PATHOLOGY AND DISCRETE TYPING UNIT ASSOCIATIONS OF *TRYPANOSOMA CRUZI* INFECTION IN COYOTES (*CANIS LATRANS*) AND RACCOONS (*PROCYON LOTOR*) OF TEXAS, USA

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ABSTRACT: *Trypanosoma cruzi* is a vector-borne, protozoal parasite of mammals. Infected humans, dogs (*Canis lupus familiaris*), and nonhuman primates may remain asymptomatic or may develop Chagas disease, most commonly characterized by lymphoplasmacytic myocarditis with myocardial degeneration and fibrosis, ultimately resulting in heart failure. Although wildlife species have important roles as sylvatic reservoirs, investigations into the pathology of *T. cruzi* in wildlife are limited to a few studies documenting histologic lesions in opossums (*Didelphis* spp.) and raccoons (*Procyon lotor*). Pathology in coyotes (*Canis latrans*) has not, to our knowledge, been described, despite their recognition as a reservoir and close genetic relationship to domestic dogs. Our objectives were to perform a detailed, comparative cardiac pathology study of sympatric, naturally infected coyotes and raccoons, to characterize the overall *T. cruzi* infection prevalence in the heart and blood of each species via PCR, and to identify infecting discrete typing units (DTUs). We sampled hunter-harvested coyotes (*n*=120) and raccoons (*n*=24) in a 28 county region of central and south Texas, US. Raccoons were significantly more likely to have positive PCR results (*P*<0.001) with a prevalence of 62% (15/24), comprising DTU TcIV exclusively, with mild to no evidence of cardiac pathology. In contrast, coyotes had a lower infection prevalence (8%, 10/120), comprising DTU TcI exclusively, with lymphoplasmacytic myocarditis observed in four of the six PCR-positive animals. Many raccoons had PCR-positive blood and heart tissue simultaneously, supporting previous reports that raccoons maintain parasitemia into chronic stages of infection; in contrast, none of the PCR-positive coyotes were positive in both heart and blood. Our findings demonstrate marked differences in *T. cruzi* infection dynamics between coyotes and raccoons, with important implications for reservoir potential and their role in transmission cycles.

Key words: American trypanosomiasis, Chagas, coyotes, myocarditis, pathology, raccoons, *Trypanosoma cruzi*.

INTRODUCTION

Trypanosoma cruzi, the vector-borne protozoal agent of Chagas disease, is endemic across much of Latin America and is capable of infecting more than 200 mammalian species (Hoare 1972). The parasite multiplies in the hindgut of triatomine insect vectors (family Reduviidae, subfamily Triatominae), which pass infectious trypomastigotes in their feces. Chagas disease is a major public-health problem in endemic areas and is increasingly recognized as a threat to human and veterinary public health across the southern US, where sylvatic transmission cycles among

vectors and wildlife reservoirs have been recognized for decades (Kofoid and McCulloch 1916; Kofoid and Donat 1933). Although many infected hosts may remain asymptomatic, some infected humans, dogs, and nonhuman primates develop cardiac disease, leading to sudden death or congestive heart failure (Rassi et al. 2010; Bern et al. 2011; Snowden and Kjos 2013).

Across the Americas, *T. cruzi* is maintained in complex transmission cycles involving diverse mammalian reservoir species and triatomine vector species. The complexity of those cycles and regional heterogeneity is one of the major challenges in Chagas disease

control and prevention. In the US, more than 30 wildlife species have been identified as susceptible hosts, but the relative importance of those species as reservoirs (i.e., their contribution to the transmission and maintenance of the parasite in nature by serving as sources of infection to vectors) has been understudied (Hodo and Hamer 2017). Additionally, investigations into the pathology of *T. cruzi* in naturally infected wildlife have been conducted on only a limited basis and in only a few species (Packchanian 1942; Ryan et al. 1985; Barr et al. 1991a; Pietrzak and Pung 1998). An understanding of the degree to which various wildlife reservoirs are clinically affected by *T. cruzi* infection (i.e., their position on the spectrum from unaffected carriers of the parasite to severely diseased hosts) is necessary for predicting population-level effects of infection as well as for targeting interventions to manage zoonotic risk.

Trypanosoma cruzi is genotypically heterogeneous and is divided into seven discrete typing units (DTUs): TcI–TcVI and TcBat, which are associated with different geographical regions (Marcili et al. 2009; Zingales et al. 2012), mammalian hosts (Jansen et al. 2017), and vector species (Brenière et al. 2016). Further, there is evidence for associations between DTU and varying clinical outcomes in humans (Ramírez et al. 2010) and dogs (Barr et al. 1991b; Duz et al. 2014) as well as in other experimental animal models (Lisboa et al. 2007; Roellig et al. 2009b). The DTUs TcI and TcIV predominate in the US (Roellig et al. 2008; Bern et al. 2011; Hodo and Hamer 2017), and DTUs TcII, TcIV, TcV, and TcVI were identified in a few rodents in Louisiana (Herrera et al. 2015; Pronovost et al. 2019). In the US, only DTU TcI and an unresolved DTU TcII/V/VI group have been associated with autochthonous human infection (Roellig et al. 2013; Garcia et al. 2017). Regarding vertebrate host associations in the US, most sampled raccoons (*Procyon lotor*) were infected with DTU TcIV, whereas opossums (*Didelphis* spp.) were almost exclusively infected with DTU TcI (Roellig et al. 2008; Bern et al. 2011; Hodo and Hamer 2017).

Both DTUs TcI and TcIV have been identified in skunks (*Mephitis mephitis*), armadillos (*Dasypus novemcinctus*), woodrats (*Neotoma* sp.), and domestic dogs (*Canis lupus familiaris*; Roellig et al. 2008; Charles et al. 2013; Curtis-Robles et al. 2017a; Hodo and Hamer 2017). These DTU associations may be important in classifying wildlife species as important reservoirs of infection. Additionally, associations between strain type and pathology or clinical outcome in wildlife may be translatable for human and domestic animal health.

Raccoons are perhaps the best-studied reservoir species in the US, and *T. cruzi* infection dynamics and pathology are described in both naturally infected and experimentally infected animals (Pietrzak and Pung 1998; Roellig et al. 2009b; Bern et al. 2011). In contrast, coyotes (*Canis latrans*) are a relatively understudied host species, for which most previous studies report seroprevalence only (Hodo and Hamer 2017) and for which, to our knowledge, only a single, limited pathology study exists (Curtis-Robles et al. 2016). Raccoons and coyotes are abundant in both rural and urban settings, having the potential to bridge parasite infections from sylvatic to peridomestic habitats. Our objectives were to perform a detailed comparative cardiac-pathology study of naturally infected coyotes in central and south Texas and sympatric raccoons in central Texas and to characterize the overall *T. cruzi* infection prevalence in the heart and blood of each species via PCR with identification of infecting DTU.

MATERIALS AND METHODS

Sampling

We conducted a cross-sectional sampling effort in January 2016 at an annual recreational nuisance-animal hunt organized by private landowners in central Texas. Animals from that hunt have been studied in prior years in the context of *T. cruzi* infection, such that we anticipated finding infected animals (Curtis-Robles et al. 2016). Animals legally harvested under recreational permits by teams of hunters during a 24-h period were brought to a central check station. The area
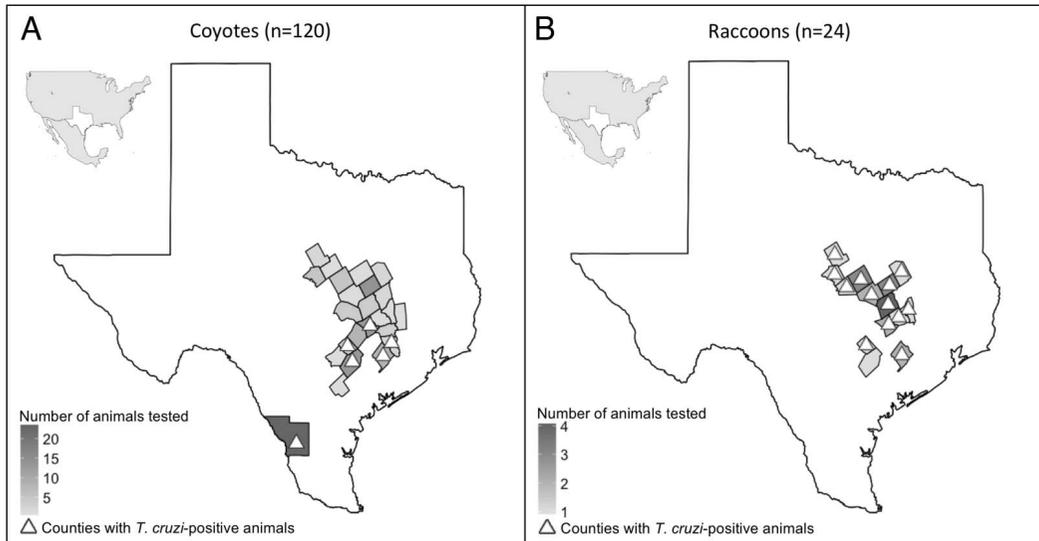
FIGURE 1. Sampling locations of (A) coyotes (*Canis latrans*) and (B) raccoons (*Procyon lotor*) by county in Texas, USA, during January and March 2016. Hearts and blood of these hunter-harvested animals were tested via PCR for presence of *Trypanosoma cruzi*. Triangles mark counties from which *T. cruzi* PCR-positive animals were collected.

of harvest encompassed 25 counties in central Texas (Fig. 1) spanning five different ecoregions (Gould et al. 1960). Our team collected samples from coyotes and raccoons for which the county of harvest was known. Hearts and blood (when available) were collected in the field within 24 h of death. At the time of collection, each heart was briefly examined for gross lesions. Blood was collected from the axillary vasculature or the thoracic cavity, as available, during heart collection. Blood and hearts were transported to the laboratory on ice.

In the laboratory, blood samples were centrifuged, and serum was collected from those samples without extensive hemolysis. A 500-μL volume of a blood clot or whole, hemolyzed blood was subsampled from each blood sample and stored at −20 C until DNA extraction. Hearts were stored at −80 C for 4–5 mo, then thawed and dissected to examine right and left atria and ventricles. Two tissue samples were taken from each of the four chambers. One section from each chamber was stored in 10% neutral-buffered formalin for histology, and the other section was minced. Minced samples from all chambers for each animal were pooled together for DNA extraction.

We also received samples from coyotes collected during an oral rabies-vaccine program surveillance in Webb County (south Texas) by the Texas Department of State Health Services in coordination with US Department of Agriculture– Animal and Plant Health Inspection, Wildlife

Services, Texas branch, from 29 February 2016 to 1 March 2016 (Fig. 1). Heart apex, blood-soaked Nobuto filter paper (Advantec MFS, Dublin, California, USA), and whole blood (when available) were collected by Department of State Health Services personnel in the field. A section of the heart apex was subsampled and minced for DNA extraction.

Molecular work

From each animal, as available, one approximately 0.5-cm^3 sample from each heart (representing all four chambers from central Texas animals and the apex only from south Texas animals), 500 μL of blood, and one Nobuto strip were subjected to DNA extraction with the Omega® E.Z.N.A.® Tissue Extraction Kit (Omega Bio-tek, Norcross, Georgia, USA) according to the manufacturer's protocol for tissue extraction with an overnight lysis for the hearts and Nobuto strips and ≥3 h lysis for blood.

After DNA extraction, samples were tested for infection with *T. cruzi* with a highly sensitive quantitative PCR to amplify a 166-base pair fragment of *T. cruzi* satellite DNA, as previously described (Piron et al. 2007; Curtis-Robles et al. 2016). DNA-negative controls (water) and a positive control of DNA extracted from a pure culture of Sylvio X10 CL4 (ATCC 50800, ATCC, Manassas, Virginia, USA; DTU TcI) were included in all reactions. Samples with a cycle threshold (Ct) value less than 36 were considered suspect positive

and subjected to a multiplex quantitative PCR targeting the spliced leader intergenic region to confirm positivity and for determination of DTU, according to previously described protocols (Cura et al. 2015; Curtis-Robles et al. 2017a). The criterion for considering a sample positive on this assay was the detection of specific fluorescence to one or more DTU-specific probes within 40 cycles in a 45-cycle assay. Negative controls (water) and positive controls of DNA extracted from *T. cruzi* strain Sylvio X10 CL4 (DTU TcI, details presented earlier) and *T. cruzi*-infected *Triatoma sanguisuga* from Texas (DTU TcIV) were included in all reactions, and the positive control of DNA extracted from *T. cruzi* Y-strain (ATCC 50832, ATCC; DTU TcII) was added for reactions run later in the study. Samples were considered positive if they generated Ct values below the respective thresholds on both PCR assays, and animals were considered PCR positive if either blood or heart samples were positive.

Histopathology

Formalin-fixed heart tissue from central Texas coyotes and raccoons was processed routinely for histopathology and stained with H&E. Two slides from each animal, representing right and left atria and ventricles, were examined by light microscopy by a board-certified veterinary pathologist, blinded to the PCR status. Inflammation was semiquantitatively scored for each heart chamber on a numeric scale as normal (0), minimal (1), mild (2), moderate (3), or severe/marked (4). Additionally, the presence of fibrosis, cardiomyocyte degeneration, or necrosis and the distribution (focal, multifocal, focally extensive) and location (interstitial, myocardial, epicardial) of lesions were recorded. An inflammation index for each animal was calculated by adding the inflammation scores for each chamber. For analysis, animals were dichotomized by pathology status (significant lesions present or absent), in which significant was defined as an inflammation score ≥ 3. The inflammation cutoff of 3 was chosen because it represented at least minimal inflammation in three heart chambers, at least mild inflammation in one section and minimal in another, or at least moderate inflammation in any one heart chamber. Slides from animals with an inflammation score ≥ 3 were reexamined, and a descriptive morphologic diagnosis was recorded. Animals with lymphoplasmacytic myocarditis (consistent with expected lesions of *T. cruzi* infection) were included in the statistical analyses.

Statistical analysis

We tested for significant differences between the presence of *T. cruzi* DNA in samples (PCR status of heart and blood) and host attributes of species and sex using a Fisher's exact test. Further, we used the Fisher's exact text to compare the presence of *T. cruzi* DNA in samples with the presence of lymphoplasmacytic myocarditis for each species separately. Finally, the Mann-Whitney-Wilcoxon test was used to determine whether the inflammation scores differed between *T. cruzi*–positive coyotes and raccoons and whether the Ct values of PCR-positive blood samples were different between species. Statistical analyses were performed in R software (R Core Team 2018).

RESULTS

Sample population

We sampled 120 coyotes from 24 Texas counties and 24 raccoons from 14 counties (Fig. 1). Both males and females of each species were sampled. We collected hearts from 97 central Texas coyotes and 23 raccoons and heart apex from 23 south Texas coyotes. Blood was available for 92 coyotes and 18 raccoons from central Texas and 21 coyotes from south Texas. We also received blood-soaked Nobuto filter paper strips from all 23 south-Texas coyotes.

PCR results

A total of 8% (10/120) of coyotes and 62% (15/24) of raccoons were confirmed to have positive PCR results for *T. cruzi* on two separate quantitative PCRs of either heart or blood (Table 1). Raccoons were significantly more likely to be *T. cruzi*–positive than were coyotes ($P < 0.001$). Sex was not associated with PCR status within either species. One coyote with PCR-positive blood was based on testing of a Nobuto strip (whole blood was not available for that animal). All other Nobuto strips tested had negative results, including three from coyotes with PCR-positive whole blood. The Ct values in *T. cruzi*–positive blood were significantly less for raccoons (mean=24.5, median=25.2, range, 18.7–27.4) than coyotes (mean=32.6, median=33.2, range, 31.1–33.86; P=0.002); thus, infected raccoons had a higher concentration of parasite DNA in blood than did coyotes did. All infected coyotes harbored DTU TcI,

TABLE 1.   Results of *Trypanosoma cruzi* PCR testing of heart and blood from hunter-harvested coyotes (*Canis latrans*) and raccoons (*Procyon lotor*) from central and south Texas, USA, collected in January and March 2016.

| | | Samples positive by PCR for *Trypanosoma cruzi*[a] | | | | | |
| | | Heart | | Blood | | Total | |
Species	No.	%	Frequency	%	Frequency	%	Frequency
Coyotes	120	4	5/120	4	5/113	8	10/120
Raccoons	24	52	12/23	44	8/18	62	15/24

[a] Frequency = no. positive/no. tested.

whereas all infected raccoons harbored DTU TcIV. No individual animals were found to harbor more than one DTU. Positive raccoons were identified in 13 of 14 sampled counties, and positive coyotes originated from five of 25 sampled counties (Fig. 1).

Pathology

Significant gross lesions, other than gunshot wound–associated trauma, were not observed in any of the hearts. Incidentally, adult heartworms (*Dirofilaria immitis*) were observed in six coyotes. Histologically, 62 of 120 animals had no lesions, 41 of 120 had minimal findings that were not considered significant (inflammation score <3), and 15 of 120 animals (11 coyotes and four raccoons) had lesions considered to be significant (inflammation score ≥3) for further characterization and inclusion in the significant-lesions group for analysis. One coyote was excluded from the histopathology analysis because of severe autolysis across all tissue sections examined. Four other coyotes that had severe autolysis of all sections, except for the left ventricle, were retained in the analysis and had no significant lesions in the assessed region of left ventricle.

Of the 11 coyotes with significant lesions, six (including four *T. cruzi*–positive animals) had mild to moderate, multifocal, lymphoplasmacytic myocarditis with varying degrees of myocardial degeneration and fibrosis (Fig. 2A–C), consistent with that described for *T. cruzi* infection in other species (Barr et al. 1991c; Andrade et al. 2009; Pisharath et al. 2013; Snowden and Kjos 2013). Another four of the 11 coyotes with significant lesions had severe locally extensive inflammation that was

primarily histiocytic or pyogranulomatous, occasionally with visible intraleukocytic zoites, most consistent with *Hepatozoon americanum* infection (Davis et al. 1978). None of these animals had positive PCR results for *T. cruzi*. Mature cysts of *H. americanum* were observed in two of these animals, as well as in other coyotes without inflammation. Another coyote had mild, multifocal pyogranulomatous inflammation associated with microfilariae of *D. immitis*. Cysts of *Sarcocystis* sp. were observed in two coyote hearts, with no accompanying inflammation. Two of the six *T. cruzi*–positive coyotes had no apparent lesions in the sections examined.

Of the five raccoons with significant histologic lesions (including three *T. cruzi*–positive animals), three (two of which were *T. cruzi* positive) had minimal to mild lymphoplasmacytic myocarditis (Fig. 2D) considered possibly consistent with *T. cruzi* infection, which was accompanied by fibrosis in one animal. In a fourth, *T. cruzi*–positive raccoon, lymphoplasmacytic inflammation was accompanied by abundant eosinophils, which is not typically associated with *T. cruzi*. The fifth, *T. cruzi*–negative raccoon with lesions had moderate-to-severe multifocal histiocytic and lymphoplasmacytic perivascular myocarditis and epicarditis. A *Sarcocystis* sp. cyst was also observed in that animal but not in the area of inflammation. *Trypanosoma cruzi* amastigotes were not observed in any of the sections examined for either species. A total of 80% (12/15) of the *T. cruzi*–positive raccoons had no significant inflammation in the heart sections examined.
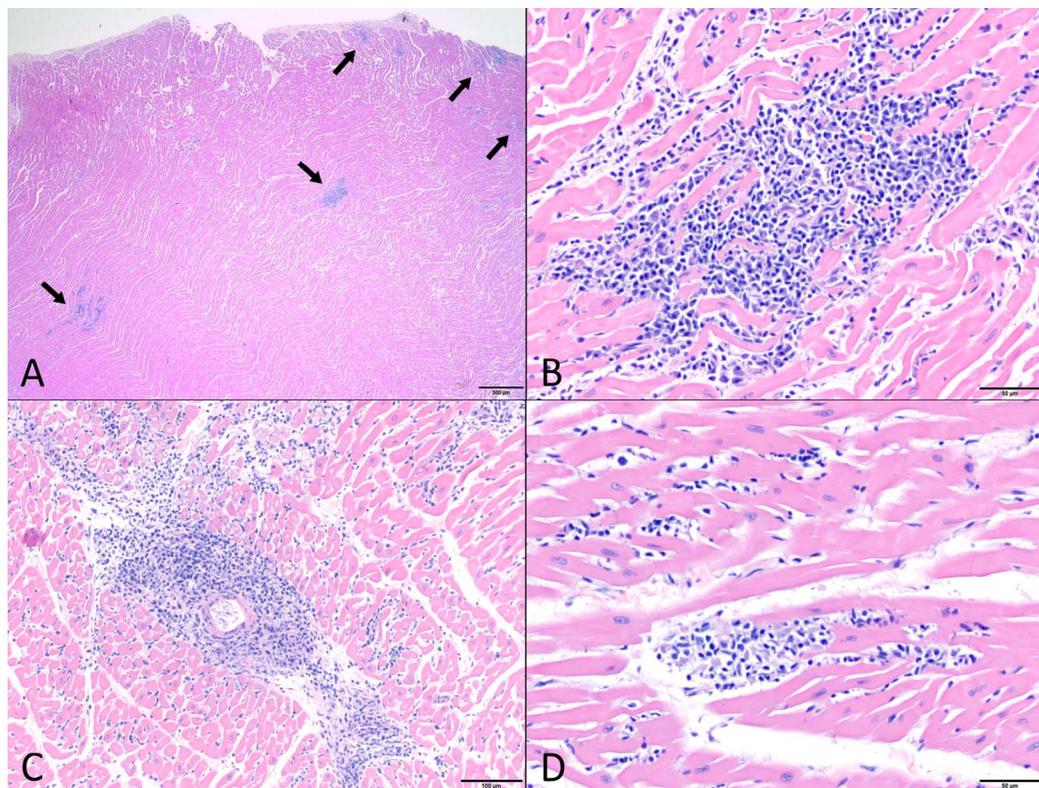
FIGURE 2.   Photomicrographs showing myocarditis in coyotes (*Canis latrans*) and raccoons (*Procyon lotor*) collected from central Texas, USA in 2016, H&E stain. (A) Scale bar=500 μm, coyote C16-38, left ventricle; inflammation is present in multiple areas across the section. (B) Scale bar=50 μm, higher-magnification view of (A); cardiac myofibers are separated by a moderate amount of inflammation composed primarily of lymphocytes and plasma cells, with myocellular degeneration and loss. (C) Scale bar=100 μm, right ventricle, coyote C16-75; moderate lymphoplasmacytic inflammation is perivascular and infiltrates between cardiac myofibers, disrupting normal architecture. (D) Scale bar=50 μm, raccoon R16-23, left ventricle; mild lymphoplasmacytic myocarditis.

Table 2 shows the number of animals with lymphoplasmacytic myocarditis compared with *T. cruzi* status. The presence of lymphoplasmacytic myocarditis was significantly associated with *T. cruzi* positivity in coyotes ($P<0.001$) but not in raccoons ($P=0.713$). Additionally, the severity of lymphoplasmacytic myocarditis (as measured by the combined inflammation score) was greater for *T. cruzi*–positive coyotes than for negative coyotes ($P<0.001$) when other types of inflammation (attributed to other etiologic agents) were excluded.

DISCUSSION

In a region of central and south Texas known as a hotspot for *T. cruzi* transmission (Curtis-Robles et al. 2016, 2017b, 2018), both raccoons and coyotes had *T. cruzi*–positive blood and cardiac tissue, sometimes associated with myocarditis. Thus, these species may not only be involved in the sylvatic transmission cycle, but also be negatively affected by the infection in some cases, especially coyotes. Although the overall *T. cruzi* prevalence was significantly higher in raccoons (62%; 15/24) than in coyotes (8%; 10/120), the infected coyotes exhibited more-severe histologic lesions than did infected raccoons. Finally, we found an association between parasite DTU and host taxa; all 10 coyotes for which DTU was determined harbored DTU TcI, whereas all 15 raccoons harbored DTU TcIV.

TABLE 2.   Two-by-two comparison of *Trypanosoma cruzi* PCR status and presence or absence of lymphoplasmacytic myocarditis in hunter-harvested coyotes (*Canis latrans*) and raccoons (*Procyon lotor*) collected from central Texas, USA, in January and March 2016.

| Species | PCR results | No. | Presence of lesions[a] | | | | |
| | | | Lymphoplasmacytic myocarditis | | No myocarditis, or with other etiology | | |
			%	Frequency	%	Frequency
Coyotes	Positive	6	67	4/6	33	2/6
	Negative	90	2	2/90	98	88/90
	Total	96	6	6/96	94	90/96
Raccoons	Positive	15	20	3/15	80	12/15
	Negative	8	25	2/8	75	6/8
	Total	23	22	5/23	78	18/23

[a] Frequency = no. positive/no. tested.

The infection prevalence we found was consistent with previous reports in both raccoons and coyotes, where prevalences range from 20% to 90% in raccoons across the southern states (Hodo and Hamer 2017), and from 4% to 14% in coyotes in several southern states (Hodo and Hamer 2017). Specifically, a study that sampled animals during a previous year of the recreational hunt across many of the same central Texas counties reported prevalences of 70.0% in raccoons and 14.3% in coyotes (Curtis-Robles et al. 2016). The likely route of transmission in these animals is oral, through ingestion of infected kissing bug vectors. This is considered an important route in dogs (Barr 2009) and was effective in experimentally infecting raccoons and skunks (Davis et al. 1980; Roellig et al. 2009a). Transmission may also occur through ingestion of parasitemic vertebrates (Thomas et al. 2007; Rocha et al. 2013), although ingestion of infected meat did not infect raccoons (Roellig et al. 2009a).

Only minimal to mild lesions have been reported from natural infection with *T. cruzi* in raccoons (Pietrzak and Pung 1998; Charles et al. 2013; Curtis-Robles et al. 2016), consistent with our findings. Experimentally infected raccoons exhibited mild to severe lesions that varied with acuteness of infection and infecting DTU, exhibiting more-severe cardiac lesions during the acute stages of infection with DTUs TcI and TcII than with DTU TcIV (Roellig et al. 2009b). The specific lesions described in previous studies are very similar to those we observed: inflammation composed primarily of lymphocytes and plasma cells, with occasional myocardial degeneration or necrosis and infrequent observations of intramyocellular *T. cruzi* amastigotes. Significant histopathologic lesions in *T. cruzi*–infected coyotes have not previously been described, but our findings of multifocal lymphoplasmacytic inflammation with destruction of cardiomyocytes were consistent with lesions reported in infected dogs (Barr et al. 1991c; Snowden and Kjos 2013). A limitation of our study was that severely affected animals with advanced heart disease, including animals that died, are not represented in our study population because they would not be available for hunters to harvest.

Autolysis hindered interpretation of some histologic sections, but less than 5% of examined sections were affected to the extent that we could not determine the presence or absence of inflammation. Overall, only minimal artifactual change resulted from the single freeze-thaw cycle at −80 C, which was manifested mainly by artifactual separation of myofibers. The relative lack of freeze-thaw artifact observed should be considered in future sampling efforts for which histopathology may have otherwise been discounted because logistics would require freezing of samples.

Definitive diagnosis of *T. cruzi* based solely on histopathology is often difficult because the intracellular amastigote form is not commonly observed in chronic infections. Indeed, in our study, we did not observe any amastigotes in histologic sections. However, although not pathognomonic, the multifocal and infiltrative pattern of lymphoplasmacytic myocarditis is supportive of *T. cruzi* infection, especially when accompanied by positive PCR or serologic results. Studies in nonhuman primates identified an association between nonspecific lymphocytic myocarditis and the presence of *T. cruzi* DNA (Andrade et al. 2009; Mubiru et al. 2014). In our study, lymphoplasmacytic inflammation was associated with *T. cruzi*–positive PCR status in coyotes but not in raccoons. Inflammation was observed in the hearts of several PCR-negative coyotes and, in some cases, was explained by the presence of other parasites, including *H. americanum* and *D. immitis* and was characterized by the presence of macrophages and neutrophils. Other differentials for lymphoplasmacytic myocarditis in these animals included *Bartonella* sp. (Chomel et al. 2006) or *Borrelia burgdorferi* (Janus et al. 2014), although *Bartonella* sp. is most often associated with valvular endocarditis (Pesavento et al. 2005), and *B. burgdorferi* is uncommon in Texas (Bowman et al. 2009; Mitchell et al. 2016; Hodo et al. 2019).

Additionally, *T. cruzi*–negative status in the face of lesions suggestive of *T. cruzi* could also be explained by false-negative PCR results. Testing results from a single blood sample or small pieces of heart tissue do not necessarily reflect the true infection status of the individual. Thus, false-negative results could have resulted from sampling error because of the multifocal nature of the parasite distribution. This was supported by our histologic findings in which inflammation was focal to multifocal and not diffusely distributed throughout all sections. Other raccoon studies have reported PCR-positive results in some, but not all, sections of heart from the same animal (James et al. 2002; Curtis-Robles et al. 2016).

Eight of 11 *T. cruzi*–infected raccoons had PCR-positive blood, and all but one of those also had positive heart tissue (Table 1), suggesting chronic infection. Although PCR cannot confirm the presence of whole, viable parasites, the presence of parasite DNA in blood is suggestive of parasitemia and likely infectiousness to vectors. In contrast, we only detected *T. cruzi* DNA in the blood of five of 10 infected coyotes, and none of these had PCR-positive heart tissue (Table 1). This suggested the possibility that, although raccoons appear to maintain long-term parasitemia with DTU TcIV, coyotes may only circulate DTU TcI during the acute stage of infection before the parasite localizes in tissues.

We found exclusive associations between host taxa and parasite DTU, in which raccoons were only infected with DTU TcIV and coyotes only with DTU TcI. The difference in degree of pathology observed between coyotes and raccoons could be explained by host species-level or DTU-level differences. In domestic dogs, experimental evidence supports differences in pathology related to different parasite strains. For example, dogs infected with *T. cruzi* isolates from an armadillo and opossum developed acute and chronic myocarditis, whereas dogs infected with an isolate from a dog did not develop disease (Barr et al. 1991c). In another study, DTU TcI infection resulted in more inflammatory cells in the hearts of infected dogs compared with DTU TcII infections (Duz et al. 2014). Raccoons are almost exclusively infected with DTU TcIV across multiple studies, with few reports of natural DTU TcI infection (Roellig et al. 2008; Bern et al. 2011; Curtis-Robles et al. 2016). Experimentally, raccoons were successfully infected with DTUs TcIV, TcI, and TcII, but developed longer-lasting parasitemia with DTU TcIV and more-severe cardiac lesions with DTUs TcI and TcII infections (Roellig et al. 2009b). *Trypanosoma cruzi* DTU TcIV may be host adapted to raccoons, supported by their high infection prevalence and lack of obvious pathology (Roellig et al. 2009b). No such association has been suggested for coyotes, and our findings of DTU TcI infection

with cardiac pathology may be evidence that DTU TcI is fundamentally a more cardiopathogenic strain than is DTU TcIV or simply that coyotes are more susceptible than raccoons to cardiac disease resulting from *T. cruzi* infection. In domestic dogs in the US, both DTU TcI and TcIV infections have been reported (Roellig et al. 2008; Hodo et al. 2019). Concerning other wildlife in central and south Texas, opossums harbor DTU TcI (Hodo et al. 2018), whereas both DTUs TcI and TcIV are found in skunks (Hodo et al. 2018) and woodrats (Charles et al. 2013). Interestingly, DTU TcIV has not been detected in autochthonous human infections of *T. cruzi* in the US (Roellig et al. 2008; Garcia et al. 2017), despite its widespread presence in triatomine vectors (Curtis-Robles et al. 2018). More research is needed on the comparative pathology of the different DTUs in canines as well as in humans. Although part of the response to *T. cruzi* infection is likely dependent on the individual host, evidence is accumulating that DTU may also have a role.

In conclusion, although coyotes had a lower *T. cruzi* infection prevalence than raccoons did, infection in coyotes was associated with more-severe lesions and with DTU TcI. These findings may have important implications for the association of *T. cruzi* DTU with resulting pathology as well as for the reservoir potential of coyotes and raccoons. We also provide further support for the association of DTU TcIV with raccoons in the US. Although raccoons are known to maintain high levels of parasitemia into the chronic stages of infection (Roellig et al. 2009b), further investigation of parasitemia dynamics of coyotes is needed to determine their contribution to the reservoir community for *T. cruzi*. Additional considerations necessitating further research are the risks posed to hunters exposed to infectious wildlife as well as the effect of infection on wildlife populations.

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