentration of 2.4 µg/mL, which makes it difficult or impossible to maintain the recommended trypanocidal concentration of 3 to 6 µg/mL.[63] Benznidazole reaches concentrations in target tissues that are much lower than those in plasma, with reported concentrations in the heart and spleen that are 32% and 27%, respectively, of concentrations achieved in plasma; these sites receive an amount of the drug that is far below the minimum inhibitory concentration values, and the strain of *T cruzi* also impacts the efficacy of benznidazole.[35,64,65] The *T cruzi* DTU I strain is the only strain that has been shown to infect people autochthonously in the United States[66] and is a major variant that causes American trypanosomiasis in dogs in the United States.[67] This strain is inherently resistant to benznidazole, which failed to resolve

parasitemia in 4 of 4 acutely experimentally infected dogs treated for 45 days after inoculation with the *T cruzi* DTU I strain.[68] Other limitations include poor drug availability in the US market and toxic effects for a total dose of 18 g reported in the human medical literature,[69] although most dogs seem to tolerate a 45- to 60-day treatment regimen fairly well.[70] Assessment of therapeutic effectiveness of a treatment for *T cruzi* can be challenging because the disease develops slowly and because anti–*T cruzi* antibodies persist in a large percentage of patients for many years after treatment. Although some authors have reported a decrease in specific antibodies following treatment and suggested that this could be used as an indicator of cure, different serologic profiles are detected with ELISA, IFA, and Western blotting in chronically infected treated dogs, which indicates these methods alone are not optimal. The expensive and lengthy patient follow-up required with these methods places an unrealistic burden on clinicians and clients. However, PCR assays have high sensitivity for detection of circulating trypomastigotes and, despite the intermittent shedding of the organism in peripheral blood, have been shown to be useful for monitoring clearance of the parasite.[68] In our study, we used serial PCR assay evaluations with multiple targets to increase our ability to detect the organism during and after treatment. Even though PCR assay is not historically considered a reliable indicator of cure because of its low sensitivity for detection of *T cruzi* in patients with chronic infection,[71] the finding of negative PCR assay results in nearly all tested dogs of the treatment group beginning 6 months after treatment was initiated (with a single positive test at the 12-month time point) supported that a cure was achieved in most dogs. Moreover, the value of persistent negative PCR assay results in monitoring of treatment has been suggested by other investigators.[72]

Our investigation of clinical outcomes revealed that the proportion of dogs in the treatment group with deaths attributable to *T cruzi* before the end of the study was significantly less than that of the control group dogs (5/105 [4.8%] vs 6/15 [40%]). Survival distributions of dogs with death attributed to *T cruzi* infection differed significantly between the treatment and control groups, with mean survival times of 23.19 and 15.64 months, respectively. Furthermore, improvement in ≥ 1 clinical sign by the last follow-up visit was significantly more common for dogs of the treatment group (53/54 [98%]) than for dogs of the control group (0/10). One of the most profound results was the previously mentioned significant difference in ECG results over time. Conduction abnormalities in 25 of 25 (100%) treatment group dogs had resolved by the time of last follow-up, whereas 3 of 9 control group dogs had no improvement in this variable and 3 additional dogs in this group developed conduction abnormalities by the last follow-up, supporting that the combined amiodarone and itraconazole treatment improved the cardiac status of treated dogs. These findings were important, given that untreated chronically infected dogs with a mean age of 4.5 years have been reported to survive for a maximum of 5 months after diagnosis of *T cruzi* infection.[21]

Acute-phase manifestations of *T cruzi* infection in dogs are usually missed because of the subtle degree and short duration of signs, such as anorexia for 1 to 2 days or acute self-limiting diarrhea, so most cases are not diagnosed until clinical signs are observed in the chronic stage unless detected incidentally during the chronic phase without overt clinical signs by routine serologic screening.[13,14] Historically, specific antiparasitic treatment was likely to be ineffective during the chronic stage because of fibrosis and cardiac damage, and treatment was mainly supportive and directed toward management of cardiac signs.[13,36,40] The clinical relevance of our findings was that successful treatment was achieved by exploiting the synergistic effects of amiodarone and itraconazole, suggesting that this combined drug treatment has efficacy against *T cruzi* in dogs with naturally acquired disease.

A limitation of the present study was that identification of the *T cruzi* strains by DTU identification was not performed in the study dogs. This is important, given that the DTU classifications have clinicopathologic implications with regard to the biological features and progression of disease as well as drug susceptibility.[32,73] Other limitations include the small size of the control group, lack of ECG and echocardiography data for all dogs, lack of blinding during clinical evaluations and assessment of ECG and echocardiography data, and inability to evaluate success rates on the basis of disease phase in the dogs.

Treatment of *T cruzi*–infected dogs that do not have signs of disease may have an important part to play in public health by reducing the risk of potential transmission through breeding[66] or exposure of infected animals to feeding vectors that can transmit the disease to other dogs. Reduction of parasitemia inhibits the spread of the disease by eliminating trypomastigotes available for a blood meal by a triatomine insect and could reduce the risk of zoonotic transmission, especially in endemic areas. Because *T cruzi* infection in animals is not a reportable condition in Texas or in other southern parts of the United States,[9,74] the number of reported cases is likely a substantial underestimate of the true incidence. The coexistence of important disease vectors, such as *Triatoma sanguisuga*, *Triatoma gerstaeckeri*, and *Triatoma lecticularia*,[74-76] and the variety of susceptible mammalian reservoirs[9,74] in a broad spectrum of ecological regions could favor occurrence of this disease at epidemic proportions in the southern United States.

Veterinarians and pet owners should be aware of the potential threat of *T cruzi* infection in domestic dogs and be familiar with the clinical signs of the disease as well as the emerging alternatives to treat it. The present study represented an effective international collaborative effort between physicians, veterinarians, and biologists and was an example of the One Health Initiative supporting the vision of improv-

ing animal and human health globally through such collaboration to address critical needs.[77]

Acknowledgments

No third-party funding or support was received in connection with this study or the writing or publication of the manuscript. The authors declare that there were no conflicts of interest.

The authors thank Dawn Boothe, Clinical Pharmacology Laboratory, Auburn University College of Veterinary Medicine for technical expertise.

Footnotes

a. Random number generator. Available at: www.randomizer.org. Accessed Sep 21, 2018.
b. Cadila Healthcare Ltd, Gujarat, India.
c. Upsher-Smith Laboratories, Maple Grove, Minn.
d. Janssen Pharmaceuticals, Beerse, Belgium.
e. Patriot Pharmaceuticals, Gurabo, Puerto Rico.
f. AxyPrep Blood Genomic DNA Miniprep kit, Axygen Biosciences, Union City, Calif.
g. 5331 Gradient MasterCycler, Eppendorf, Hamburg, Germany.
h. Clontech, Mountain View, Calif.
i. Green GoTaq Flexi buffer, Promega Corp, Madison, Wis.
j. GoTaq Flexi DNA Polymerase, Promega Corp, Madison, Wis.
k. Texas Veterinary Medical Diagnostic Laboratory, Texas A&M University, College Station, Tex.
l. DoD Food Analysis & Diagnostic Testing Laboratory, Public Command Region-South, Joint Base San Antonio-Fort Sam Houston, Tex.
m. Trinity Biotech, Bray, Ireland.
n. Seracare Life Sciences Inc, Milford, Mass.
o. Clinical Pharmacology Laboratory, College of Veterinary Medicine, Auburn University, Auburn, Ala.
p. Texas Veterinary Pathology, Spring Branch, Tex.
q. The Joint Pathology Center, Silver Springs, Md.
r. Stata, version 13.0, StataCorp, College Station, Tex.

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