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TRYPANOSOMA CRUZI IN FREE-RANGING MAMMALIAN POPULATIONS IN SOUTH TEXAS

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ABSTRACT: Chagas disease, also known as American trypanosomiasis, is caused by the flagellate protozoan *Trypanosoma cruzi*. It is a significant health concern in South and Central America, where millions of people are infected or at risk of infection, and is an emerging health concern in the United States. The occurrence of Chagas disease in natural environments is supported by mammal host species, but those primary species may vary based on geographic location. In South Texas, the primary host species for the disease is poorly understood, and required a field study to determine the spatial distribution of *T. cruzi* prevalence in free-ranging mammals. Our study objectives were to determine the spatial distribution and prevalence of *T. cruzi* parasites in free-ranging mammals. We compared *T. cruzi* prevalence among species, among vegetative communities, and among different topographies (i.e., floodplain versus upland). From December 2011 through December 2013, 450 blood and tissue samples from geolocated free-ranging wildlife mammal species were analyzed with the use of polymerase chain reaction to detect protozoan *T. cruzi* DNA. We also calculated mammal abundance with the use of mark–recapture methodology and recorded capture-site characteristics such as vegetation structure. We found that animals in grasslands had a significantly lower infection rate when summed across all species compared with animals in dense hardwoods and semi-improved woodlands (P=0.001). A higher percentage of infections were found in the lower-elevation floodplain—65% (28/43) of animals sampled, compared to upland areas—25% (9/36) of animals sampled. Our study suggested that common free-ranging meso-mammals supported *T. cruzi* in natural environments and are of public health concern in South Texas. Mitigation strategies should consider a range of management activities to include vegetation management, selective application of insecticides, and changes in human behavior in high-risk areas.

Key words: Chagas disease, distribution, free-ranging mammals, triatomine insects.

INTRODUCTION

Chagas disease, caused by the flagellate protozoan *Trypanosoma cruzi*, is considered a significant human health problem in Central and South America, where 8–11 million people are infected (Centers for Disease Control and Prevention [CDC] 2011). Although there have been few cases reported in the United States, the CDC estimates that ≥300,000 immigrant people are or have been infected (Bern et al. 2011). The primary vector of Chagas infection is kissing bugs (*Triatoma* spp.), which are found from South America through the southern United States. Eleven species of triatomines are known to occur in North America (Bern et al. 2011), with the most common vectors being *Triatoma rubida* and *Triatoma protacta* in Arizona and California, and *Triatoma gerstaeckeri* and *Triatoma sanguisuga*, primarily found in Texas and New Mexico (Sarkar et al. 2010).

Infection with *Trypanosoma cruzi* occurs in free-ranging mammal species when fecal material from infected kissing bugs, containing infective *T. cruzi* protozoal trypomastigotes, is rubbed or introduced into the feeding bite wound or mucous membranes, or when feces contaminate food or water consumed by mammals (Lent and Wygodzinky 1979; Rozas et al. 2005; Kjos 2007). Triatomines are capable of transmitting the pathogen through

contact with infected blood and tissue, transplacentally, by carnivory, and through mammals feeding directly on infected triatomines (Roellig et al. 2009a).

We expanded on previous work (Kramm 2015) reporting effective maintenance of Chagas disease by meso-mammal populations, particularly raccoons (*Procyon lotor*) and Virginia opossums (*Didelphis virginiana*), in South Texas. The spatial distribution of *T. cruzi* in free-ranging mammalian populations is poorly understood (Bosseno et al. 2009; Brown et al. 2010), particularly in the United States (Sarkar et al. 2010). Control and risk management of *T. cruzi* requires an understanding of the reservoir host species and basic *T. cruzi* distribution at the local level to aid in developing prevention programs (Noireau et al. 2009; Brown et al. 2010; Lescure et al. 2010). An improved understanding of the impact of local geographic distribution (e.g., vegetative community, urban development) on *T. cruzi* prevalence will also support directed remediation (e.g., vegetation alteration, selective insecticide application). This is especially important as recent research suggests that Chagas disease may be more widespread in the United States than previously reported (Sarkar et al. 2010). The goal of our project was to understand better the current parasite–vector–host association within a geospatial context in South Texas. Specifically, our study objectives were to determine the spatial distribution of *T. cruzi* parasites in free-ranging mammalian species, and to determine the prevalence of *T. cruzi* in free-ranging mammals. This information can be used to provide a better understanding into the potential disease risk to South Texas communities, and in framing control or risk prevention strategies.

MATERIALS AND METHODS

Study area

Our study was conducted on Joint Base San Antonio (JBSA)–Lackland Annex (29°22′36"N, 98°39′36"W) Bexar County, Texas (Fig. 1). The 1,619-ha military installation supports multiple uses that include military mission and training activities, and is comprised of grasslands, managed woodlands, and deciduous riparian upland woodlands. Herbaceous cover primarily includes King Ranch bluestem (*Bothriochloa ischaemum* var. *songarica*), buffalograss (*Buchloe dactyloides*), and Texas wintergrass (*Stipa leucotricha*). Managed woodlands are similar to deciduous woodlands but differ in the relative openness of the areas due to periodic mowing and selective harvest of canopy species. Woodland plant species include mesquite (*Prosopis glandulosa*), sugarberry (*Celtis laevigata*), cedar elm (*Ulmus crassifolia*), annual sunflower (*Helianthus annuus*), and ashy sunflower (*Helianthus mollis*). Riparian woodland plant species include black willow (*Salix nigra*), green ash (*Fraxinus pennsylvanica*), basswood (*Tilia caroliniana*), sugarberry (*Celtis laevigata*), chinaberry (*Melia azedarach*), giant ragweed (*Ambrosia trifida*), and morning glory (*Ipomoea* sp.). The climate of this area is subtropical, with mild winters and hot summers (mean temperatures=14 C and 27 C, respectively). Precipitation is variable, but averages 835 mm annually, with peaks in spring and fall.

We tested for *T. cruzi* in 192 free-ranging meso-mammals and large mammals that were either purposefully captured (n=79) or were opportunistically collected in a concurrent study (n=113). We captured a total of 86 individual small and meso-mammals, including 17 raccoons, 29 Virginia opossums, 13 striped skunks (*Mephitis mephitis*), 14 hispid cotton rats (*Sigmodon hispidus*), one southern plains wood rat (*Neotoma micropus*), five white-ankle mice (*Peromyscus pectoralis*), four plains harvest mice (*Reithrodontomys montanus*), two northern pygmy mice (*Baiomys taylori*), and one fox squirrel (*Sciurus niger*). We could not test seven individuals because of various circumstances. Additional meso-mammal and large mammal samples were taken from three hispid cotton rats, 14 raccoons, 20 striped skunks, 18 white-tailed deer (*Odocoileus virginianus*), one feral swine (*Sus scrofa*), 11 Virginia opossums, four plains harvest mice, one northern pygmy mouse, and one fox squirrel, obtained from unrelated but concurrent management efforts and also tested for *T. cruzi*. We did not have spatial data for these opportunistically collected samples, which were not included in the spatial analyses.

Trypanosoma cruzi spatial distribution

We identified two potential indicators of *T. cruzi* spatial distribution based on vegetative types and floodplain extent. Our study area was characterized by three distinct vegetative communities: 1) grasslands dominated by herbaceous species and absence of canopy cover, 2) semi-improved woodlands (i.e., park-like) made up of widely dispersed canopy trees, and 3) deciduous woodlands defined by largely unman-

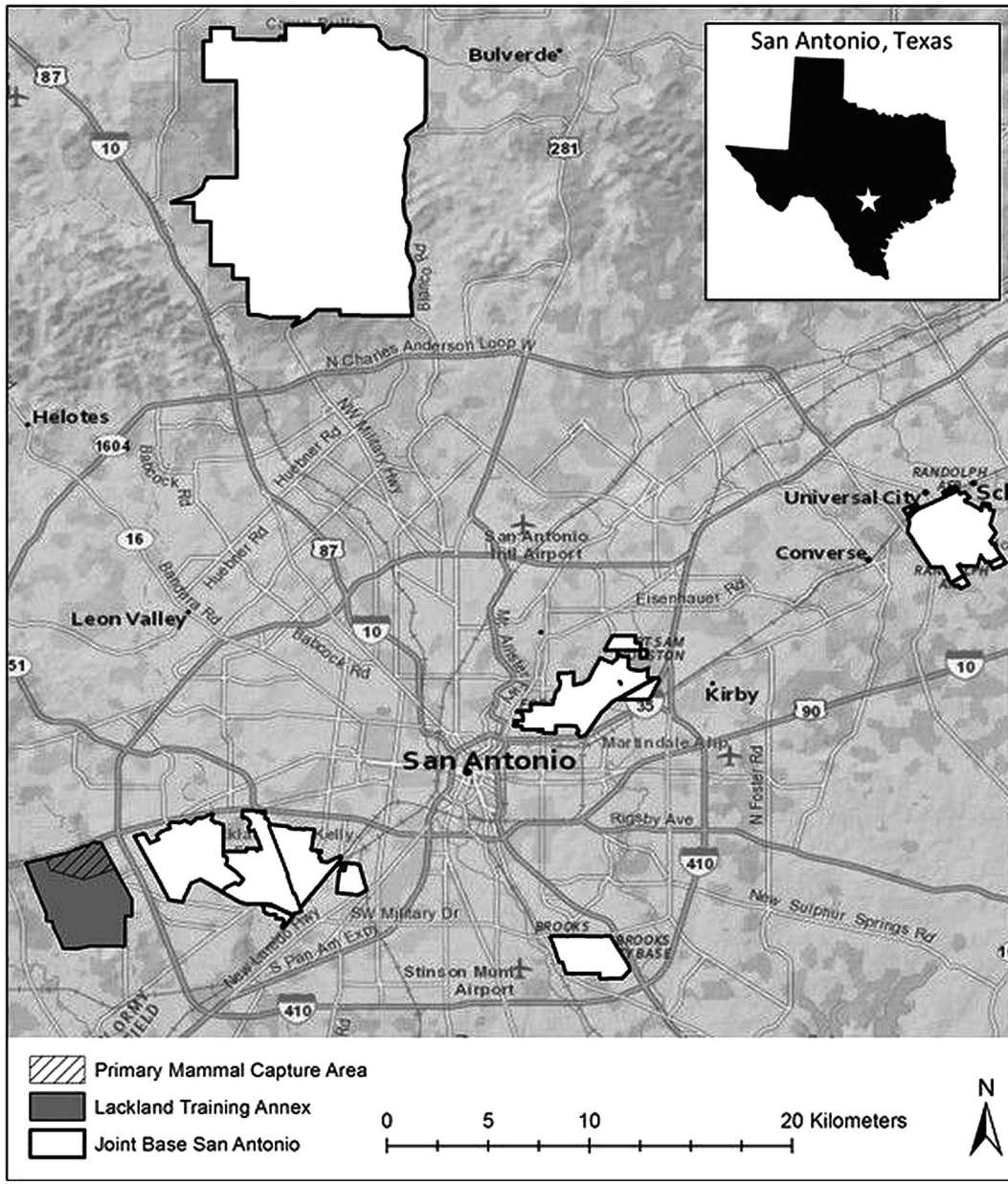

FIGURE 1. The *Trypanosoma cruzi* study area on Lackland Training Annex, a part of the multi-installation Joint Base San Antonio in San Antonio, Texas (base map; Environmental Systems Research Institute 2017). Blood and tissue samples were obtained from small, midsized, and large mammals with the use of a combination of box traps for live capture and samples sourced from concurrent research and management studies (2012–2013). Samples from 192 different individuals were designated as *T. cruzi* positive (n=51) based on the results of two separate polymerase chain reaction analyses coinciding with expected 330–base pair (bp) kDNA and 188-bp nDNA.

aged tree stands. The study area was divided by Medio Creek running north–south with an approximately 600-m-wide floodplain. We used a total of nine transects with three transects in each vegetative community (Fig. 2). Four of these transects were in the floodplain and riparian areas and five of the transects were in upland areas.

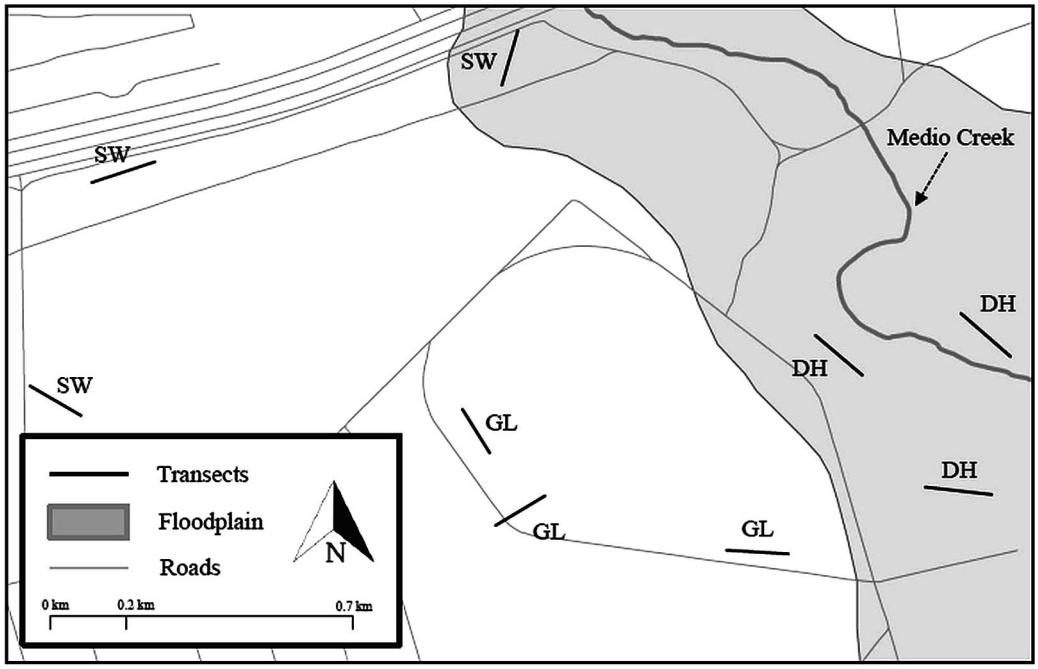

FIGURE 2. Meso-mammal capture and sampling transects for *Trypanosoma cruzi* study segregated by vegetation types (SW: semi-improved woodlands, GL: grasslands, DH: dense hardwoods) and topography (upland and floodplain) on Lackland Training Annex, a part of the multi-installation Joint Base San Antonio in San Antonio, Texas. Blood and tissue samples were obtained from small and midsized mammals captured with box traps (n=90) placed along the transects (2012–2013). Samples (n=79) were designated as *T. cruzi* positive (n=37) based on the results of two separate polymerase chain reaction analyses coinciding with expected 330–base pair (bp) kDNA and 188-bp nDNA.

Mammal sampling

We conducted seasonal (December 2011–December 2013) large and meso-mammal (body mass of 2.5–25 kg) population surveys and trapping to determine *T. cruzi* incidence in various vegetative community types (i.e., grasslands, semi-improved woodlands, deciduous woodlands) and topographies such as riparian, floodplain, and upland) in our study area.

We trapped small mammals with the use of 10 Sherman live traps (7.6×9×23 cm; H.B. Sherman Traps, Tallahassee, Florida, USA) placed at 10-m intervals along the nine individual 100-m transects (n=90 total traps) described previously. Meso-mammals were trapped with five live traps (48×15×15 cm; Tomahawk Live Traps, Tomahawk, Wisconsin, USA) randomly placed along each 100-m transect (15 total traps). Traps were baited with oats and cat food and set between dusk and dawn for four or five consecutive nights. Trapping events were conducted two to four times per season, annually during the 2-yr study. We characterized mammals by species, sex, age, weight, vegetation type found at the capture site, and transect/trap number. Individuals were ear-tagged or paint-marked for recapture identification.

Disease data collection and prevalence analysis

We collected blood and tissue samples from small and meso-mammals during density estimation captures (in a concurrent study), and from additional lethal captures (medical-grade carbon dioxide gas, Institutional Animal Care and Use Committee Approval 2011-294). Additionally, we secured blood and tissue samples from concurrent white-tailed deer research and feral hog control efforts conducted on JBSA. We extracted whole blood from the leg veins of captured and released white-tailed deer. Finally, we opportunistically collected whole blood and tissue samples from euthanized feral hogs caught in circular style corral traps by military support personnel. Collected whole blood specimens were placed in BD Vacutainer® tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA), and

TABLE 1. Captured mammals tested and prevalence of *Trypanosoma cruzi* at Lackland Training Annex a part of the multi-installation Joint Base San Antonio in San Antonio, Texas, 2012–2013. Captures were delineated according to location: vegetation type (grasslands, semi-improved woodlands, dense hardwoods) and topography (upland, floodplain). Blood and tissue samples were obtained from small and midsized mammals captured with box traps (n=90). Samples (n=79) were designated as *T. cruzi* positive (n=37) based on the results of two separate polymerase chain reaction analyses coinciding with expected 330–base pair (bp) kDNA and 188-bp nDNA.

		Number of positive samples				
		Vegetation			Topography	
Mammal species	Total tested	Grasslands	Semi-improved woodlands	Dense hardwoods	Upland	Floodplain
Hispid cotton rat (*Sigmodon hispidus*)	14	1	0	0	1	0
White-ankle pygmy mouse (*Peromyscus pectoralis*)	5	0	0	0	0	0
Fox squirrel (*Sciurus niger*)	1	0	0	0	0	0
Virginia opossum (*Didelphis virginiana*)	29	0	9	12	5	16
Raccoon (*Procyon lotor*)	17	0	2	5	0	7
Striped skunk (*Mephitis mephitis*)	13	3	0	5	3	5
Totals	79	4	11	22	9	28

tissue excisions, which included heart, liver, lung, and muscle, were placed in zip-closure plastic bags; specimens were stored in a cooler with dry ice for transport to the laboratory.

Differing parasitemia levels occur between the trypomastigote (extracellular, nondividing form) acute and amastigote (binary fission replicating form) chronic disease phases, making the detection of protozoan gene DNA sequences or host antibodies challenging, depending on the analysis techniques that are used (Brasil et al. 2010). Polymerase chain reaction (PCR) was applied in our study to detect specific parasite gene sequence molecules from meso-mammal blood and tissue samples, and for further validation of acute or chronic infection. The Department of the Army Public Health Command Region–South (JBSA–Ft. Sam Houston, San Antonio, Texas) performed PCR analyses (Kramm 2015). Samples were designated as *T. cruzi*–positive based on the results of two separate PCR analyses coinciding with expected 330–base pair (bp) kDNA and 188-bp nDNA (Reisenman et al. 2010; Kramm 2015; Kramm et al. 2016). We compared *T. cruzi* prevalence among species, among vegetative communities, and among different topographies (i.e., floodplain versus upland) with the use of chi-square tests (α=0.05).

RESULTS

Trypanosoma cruzi spatial distribution and prevalence

We found *T. cruzi* DNA in four species with significant differences in *T. cruzi* prevalence among species (χ^2=18.113, df=3, P<0.001). Prevalence in each of the four positive species was Virginia opossums, 65% (26/40); striped skunks, 27% (9/33) of sampled; raccoons, 45% (14/31) of sampled; and hispid cotton rats, 12% (2/17). The preponderance of *T. cruzi* infections found in verified locations (from captured animals) occurred in the lower-elevation floodplain (35%, 28/79) as compared to the higher-elevation uplands (11%, 9/79; Table 1). These also represented significantly higher infection rates in animals in the floodplain than in the uplands (χ^2=9.849, df=1, P=0.002). Additionally, we found the prevalence to be higher in animals from the dense hardwoods vegetation community (28%, 22/79), than semi-improved woodlands (14%, 11/79), or grasslands (5%, 4/79). A greater percentage of captured animals were infected in the semi-improved woodlands (61%, 11/18) when compared to dense hardwoods (60%, 22/37) or in grasslands (17%, 4/24; Table 1). Animals in grasslands had a significantly lower infection rate when summed across all species compared with dense hardwoods and semi-improved woodlands (χ^2=13.078, df=2, P=0.001). Animals found in dense hardwoods and semi-improved woodlands did not differ

significantly in infection rates (χ^2=0.495, df=1, P=0.481).

DISCUSSION

In our study, we found core areas for Chagas disease infection were primarily within floodplain or riparian deciduous forests. The Chagas disease–positive species included raccoons, Virginia opossums, striped skunks, and hispid cotton rats, which are typically generalists with the ability to live in human-dominated areas, but that also naturally gravitate towards riparian zones. We suspect the animals tested are the primary hosts for the pathogen and that the low numbers of positive animals in grasslands or semi-improved woodlands were reflective of habitat preferences for these species. This finding is supported by research that indicates triatomines are located in a variety of habitats, including grasslands, woodlands, and human-dominated areas such as houses, and often feed on mammals (Bern et al. 2011). Mammals in our study that rarely if ever utilized burrows (e.g., white-tailed deer and feral hogs) showed no measurable level of infection. Our investigation recognized meso-mammals as primary *T. cruzi* reservoirs, which supported the hypothesis that these common mammals helped to maintain and transmit the parasite in association with the triatomine vector. Furthermore, despite the different characteristics of the two types of woodlands we studied, there were significantly higher rates of infection of the mammals in them compared to mammals in the upland grasslands. This finding lent credence that these species (woodland and riparian generalists) were important in pathogen persistence.

The lack of evidence of *T. cruzi* in all rodents except in hispid cotton rats was contrary to the findings of Pinto et al. (2010), and may be attributed to differences between the protozoal pathogen and the diversified mammal composition found within specific vegetation communities (Roellig et al. 2009b). Beard et al. (2003) reported that *T. cruzi* infection of canine species in South Texas may be endemic. A recent study in Mexico identified the blood meal origins of 47 triatomines and found that raccoons and armadillos (*Dasypus* spp.) were the main blood meal hosts (Bosseno et al. 2009). A study in the US reported *T. cruzi* infection among 11 reservoir species from six southern states, and detected a higher incidence of the infection in Virginia opossums and raccoons (Brown et al. 2010). These studies suggest that *T. cruzi* prevalence varies in its preferred host species over geographic regions.

The distribution of *T. cruzi* prevalence in mammal species has implications for managing wildlife populations to limit disease transmission to humans. These species prefer deciduous forests located near bodies of water and actively utilize burrows, hollow logs, and other enclosed areas. Based on our study findings, no management action is recommended for the majority of rodent species and larger game species. Instead, management minimizing the risk of exposure to target host species should focus on meso-mammals, specifically raccoons, Virginia opossums, and skunks. Because of the high incidence of *T. cruzi* among these animals, however, their removal should be applied with caution because the kissing bugs typically associated with them could become displaced and search for alternative blood meals from pets and people (Barr 2009). Also, removal or large population reduction of meso-mammal species may lead to unpredictable trophic cascades or species adaptations (e.g., reduction of raccoons may allow Virginia opossum populations to increase). Modeling of meso-mammal population dynamics is proposed to evaluate various management actions a priori, to include management of vegetative communities having relatively high prevalence of *T. cruzi* infection. Peridomestic meso-mammals are typically not constrained by natural predators, and occupy South Texas microhabitat regimes that might increase the threat of zoonotic diseases through increased human interactions. Managing vector and reservoir movements will require an integrated control approach that includes identifying harborage

locations, select application of spatial insecticides, mechanical or chemical management of vegetation to discourage vectors and reservoirs, and the use of mammal bait formulations approved by the Environmental Protection Agency impregnated with systemic insecticide (McPhatter et al. 2012) that will produce the deaths of hematophagous triatomines without deleterious effects on vertebrate hosts.

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