


STANDARD ARTICLE

Repeated cross-sectional study of *Trypanosoma cruzi* in shelter dogs in Texas, in the context of *Dirofilaria immitis* and tick-borne pathogen prevalence

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Background: Vector-borne diseases have an adverse impact on health of dogs, and infected dogs can be sentinels for human infection. Infection with *Trypanosoma cruzi*, an agent of Chagas disease, causes fatal heart disease in dogs across the southern United States but has been neglected from wide-scale prevalence studies.

Objectives: To determine the prevalence of exposure to *T. cruzi*, *Ehrlichia* spp., *Anaplasma* spp., *Borrelia burgdorferi*, and infection with *Dirofilaria immitis* among dogs in shelters across Texas and to identify risk factors for *T. cruzi* seropositivity.

Animals: Six hundred and eight dogs.

Methods: This repeated cross-sectional study was performed by collecting blood from ~30 dogs during each of the 3 visits to 7 shelters. We tested serum for antibodies to *T. cruzi* using 2 tests in series and for antibodies to *Ehrlichia* spp., *Anaplasma* spp., and *B. burgdorferi* and *D. immitis* antigen using the IDEXX SNAP 4DX Plus point-of-care test. DNA was extracted from blood clots and tested for *T. cruzi* DNA and strain type via quantitative polymerase chain reactions (qPCR). We used logistic regression to assess risk factors.

Results: One hundred ten (18.1%) of 608 dogs were seropositive for *T. cruzi*. Prevalence of exposure to the other vector-borne agents was: *Ehrlichia* spp. 3.6%; *Anaplasma* spp. 6.9%; *B. burgdorferi* 0.2%; and *D. immitis* infection 16.0%. Six of 559 (1.1%) dogs were qPCR-positive for *T. cruzi*.

Conclusions and Clinical Importance: *T. cruzi* seroprevalence was comparable to *D. immitis* prevalence and higher than seroprevalence of the tick-borne pathogens. *T. cruzi* is an underrecognized health threat to dogs across Texas and possibly other southern states where triatomine vectors are endemic.

KEYWORDS

Chagas disease, heartworm, tick-borne disease, vector-borne disease

1 | INTRODUCTION

Vector-borne diseases have a serious impact on health of dogs in the United States, and many vector-borne pathogens are also of concern

Abbreviations: AKC, American Kennel Club; CDP, Chagas Detect Plus; DTU, discrete typing unit; EDTA, ethylenediaminetetraacetic acid; IFAT, indirect fluorescent antibody test.

to human health. Dogs are useful sentinels for many zoonotic diseases, both in areas of high and low prevalence^{1,2} and in areas of disease emergence.³ The most well-known vector-borne infections of dogs in the United States are the mosquito-borne nematode *Dirofilaria immitis* (heartworm) and the tick-borne organisms, including *Borrelia burgdorferi* (agent of Lyme disease), *Anaplasma* spp. (*A. phagocytophilum* and *A. platys*), and *Ehrlichia* spp. (*E. canis* and *E. ewingii*). Veterinarians and pet owners generally recognize the importance of these

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pathogens, and recommendations include routine testing and administration of preventive medications and ectoparasiticides in areas where transmission occurs.^{4,5}

A less recognized vector-borne pathogen of increasing concern across the southern United States is *Trypanosoma cruzi*, the agent of Chagas disease (American trypanosomiasis). This zoonotic protozoan parasite is a well-known human health threat across Latin America and exists in robust sylvatic cycles in the southern United States, where the triatomine insect vectors (known colloquially as “kissing bugs” or “cone-nose bugs”) are widespread.⁶ *T. cruzi* is divided into discrete typing units (DTUs TcI-TcVI),⁷ which are associated with different geographical regions and reservoir hosts and with different disease manifestations in animals and possibly humans.⁷⁻¹⁰

T. cruzi has been documented in dogs throughout many southern states^{6,11-16} but has been relatively neglected in wide-scale disease surveillance surveys. Infected dogs can develop acute or chronic myocarditis with resulting heart failure¹⁷ or can remain subclinically infected for life. Currently, there are no good prognostic markers to predict the outcome of infection. The diagnostic tests used to detect *T. cruzi* in dogs are imperfect with no accepted gold standard. The most widely used test in veterinary diagnostics is indirect fluorescent antibody test (IFAT), with additional tools available for research purposes, but discordant results are common.^{18,19} Furthermore, there is neither a vaccine nor an approved antiparasitic treatment for *T. cruzi* in dogs, creating challenges for veterinarians attempting to make recommendations regarding infected dogs.

Defining the current burden of *T. cruzi* in dogs compared with prevalences of more well-recognized infections is essential for driving efforts to develop vaccines, therapeutics, and improved diagnostics. Furthermore, in some regions, dogs are considered sentinels for human risk of Chagas disease,^{15,20-22} and thus information on

exposure in dogs could be useful for guiding broader public health efforts. The objectives of our study were to determine the prevalence of *T. cruzi* and other vector-borne infections in dogs in shelters across Texas and to identify risk factors for infection. In addition, we measured circulating *T. cruzi* DNA to infer infectivity risk and characterized the infecting *T. cruzi* genetic strain type. Here, we present our findings of widespread *T. cruzi* seropositivity and *D. immitis* antigenemia among Texas shelter dogs, regional distributions of *Ehrlichia* spp. and *Anaplasma* spp. seropositivity, and *B. burgdorferi* seropositivity in only a single dog with an unknown travel history.

2 | MATERIALS AND METHODS

2.1 | Study design and sample collection

In our repeated cross-sectional study, we sampled dogs at 7 shelters across 7 different Gould ecoregions of Texas,²³ visiting each shelter 3 times over an 18-month period from May 2013 to December 2014. Shelters located in the cities of Bryan/College Station, Dallas, Edinburg, El Paso, Fort Worth, Houston, and San Antonio (Figure 1) responded to a request for participation and were selected for inclusion in the study. The shelters in Dallas and Fort Worth were municipal animal control facilities; shelters in Edinburg and Bryan/College station were nonprofit shelters with contracts to house animals from the neighboring municipalities where no municipal animal control facility exists; and the shelters in El Paso, Houston, and San Antonio were private nonprofit shelters in areas that were also served by separate municipal animal control agencies. To estimate the prevalence of *T. cruzi* (the primary outcome of interest) within $\pm 2.5\%$ with 95% confidence interval (CI) (using an estimated true prevalence of 10% based

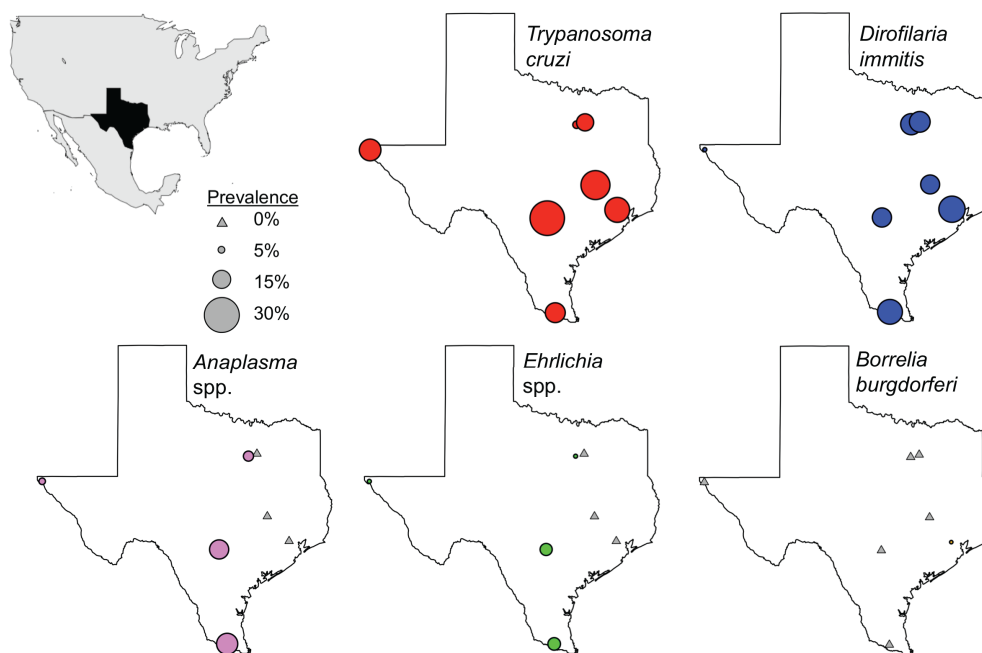


FIGURE 1 Maps indicating shelter locations and seroprevalences among dogs in each shelter for each of the 5 vector-borne pathogens. Circle size is relative to prevalence as illustrated in the legend, and triangles mark shelters where no positive dogs were detected. The map was created in R Statistical Software³⁵

on a pilot study¹⁵), the target was >550 samples. Thus, approximately 30 dogs were sampled from each shelter during each visit. Dogs aged 6 months and older with fresh feces available at the time of sampling (for separate projects^{24,25}) were eligible for inclusion. We preferentially sampled dogs admitted to the shelter within the previous 14 days so that test results would be most likely to reflect exposure in the dog's original environment before entering the shelter, although to meet the sample size, not all sampled dogs met these criteria. Many of the shelters routinely treated dogs with anthelmintic drugs (eg, fenbendazole, pyrantel pamoate) upon intake, but these drugs are not known to be effective against *T. cruzi*, *D. immitis* adults, or tick-borne pathogens, and were not expected to impact the outcome of our study. Demographic data (approximate age, sex, predominant American Kennel Club [AKC] breed group) were estimated based on shelter records, the investigators' assessments of the animal, or both.

During each visit, up to 5 mL of blood was collected from each dog in accordance with client-owned animal use protocols approved by the Texas A&M University Institutional Animal Care and Use Committee (AUP 2015-0289). Blood was collected into a tube with no additive and a tube containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). In the laboratory, the tubes were centrifuged, and the blood was separated into the following components: serum, clot, plasma, buffy coat, and packed cells. Serum was refrigerated until the initial serologic screening test was completed within 3 days of collection. Remaining serum and other blood components were frozen at -20°C or -80°C , and molecular and additional serologic testing were performed on aliquots over the next 3 years. All dogs were examined for ticks at the time of blood collection; ticks were collected and identified morphologically by examination under a dissecting microscope and comparison to standard morphological keys.²⁶

2.2 | Serology

To detect anti-*T. cruzi* antibodies, serum samples were 1st tested using a commercially available rapid immunochromatographic test (Chagas Stat-Pak; Chembio Diagnostic Systems, Inc., Medford, New York) developed for use in humans using 3 recombinant antigens and validated using human sera from South and Central America.²⁷ The Stat-Pak has also been used in dogs, with reported high sensitivity and specificity when compared to IFAT.²⁸ We ran the test according to manufacturer's instructions in which all samples that generated a band, irrespective of the intensity of color, were considered positive on this test.

All of the Stat-Pak positive samples and a subset of negatives (comprised systematic random selection of 10% of the Stat-Pak negatives plus an additional 50% of the PCR suspect-positive samples [see below], for a total of 81 Stat-Pak negative samples) were subjected to a 2nd, independent rapid immunochromatographic test (CDP, Chagas Detect Plus Rapid Test; InBios International, Inc., Seattle, Washington) that uses a multi-epitope recombinant antigen derived from antigens specific to North American *T. cruzi* strains as well as those from Central and South America.²⁹ Tests were run according to manufacturer's instructions. Samples positive on both the Stat-Pak and the CDP tests were considered seropositive in the calculation of seroprevalence and in statistical analyses for the

identification of risk factors. As infection appears to be lifelong in both untreated humans and dogs,^{6,30} seropositive dogs were considered currently infected.

For the other vector-borne pathogens, we used the SNAP 4Dx Plus (IDEXX, Westbrook, Maine), a commercially available ELISA which affords simultaneous detection of canine antibodies to *E. canis*, *E. ewingii*, *A. phagocytophilum*, *A. platys*, and *B. burgdorferi* and antigen of *D. immitis*. This point-of-care rapid diagnostic test kit is widely used in clinical settings in the United States. This test was run using whole blood anticoagulated with EDTA according to manufacturer's instructions.

2.3 | Molecular detection of *T. cruzi* DNA

DNA was extracted from approximately 250 μL of blood clot using a commercial spin-column-based kit (E.Z.N.A. Tissue DNA kit; Omega Bio-Tek, Norcross, Georgia). Each set of DNA extractions included a no-template negative control. Clots from all sampled dogs were able to be tested except for samples from the first 2 visits to the Bryan/College Station shelter, which were regrettably not retained for this analysis. We performed an initial screening with a real-time quantitative PCR (qPCR) using Cruzi 1, 2 primer set and Cruzi 3 probe as previously reported.³¹ This qPCR amplifies a 166-bp region of a repetitive satellite DNA sequence and is sensitive and specific for *T. cruzi* when compared to other PCR techniques.³² Based on internal laboratory validations, samples with a cycle threshold (Ct) value of ≤ 34 were considered suspect positive. Suspect positive samples were subjected to a multiplex qPCR targeting the spliced-leader intergenic region to confirm positivity and for determination of strain type, according to previously described protocols.^{33,34} Positive (DNA extracted from *T. cruzi* Sylvio X10 clone 4, American Type Culture Collection, ATCC #50800) and negative (water) controls were included in each PCR batch.

2.4 | Statistical methods

Statistical analyses were performed in R version 3.3.0.³⁵ To determine whether dogs with a longer duration of stay in the shelter were more likely to be positive for any of the pathogens, we used Welch's *t*-test to compare the average length of shelter stay (days) of positive versus negative dogs for all pathogens except for *B. burgdorferi*. Variables assessed as putative risk factors were estimated dog age (<1 year old or ≥ 1 year old), origin (stray versus owner-relinquished), sex, season of sampling, breed group, and shelter. Many dogs were of mixed breed but were classified into AKC breed groups based on the most dominant breed features. For each infectious agent except *B. burgdorferi* (because of the low number of positive dogs [$n = 1$]), bivariable analysis using chi-squared or Fisher's exact tests was performed to evaluate the relationship between each of the putative risk factors and test outcome, excluding dogs with unknown status for each variable. For *T. cruzi* seropositivity and *D. immitis* antigen-positivity, risk factors with *P* value $\leq .25$ in bivariable analysis were further investigated with logistic regression using mixed-effects models in R package "lme4," controlling for shelter as a random effect. Generalized linear mixed models were used, and factors with *P* values $\leq .05$

were considered significant. Odds ratios and their 95% CIs were calculated. Logistic regression was not performed for *Anaplasma* spp. and *Ehrlichia* spp. because of low numbers of positive dogs resulting in zero values for several variables.

We were also interested in dogs that tested positive for more than 1 vector-borne agent, suggesting coinfection or coexposure. We used chi-squared tests to determine whether the frequency of coinfection or coexposure was higher than would be expected because of the chance for each possible combination of 2 infectious agents. In addition, Cohen's kappa (R package "fmsb") was used to assess the agreement between *T. cruzi* serologic status and PCR status.

3 | RESULTS

3.1 | Population data

The study included 608 dogs; demographic data are reported in Table 1. Sampling took place over 3 periods: summer 2013 (May-August), winter 2013-2014 (December-February), and fall 2014 (September-December). Number of dogs sampled from each shelter ranged from 64 (Bryan/College Station) to 95 (San Antonio). For dogs for which admission date was known, duration of shelter stay before sampling was 14 days or less for 382 of 512 (74.6%) dogs. The median length of stay for the remaining 130 dogs was 32 days, the mean was

TABLE 1 Demographic data and results of bivariable and logistic regression analysis of potential risk factors for *Trypanosoma cruzi* seropositive status among 608 dogs at 7 animal shelters across Texas

Risk factor	No. of dogs tested	No. seropositive (%)	Bivariable analysis P-value	Logistic regression		
				Odds ratio	95% confidence interval	P-value
Shelter location			.002	RE	RE	RE
Bryan/College Station	64	15 (23.4)				
Dallas	93	13 (14.0)				
Edinburg	91	15 (16.5)				
El Paso	88	16 (18.2)				
Fort Worth	91	5 (5.5)				
Houston	86	18 (20.9)				
San Antonio	95	28 (29.5)				
Age group			.11			
<1 y	82	9 (11.0)			Reference	
≥1 y	520	99 (19.0)		1.81	0.869-3.77	.11
Unknown ^a	6	2 (33.3)				
Origin			.96	NI	NI	NI
Owner-relinquished	93	15 (16.1)				
Stray	387	59 (18.0)				
Unknown ^a	128	36 (28.1)				
Sex			.91	NI	NI	NI
Female	294	54 (18.4)				
Male	311	55 (17.7)				
Unknown ^a	3	1 (33.3)				
Sampling season			.82	NI	NI	NI
Summer (May-Aug)	203	36 (17.7)				
Winter (Dec-Feb)	206	40 (19.4)				
Fall (Sep-Dec)	199	34 (17.1)				
Breed group			.33	NI	NI	NI
Herding	122	22 (18.0)				
Hound	30	7 (23.3)				
Nonsporting	20	3 (15.0)				
Sporting	111	25 (22.5)				
Terrier	187	28 (15.0)				
Toy	58	15 (25.9)				
Working	50	6 (12.0)				
Unknown ^a	30	4 (13.3)				
Total	608	110 (18.1)				

Abbreviations: NI, not included in logistic regression model; RE, random effect; shelter was included in the mixed model as a random effect, therefore odds ratios were not generated.

^a Unknowns for each risk factor were excluded from bivariable analysis and logistic regression of that risk factor.

43 days, and the longest length of stay was 225 days. Length of stay was not associated with serostatus for any of the infectious agents (P values = .4-.7). The proportion of males versus females was approximately equal with 294 (48.4%) females and 311 (51.2%) males, while sex was not recorded for 3 dogs (0.5%). A total of 520 dogs (85.5%) were estimated to be 1 year or older, and 82 (13.5%) dogs were younger than 1 year old, with 6 (1.0%) dogs of unknown age. The origin of 387 (63.7%) dogs was classified as stray, 93 (15.3%) dogs had been relinquished by their owners, and origin was not known for 128 (21.1%) dogs. The Terrier breed group was the most well represented, with 187 (30.8%) dogs, of which 74% (138 dogs) were recorded as pit bulls or pit bull mixes, although there was a potential for misclassification of breeds based solely on subjective physical appearance. Breed was not recorded for 30 (4.9%) dogs.

3.2 | Serologic results

For *T. cruzi*, of 120 dogs positive on the Stat-Pak, 110 were also positive on the CDP. Of the randomly selected 81 dogs negative on the Stat-Pak and tested on the CDP, 39 (48%) dogs were positive on the CDP. With the criterion of being positive on both tests for the purposes of statistical analysis, 110 of 608 dogs were therefore considered *T. cruzi* seropositive, yielding an overall seroprevalence of 18.1% (95% CI = 15.1%-21.4%). *Trypanosoma cruzi* seroprevalence ranged from 5.5% in the Fort Worth shelter to 29.5% in the San Antonio shelter (Tables 1 and 2, Figure 1). Overall proportion of positivity for the other 4 vector-borne agents was as follows (Table 2): 97 *D. immitis* antigen-positive (16.0%, 95% CI = 13.1%-19.1%); 22 *Ehrlichia* spp. seropositive (3.6%, 95% CI = 2.3%-5.4%); 42 *Anaplasma* spp. seropositive (6.9%, 95% CI = 5.0%-9.2%); and 1 *B. burgdorferi* seropositive (0.2%, 95% CI = 0.004%-0.9%). The single *B. burgdorferi* seropositive dog was a 14-year-old castrated male Doberman mix in Houston with an unknown travel history.

3.3 | Coinfections

Coinfections (or coexposures) were observed for all infectious agents other than *B. burgdorferi*, with common coinfections including *T. cruzi* and *D. immitis* ($n = 19$ dogs), and *Ehrlichia* spp. and *Anaplasma* spp. ($n = 16$ dogs; Supporting Information Supplemental Figure). Three-way and 4-way coinfections were observed as well. The only coinfection observed that was statistically more frequent than would be

expected due to a chance was coinfection with *Ehrlichia* spp. and *Anaplasma* spp. ($P < .001^{**}$), and coinfecting dogs were identified at all 4 shelters where dogs tested positive for *Ehrlichia* spp. and *Anaplasma* spp.

3.4 | Risk factor assessment

In bivariable analyses of risk factors for *T. cruzi* seropositivity, only age group had a P value $< .25$ (Table 1) and was included in the mixed-effects logistic regression model, controlling for shelter as a random effect. As estimated by logistic regression, age group was not significantly associated with *T. cruzi* seropositivity (Table 1). Shelter was also significantly associated with seropositivity in bivariable analysis (P value = .002), driven by low seroprevalence at the Fort Worth shelter (5.5%).

For *D. immitis*, age group, origin, and sex had P values $< .25$ (Table 2) in bivariable analyses (Supporting Information Supplemental Table 1) and were included in the logistic regression model. As estimated by logistic regression, only age group was significantly associated with *D. immitis* antigen-positivity (Supporting Information Supplemental Table 1). Dogs aged 1 year or older were 12 times more likely to be infected than dogs less than 1 year old (OR = 12.4, 95% CI = 1.7-90.9). Shelter was also significantly associated with antigen-positive status in bivariable analysis (P value = .01), driven by low prevalence at the El Paso shelter (2.3%).

Although *T. cruzi* and *D. immitis*-positive dogs were identified in all 7 shelters, *Anaplasma* spp. and *Ehrlichia* spp. seropositive dogs were only detected in 4 shelters (Table 2, Figure 1). In bivariable analysis of risk factors for *Anaplasma* spp. and *Ehrlichia* spp. seropositivity, only shelter location was significantly associated with serostatus (P value $< .001$) (Supporting Information Supplemental Tables 2-3).

3.5 | Molecular detection of *T. cruzi* DNA

DNA extracts from blood clots of 559 dogs were tested with the *T. cruzi* screening qPCR; 53 samples were considered suspect-positive with a Ct value ≤ 34 (range 28-34) and were subjected to further testing. Of these, 6 (1.1% of total) samples were confirmed positive by the strain typing qPCR, whereas the remaining samples were negative on the secondary assay and were therefore considered *T. cruzi* negative in the analysis. Five of the PCR-positive dogs were infected with DTU TcI, and 1 dog was infected with TcIV. Two of the positive dogs

TABLE 2 Overall and within-shelter prevalence of exposure to or infection with 5 vector-borne pathogens among 608 dogs at 7 animal shelters across Texas

	N	<i>Trypanosoma cruzi</i> (%)	<i>Dirofilaria immitis</i> (%)	<i>Anaplasma</i> spp. (%)	<i>Ehrlichia</i> spp. (%)	<i>Borrelia burgdorferi</i> (%)
State-wide overall	608	110 (18.1)	97 (16.0)	42 (6.9)	22 (3.6)	1 (0.16)
Shelter location						
Bryan/College Station	64	15 (23.4)	10 (15.6)	0 (0)	0 (0)	0 (0)
Dallas	93	13 (14.0)	16 (17.2)	0 (0)	0 (0)	0 (0)
Edinburg	91	15 (16.5)	19 (20.9)	16 (17.6)	9 (9.9)	0 (0)
El Paso	88	16 (18.2)	2 (2.3)	4 (4.5)	2 (2.3)	0 (0)
Fort Worth	91	5 (5.5)	16 (17.6)	7 (7.7)	2 (2.2)	0 (0)
Houston	86	18 (20.9)	19 (22.1)	0 (0)	0 (0)	1 (1.2)
San Antonio	95	28 (29.5)	15 (15.8)	15 (15.8)	9 (9.5)	0 (0)

were from Dallas (TcI), both sampled in May; 3 from San Antonio (TcI), 1 sampled in each of July, September, and December; and 1 dog from El Paso (TcIV), sampled in January. Only 1 of these 6 PCR-positive dogs (San Antonio, December) was seropositive on both the Stat-Pak and CDP serologic tests and therefore considered seropositive in our analysis; 3 of the 6 dogs were positive on the CDP only, and 2 PCR-positive dogs were negative on both serologic tests. Of the remaining unconfirmed suspect PCR-positive dogs, 6 of 47 (11.3%) dogs were seropositive on both Stat-Pak and CDP. There was no agreement between serostatus and suspect PCR status ($\kappa = -0.02$, 95% CI = $-0.2-0.1$, $P = .62$).

3.6 | Ticks

We found ticks on 74 dogs (12.2%) at 5 shelters (Dallas, Edinburg, El Paso, Fort Worth, and San Antonio), with number of ticks per infested dog ranging from 1 to 43. No ticks were found on dogs sampled at the Bryan/College Station or Houston shelters. Tick infestation was most prevalent in the Edinburg shelter, where ticks were found on 55 of 91 dogs (60.4%). We collected and identified a total of 353 ticks, all adults. The majority ($n = 346$, 98.0%) were *Rhipicephalus sanguineus*, found at all 5 above-named shelters, with an additional 6 *Ixodes scapularis* collected from Fort Worth and 1 *Dermacentor variabilis* from Edinburg.

4 | DISCUSSION

In our study, we report an overall *T. cruzi* seroprevalence of 18.1% in dogs in shelters across Texas, with prevalence estimates in individual shelters ranging from 5.4% to 29.5%. Seroprevalence of *T. cruzi* was comparable to prevalence of *D. immitis* (16%) in the sampled population and higher than the seroprevalences of *Ehrlichia* spp. (3.6%), *Anaplasma* spp. (6.9%), and *B. burgdorferi* (0.2%). We documented coinfections or coexposures with multiple vector-borne pathogens in many dogs, but only coexposure to *Ehrlichia* spp. and *Anaplasma* spp. was significantly more frequent than would be expected due to chance. Odds of coexposure or coinfection are expected to be lower for pathogens that have different vectors (ie, mosquitoes versus kissing bugs versus different tick species)¹; however, certain risk factors such as time spent outside and lack of antiparasitic treatment might lead to increased exposure to many types of vectors.

The *T. cruzi* seroprevalence we report (18.1%) is on the high end of prevalence estimates reported in previous serosurveys of dogs from Texas, Louisiana, and Oklahoma, which ranged from 3.6% to 22%.^{11-13,28,36} Our other more targeted studies of Texas canine populations have revealed 57.6% seroprevalence ($n = 85$) in working dogs housed in outdoor kennels,³⁴ 19.6% in dogs in underserved communities in south Texas ($n = 209$),³⁶ and 7.4%-18.9% in government working dogs along the Texas-Mexico border.¹⁹ The *T. cruzi* seroprevalence obtained in our study (18.1%) is more than twice the estimated seroprevalence (8.8%, $n = 205$) we previously reported from initial spring sampling at these shelters,¹⁵ owing to revised test interpretation criteria and the availability of the additional serologic test (CDP) used in the current study. Discordance between serologic test results and the lack

of a gold standard is a major obstacle in *T. cruzi* diagnostics, as we observed in our study and has been previously discussed.^{19,37}

The 16.0% *D. immitis* infection prevalence we found is higher than the 5.5% reported in a large survey of owned dogs presented to veterinary clinics in Texas,³⁸ but similar to the 14.6% reported in a survey of shelter dogs in Florida.³⁹ The finding of higher prevalence of *D. immitis* infection in shelter dogs is expected, considering the origin of most shelter dogs as free-roaming strays or relinquished as unwanted dogs by their owners, therefore, unlikely to be receiving preventive antiparasitic drugs or ectoparasite prevention. Recent studies have illuminated the problem of false-negative results on heartworm antigen tests because of immune complex formation that sequesters antigen. Because we did not subject our serum samples to heat treatment and did not conduct tests to detect microfilaria, the prevalence we report could be an underestimate of the true prevalence by approximately 5%-10%.^{40,41}

The seroprevalence for *Ehrlichia* spp. we found (3.6%) is similar to a previous report for dogs across the Southeast including Texas (3.2%), but the *Anaplasma* spp. prevalence we found (6.9%) is higher than in that study (4.4% United States overall, 0.9% Southeast).⁴ Unfortunately, because the test used does not allow differentiation between the individual species of *Anaplasma* and *Ehrlichia*, it is difficult to draw conclusions related to the specific *Anaplasma* and *Ehrlichia* species individually. The higher level of coinfection with *Ehrlichia* and *Anaplasma* spp. could be explained by a common vector, as *R. sanguineus* is the vector of *E. canis* and is likely the principal vector of *A. platys*^{42,43} and was the most common tick species we encountered on dogs in shelters during our study. In particular, this tick species commonly infested dogs at the southernmost shelter along the Texas-Mexico border (Edinburg), where seroprevalence for *Anaplasma* spp. and *Ehrlichia* spp. was the highest. *Rhipicephalus sanguineus* is particularly problematic in shelter settings, because of the ability of the vector to complete a full life cycle indoors and infest dogs year round. Some shelters might have applied ectoparasiticides to dogs upon admission, and this would have reduced the number of ticks on dogs at those shelters; unfortunately, information on this practice was not recorded during our study.

Our results suggest a very low risk of exposure of dogs in Texas to *B. burgdorferi*, agent of Lyme disease. We found a seroprevalence of 0.2% ($n = 1$), which is the same estimate that was previously reported in Texas from the results of a national veterinary clinic-based survey.³⁸ The single-aged dog with a positive *B. burgdorferi* antibody result had an unknown history, which could have involved travel outside of Texas during his 14 years of life before presenting to the urban shelter in Houston. As dogs are considered good sentinels for *B. burgdorferi*,^{2,3} we expect this low prevalence in shelter dogs across the state is representative of a low risk of *B. burgdorferi* in Texas. This is supported by previous reports of low prevalence of this pathogen in ticks, animals, and humans across the southern United States.^{1,4,38,44-46}

The geographic patterns of canine *T. cruzi* seropositivity generally fit with previous estimates of *T. cruzi* risk distribution in Texas,^{12,47} with the exception of El Paso, where dogs had a relatively high seroprevalence in our study (18.0%) despite predicted low transmission risk in the area in previous studies.^{12,47} There is potential for shelter operational differences, source of dogs, or other unmeasured factors

to influence relative prevalence for these pathogens. Our analysis of dog origin (stray versus owner-relinquished) was hindered by a large number of dogs of unknown origin, resulting from variation among shelters in the classification of dogs and dogs that were transferred from other local shelters with their original origin unknown.

In terms of the other putative risk factors, although the sporting and working breed groups were overrepresented among *T. cruzi*-infected dogs in a previous study,¹² we did not find a difference in seropositivity among breed groups suggesting multiple breed groups share the risk of exposure. However, classification of shelter dogs into breed groups based solely on physical appearance has the potential for misclassification bias.^{48,49} We found dogs aged 1 year or older had nearly double the *T. cruzi* seroprevalence (19%) than dogs younger than 1 year old (11%), although this difference was not statistically significant. The finding of increased odds of *D. immitis* positivity in dogs 1 year or older was expected considering the long prepatent period of *D. immitis* (approximately 6 months), and that older dogs have had more time to be exposed to vectors. Lower odds of *D. immitis* positivity in El Paso are consistent with the fact that El Paso is at a higher elevation and significantly less humid than other shelter locations, both factors that can negatively influence mosquito survival.

A small percentage (1.1%) of dogs had *T. cruzi* DNA in their blood confirmed by 2 qPCR assays. Although PCR does not demonstrate the presence of whole, viable parasites, a PCR-positive blood sample suggests that the dog could be parasitemic and thus serves as a source of infection to blood-feeding kissing bug vectors. Only 1 of the 6 confirmed PCR-positive dogs was seropositive on both the Stat-Pak and CDP, although 3 were positive on the CDP only. The low frequency of PCR-positivity among seropositive dogs suggests that dogs might not sustain high parasitemia levels after the acute stage of infection, and the low PCR-positivity overall suggests that this population of dogs did not include many acutely infected individuals.

Most of the *T. cruzi* PCR-positive dogs were infected with strain type TcI, with only 1 dog infected with TcIV. Although historic reports documented primarily TcIV infection in dogs in the United States,⁵⁰ our findings are consistent with our recent studies in Texas that found predominantly TcI with fewer TcIV infections.^{34,36,51} Thus far, autochthonous human cases of *T. cruzi* infection in the United States for which the DTU has been determined have consisted of TcI or unresolved TcII/V/VI, with no findings of TcIV in humans.^{52,53} Different strain types appear to have different pathologic effects in dogs^{10,54,55} and potentially in humans,⁹ and determination of which strain type a dog is infected with could provide clinical insight or be relevant for zoonotic concerns.

Chagas disease is an underrecognized threat to canine health, and shelter dogs across Texas show high *T. cruzi* seroprevalence (18.1%), nearly equal to the prevalence of *D. immitis* (16.0%) in the same population. This serosurvey provides a baseline for understanding *T. cruzi* risk across the southern United States and reinforces the need for better options for diagnosis and treatment of infected animals, as well as for vector control and infection prevention efforts. Furthermore, our findings stress the importance of comprehensive vector-borne disease testing in dogs adopted from shelters in the Southern United States and of vector control within shelters to block transmission. In addition, movement (eg, through pet adoptions, rescues, or relocation

of owned animals) of *T. cruzi*-infected dogs from the south to other areas of the country is a growing concern. This risk is recognized for *D. immitis*, especially after movement of dogs as part of disaster response,⁵⁶ and protocols are now in place to test for heartworm before moving dogs,⁵⁷ but testing for *T. cruzi* exposure should also be included in such protocols for dogs being relocated from the southern United States. Although placement of *T. cruzi*-infected animals in northern states would be a dead-end from a transmission standpoint because of the lack of triatomine vectors, such dogs may encounter a veterinary community with little awareness for this disease. In addition, from a One Health perspective, any advances in our understanding of canine Chagas disease have the potential to also advance human health given the shared risk factors of triatomines in the environment, similar disease progression between humans and dogs, and parallel challenges with respect to suboptimal diagnostics.

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CONFLICT OF INTEREST DECLARATION

J. Rodriguez is currently employed by Zoetis, Inc. Zoetis did not sponsor this research nor was J. Rodriguez a Zoetis employee at the time the research was conducted.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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

The study was conducted in accordance with client-owned animal use protocols approved by the Texas A&M University IACUC (AUP 2012-0267).

HUMAN ETHICS APPROVAL DECLARATION

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

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SUPPORTING INFORMATION

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