A ONE HEALTH APPROACH TO STUDY THE EPIDEMIOLOGY OF *TRYPANOSOMA CRUZI* IN HUMANS, DOMESTIC ANIMALS, AND WILDLIFE IN THE RIO GRANDE VALLEY OF TEXAS ALONG THE UNITED STATES-MEXICO BORDER

A Dissertation

by

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ABSTRACT

This dissertation focused on a One Health approach to assess the prevalence of *Trypanosoma cruzi* in south Texas. We sampled from humans, domestic animal, and wildlife populations of south Texas to understand the seroprevalence and the ecology of parasite transmission in an area where infected vectors are present.

In 2015 we conducted a cross- sectional prevalence study in indigent, medically underserved human and cohabiting canine populations of seven south Texas border communities, known as colonias. Defining positivity as those samples that were positive on two or more independent tests, we found 1.3% seroprevalence in 233 humans, including one child born in the United States with only short-duration travel to Mexico. Among 209 dogs, seroprevalence was 19.6%, but adjusted to 31.6% when including those dogs positive on only one test and extrapolating potential false negatives. Parasite DNA was detected in five dogs, indicating potential parasitemia.

In 2016-2019, a cross-sectional study was conducted to quantify the prevalence of *T. cruzi* in owned dogs, including both measures of anti-*T. cruzi* antibodies and parasite DNA circulating in the blood. Through serological testing of 340 dogs using three different tests, we identified 110 (32.4%) dogs that showed positivity on at least two independent antibody tests. Using PCR assays, 10 of 300 (3.3%) dogs showed parasite DNA in their blood.

In 2017, we collected samples from 100 wild opossums and 167 cats across three seasons that were euthanized in a south Texas animal shelter for reasons unrelated to our study. Samples were screened for *T. cruzi* DNA using qPCR to amplify the 166-bp gene region with

confirmation of positive status achieved through one or more additional PCR assays, including a qPCR to determine the parasite discrete typing unit (DTU). Of 100 opossums, parasite DNA was found in blood clot (9%), heart tissue (10%), and anal gland secretions (12%). The 43 of these opossums with expanded sample collection showed infection in 16.3% of intercostal muscle and 11.6% of anal gland tissue. In total, 15 (15%) opossums had at least 1 infected tissue type, of which 9 (9%) had two or more different tissue types that tested positive. Through serological testing of 167 cats from 14 south Texas regions that arrived to a large shelter, 19 cats (11.4%) were seropositive on at least two independent tests. Three cats- of which two were seropositive- had at least one PCR-positive tissue (1.8%); infected tissues included heart, bicep femoris muscle, sciatic nerve, mesentery, and esophagus. Histopathology identified pathology, like lymphoplasmacytic inflammation, consistent with *T. cruzi* infection.

In 2018 and 2019, we received ocelot tissues from salvaged animals that were installed in the Biodiversity Research & Teaching Collections at Texas A&M University and Gladys Porter Zoo; carcasses were collected following death primarily by vehicle collision from 2010-2017 around Laguna Atascosa National Wildlife Refuge in South Texas. *T. cruzi* DNA was detected in samples of two different ocelots (9.5%) with infection found in skeletal muscle and blood clot.

Samples where the parasite discrete typing unit (DTU) was successfully ascertained showed exclusively 'TcI', the *T. cruzi* strain associated with autochthonous human disease in the US. Under a one-health framework, the identification of infection in humans, domestic animals, and wildlife is a critical prerequisite to developing public health interventions in south Texas and underserved border communities.

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CHAPTER I

CHAGAS DISEASE (AMERICAN TRYPANOSOMIASIS)

INTRODUCTION

The etiological agent of Chagas disease, *Trypanosoma cruzi*, is a single celled protozoal parasite primarily transmitted through the infected feces of blood feeding triatomine insects. The parasite was discovered in 1909 by a Brazilian scientist and physician named Carlos Chagas.[1] Infected humans and animals may suffer a spectrum of acute and chronic health issues including fatal cardiac disease. Domestic and wild mammals serve as reservoirs for the parasite. The triatomine insect vector, commonly known as a kissing bug, is widely dispersed throughout the Americas, including South America, Central America, Mexico, and the southern United States (U.S.). Chagas disease may be found in other areas of the world where the vector is not present due to migration or travel of infected individuals into non-endemic countries.[2]

Chagas disease is classified as a neglected tropical disease due to its distribution in tropical and subtropical climates, disproportionate impact on people living in poverty, and relative neglect from research and funding arenas.[3] Estimates by the World Health Organization suggest Chagas disease affects approximately 6-8 million individuals in Latin America,[4] and a more liberal estimate suggests 17 million people are infected and 100 million are at risk of contracting the parasite.[5] Of 21 Latin American countries, the majority of cases occur in Argentina, Brazil, and Mexico.[4,6] Active transmission of *T. cruzi* in Latin American countries places 13% of the Latin American population at risk for *T. cruzi* infection.[6] Vector transmission in the domestic environment has been interrupted or diminished in some Latin American countries through targeted vector interventions, but wildlife and sylvatic vectors in these areas

maintain transmission cycles.[7] The average annual economic burden of health-care cost related to Chagas disease in Latin America is approximately US$500 million.[8] In Latin America, the disease is responsible for 772,304 disability adjusted life years (DALYs) which comprises over 95% of DALYs attributed to Chagas disease worldwide.[8]

In the U.S., there is increasing recognition in the human health community for Chagas disease. The majority of human cases in the U.S. are identified through blood banks and occur in individuals who likely acquired the infection while living or visiting an endemic region in Latin America.[9] However, locally acquired infections in the U.S. are increasingly recognized.[10,11] The first documented case of locally acquired Chagas disease in the U.S. was in Corpus Christi, Texas in 1955.[12] The 2012 estimate of over 238,000 cases of *T. cruzi* infection in the U.S. may be higher when undocumented immigrant populations are considered.[13] Individuals living in impoverished and medically underserved communities along the U.S.-Mexico border may be at heightened risk for infection.[14]

VECTOR

Triatomine insects, comprised of approximately 150 species in the subfamily Triatominae, are known by many different names in Latin America and are commonly referred to as 'chinche besuconas' or 'vinchucas.'[15] In the U.S. triatomines are commonly called 'kissing bugs,' 'cone nosed bugs,' and 'reduviid bugs.' Triatomine insects mainly occur in the Americas and some Caribbean islands. One species of triatomine occurs in Africa and a few occur in Australia/Indo-Pacific region.[16,17] Species of triatomines occur in parts of southeast Asia but are not known to transmit *T. cruzi*.[18] The life cycle of the usually nocturnal insect includes five flightless nymph stages that develop into adults capable of flying. All life stages are

hematophagous, meaning they strictly feed on blood. Insect size and appearance is dependent on life stage and species (Figure 1). Early life stages are likely to be located in nesting habitats of mammals in close proximity to blood sources. Adult triatomines may approximately range from 5-44mm in length.[19] Triatomines in the U.S. range from 13-29mm in length.[19] Some triatomine species favor sylvatic environments while others favor peridomiciliary structures or houses, which influences their proximity and potentially infectivity to domestic animals and humans. For example, *Triatoma infestans* in South America is known to colonize and infest housing, which increases their contact with humans.[20] There are 11 species occurring in the U.S. (Figure 2), where *Triatoma sanguisuga* is the most widely distributed infective triatomine insect which often feeds on humans.[21] In a citizen science program in Texas, the majority of triatomines collected by members of the public were found in peridomestic environments including dog kennels, patios, garages, and the outside of houses.[22] \

Figure 1: Triatomine life stages. From left to right, egg, nymphal instars 1-5, adult female, and adult male *Triatoma gerstaeckeri*. Photographed specimens were collected in Texas. (Photo: Gabriel Hamer, TAMU- Entomology)

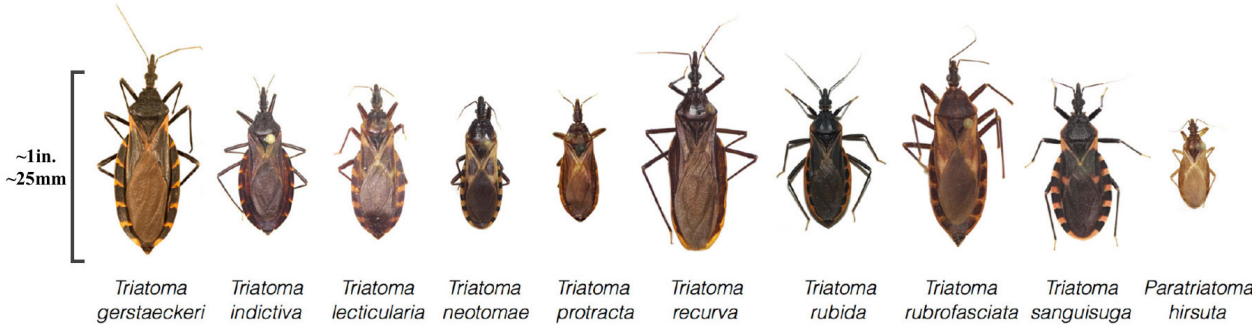

Figure 2: Adults of 10 kissing bug species that occur in the U.S. (Photo: Gabriel Hamer & Justin Bejcek, TAMU-Entomology)

The infection prevalence of triatomine vectors in the U.S. varies by species and geographic area; for example, in the western U.S. the dominant vector *Triatoma rubida* is characterized by less than 20% infection prevalence, whereas in the south central U.S. over 50% of the dominant vector *Triatoma gerstaeckeri* are infected. [22–24]

ANIMAL RESERVOIRS

There is historical evidence of *T. cruzi* infection in wild and domestic animals in the southern U.S. [12,14,25–27] Triatomines are often associated with the nests and burrows of wildlife hosts, including woodrats (*Neotoma* spp.). Studies conducted in domestic dog (*Canis lupus familiaris*) populations from the southern U.S. show exposure to *T. cruzi* (seroprevalence: 3.6%-57.6%) indicating active transmission in peridomestic environments and identifying domestic dogs as key sentinel hosts. [28,29] Additionally, several species of wildlife in the U.S., including raccoons (*Procyon lotor*), opossums (*Didelphis virginiana*), and coyotes (*Canis latrans*), serve as key reservoir hosts contributing to the infection cycle.[30–32] The impact this parasite has on animal populations is largely unknown, although cardiac pathology in infected wild animals and domestic dogs has been detected.[30,33,34] There is no approved treatment or vaccine for Chagas

disease in animals. *Trypanosoma cruzi* is unlikely to be transmitted directly from infected animals to humans; however, infected animals signal an environment where infected triatomine insects occur and may pose risk of transmission to humans.

BIOLOGY & TRANSMISSION

Trypanosoma cruzi undergoes various changes in stage within the vector and the host. When feeding, a triatomine insect ingests a flagellated form of the parasite (trypomastigote) from an infected mammalian host. Within the insect, the parasite then undergoes transformation to the epimastigote stage and multiplication occurs in the midgut of the insect. Transformation of the parasite occurs again in the hindgut of the insect, after which the metacyclic trypomastigote stage is passed in the feces; this is the infective stage of the parasite and is the primary source of infection to mammals. The stercorarian (vector-fecal) route of transmission occurs when the triatomine insect defecates infected feces on the host, which may self-inoculate by introducing the parasite into broken skin (bite, abrasion, cut) or mucous membranes (mouth, eyes, nose). The post-feeding defecation interval (amount of time between vector feeding and vector defecation) may be variable among vector species and is important for transmission, because a shorter interval suggests the vector is likely to still be in contact with the host when it defecates, resulting in increased risk of transmission.[35–37] Once the parasite enters the host, the trypomastigote circulates in the blood and infiltrates various tissues. Subsequent to tissue infiltration is transformation into the intracellular amastigote stage of parasite, which multiplies via binary fission. Eventually parasites burst out of the cell into the bloodstream for circulation and infection of other tissues.[38]

In addition to vector-fecal transmission, the parasite may be transmitted through ingestion of infected triatomine insects or foods contaminated by insects, congenitally, via infected blood transfusion or organ transplant, and through laboratory/medical accidents[39] (Figure 3). There have been outbreaks of acute Chagas disease in South America associated with the consumption of food/beverages contaminated by infected triatomine insects that had been crushed in the preparation process.[40,41] Additionally, consumption of infected insects is speculated to be an important route of transmission leading to Chagas disease in domestic dogs and wildlife.[42] Additionally, there is limited evidence of parasite transmission through breast feeding via broken skin and through sexual transmission.[43,44]

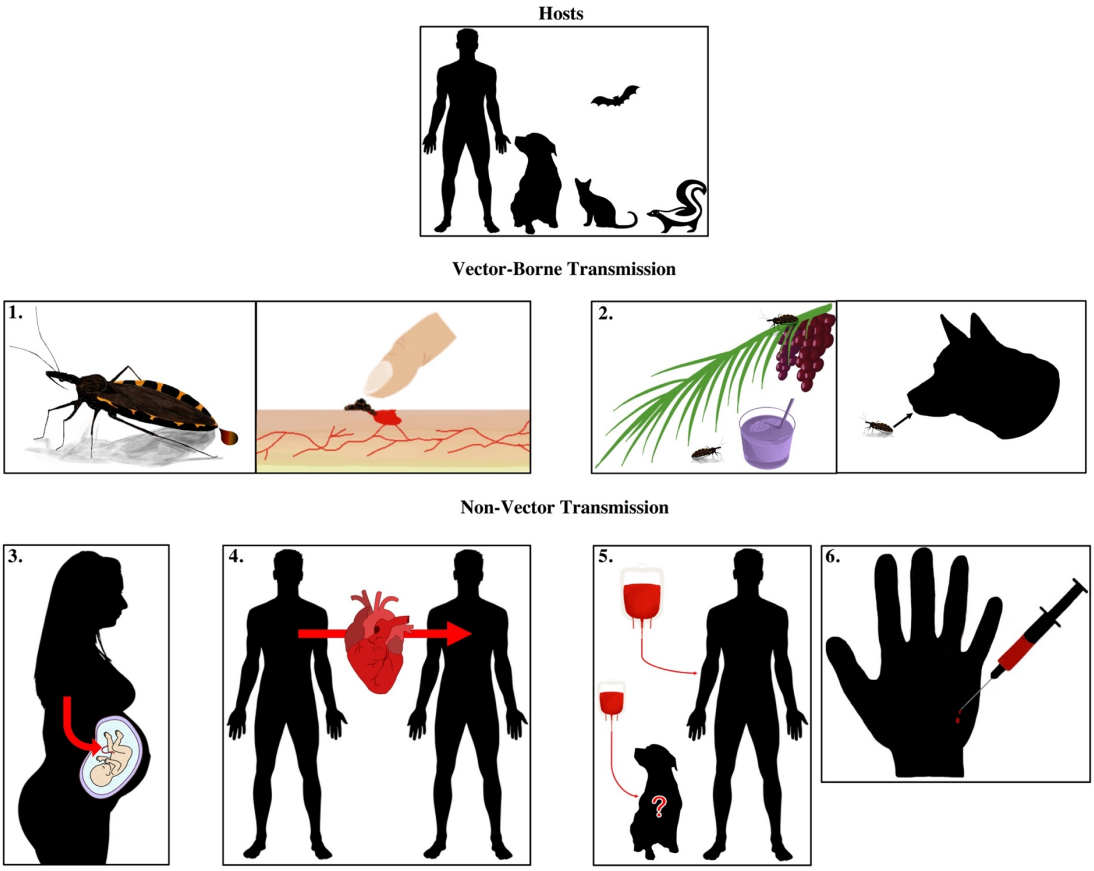

Figure 3: *Trypanosoma cruzi* transmission routes.

VECTOR-BORNE TRASNMISSION:1. Stercorarian (vector-fecal) transmission occurs via self-inoculation when contaminated feces enter broken skin or a mucous membrane of a host. 2. Oral transmission occurs when an infected triatomine insect contaminates a food source or when an infected insect is eaten directly by a host. NON-VECTOR TRANSMISSION: 3. Vertical transmission may occur if the mother is infected before or during pregnancy. 4. Transmission via organ transplant occurs when infected tissues are transplanted to an uninfected individual. 5. Transfusion transmission occurs when an individual receives blood from an infected donor. 6. Transmission via occupational and laboratory accidents may occur.

PATHOLOGY

Chagas disease occurs in three phases which may be associated with inapparent or non-specific symptoms.[38] The *acute phase* occurs when the parasite has entered the blood stream and is circulating and actively multiplying. If the host was infected via a vector, there may be swelling and inflammation at the bite site, known as a chagoma. Unilateral swelling around the eye, due to the introduction of infected triatomine feces, is the most recognizable indicator of acute Chagas disease and is commonly known as Romaña's sign.[45] Other symptoms may become present within 1-2 weeks after initial infection, including fever, loss of appetite, lethargy, malaise, and headaches. More severe symptoms of the acute phase include enlarged lymph nodes, spleen, and liver or cardiac abnormalities. Some acutely infected individuals may experience no symptoms at all. Following the acute phase, infected individuals enter the *indeterminate phase*. During this phase, which may last years to decades, the circulating parasite is diminished to low or undetectable levels, and the parasite remains in tissues with no obvious symptoms or complications. Many infected individuals may remain asymptomatic for life. Approximately 20-30% of infected individuals then enter the *chronic phase*, characterized by clinical abnormalities that may include may include severe cardiac abnormalities, such as enlargement the heart (cardiomegaly), congestive heart failure, and death.[38] Other clinical manifestations of the chronic phase include digestive complications, primarily the enlargement of the colon (megacolon) or esophagus (megaesophagus), and, less commonly, neurological complications.

The degree to which infected individuals present acute or chronic signs is likely dependent on several factors including the individual's immune system, the genetics of the infected person, and the genetics of the parasite. Coinfections, such as HIV, may increase the

severity of Chagas signs and symptoms.[46] Immunocompromised individuals such as young children, the elderly, individuals taking immunosuppressants, or those with immunosuppressive diseases may experience more severe symptoms and complications.[47] There is also evidence showing that the genetics of the host is a key factor in the presentation and severity of cardiac pathology.[48] Additionally, variation in the genetic strain of the parasite (discrete typing unit) has been attributed to variation in the clinical outcome of infected persons and animals.[49] For example, in humans, the *T. cruzi* strain type TcI is more associated with cardiac pathology in comparison to TcII/V which is associated with digestive pathology[50] In a mouse study, cardiac pathology and survival rate was dependent *T. cruzi* strain type.[51]

DIAGNOSTICS

Diagnosis of Chagas disease is made based on the patient's clinical findings, history that may put the patient at risk, and the use of multiple diagnostic tests. The approach for diagnosis of Chagas disease depends upon the phase of infection. During the acute phase when the parasite is actively circulating in the blood of the host, methods to directly detect the parasite from blood are useful. The parasite can be detected during the acute phase through microscopic assessment of blood smears. Additionally, DNA from the parasite can be amplified from the blood using polymerase chain reaction (PCR). Other diagnostic approaches that are useful during the acute phase include hemoculture (using culture media to grow parasite from a blood sample) and xenodiagnosis (in which uninfected triatomine insects feed on a host and are later assayed for infection). Unless antibody-detection assays are targeted to IgM antibodies, serological methods may be of limited utility in the acute phase, as the host may not yet have a detectable antibody response to infection.

Diagnostic tests used during the indeterminate and chronic phases are usually serological tests to detect anti-*T. cruzi* IgG antibodies, including enzyme-linked immunosorbent assays (ELISA), radioimmune precipitation assay (RIPA), indirect immunofluorescence assays (IFA), and more recently, lateral flow immunochromatographic assays. [52] However, each assay may be associated with cross-reaction, false positive or false negative results. For this reason, the WHO recommends confirming infection on the basis of reactivity in at least two conventional serological tests with independent antigenic principles.[53] Besides serological testing, biopsies, cardiac evaluations, and post mortem procedures may assist in diagnosis of Chagas disease in the indeterminate and chronic phases. Muscle biopsies may reveal intracellular amastigotes or inflammation when histologically observed.[54] Electrocardiograms and radiographs are often used in suspect Chagas cases to evaluate cardiac abnormalities, including arrhythmias and cardiomegaly. Post mortem gross abnormalities and histological assessments of tissues, including the heart, may confirm the presence of *T. cruzi* or associated cardiomyopathies such as lymphoplasmacytic inflammation.

TREATMENT

Two antitrypanosomal drugs- nifurtimox and benznidazole- are available for treating human Chagas disease. In 2017, the Food and Drug Administration (FDA) approved benznidazole for use in infected children (ages 2-12).[55] Nifurtimox is considered to produce more toxic side effects than benznidazole, but has shown similar effectiveness.[56] In the U.S., nifurtimox is not FDA approved, but may be obtained through CDC using an investigational protocol. Both medications are used in Latin America, where they have been noted to provide significant cure rates in infants and acutely infected individuals.[57,58] Antiparasitic treatment

during the chronic phase is less effective. Once the parasite causes irreparable damage to the heart and other tissues, symptomatic treatment is administered that may include pacemaker implants. The international clinical trial (BENznidazole Evaluation For Interrupting Trypanosomiasis [BENEFIT]) showed benznidazole failed to halt disease progression in patients with chronic Chagas cardiomyopathy.[59,60]

DISEASE PREVENTION & CONTROL

There is no vaccine against Chagas disease. Vector control can reduce the risk of Chagas disease in humans and domestic animals. The Southern Cone Initiative (SCI), a coalition of South American countries (Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay), was designed to interrupt transmission by eliminating or reducing *Triatoma infestans* vectors in at-risk communities through insecticide treatment and community engagement.[7] Residual pesticide interventions were shown to reduce or deter insects in peridomestic habitats, thus reducing the risk to domestic animals and humans.[61,62] SCI insecticide interventions were especially effective when combined with housing improvements.[61] Improvements to homes with thatched roofs and abode exteriors in endemic countries showed a reduction in triatomine infestations.[61,63] Homes with modern construction may not be as prone to triatomine infestations. However, older homes with inadequate window screening or homes with entry points due to compromised structures may allow triatomine intrusion or domiciliation.[64] Since triatomine insects are attracted to artificial light, reduction of light sources around peridomiciliary structures and homes reduce their attraction to the vectors.[65] Reduction of triatomine habitat and habitats of sylvatic hosts including wood and debris piles around homes may also reduce insect numbers.[66] Also, structural updates to compromised housing provide protection from entry by triatomines.[67]

The SCI also focused on eliminating transmission via blood product transfusions through the development of patient and product screenings. This portion of the initiative lacked compliance in some countries with high prevalence rates, such as Bolivia, where an estimated 50% of blood banks still lack serological screenings.[7] In the U.S., first time blood donors are tested for anti-*T. cruzi* antibodies, but not upon subsequent donations, since the risk of local transmission is thought to be minimal. Pregnant women who were born or lived in endemic countries, as well as women who suspect having been bitten by a triatomine insect, should be screened to manage congenital transmission as early detection and treatment of congenital infections is associated with high rates of cure. [68,69]

Individuals traveling to endemic areas where triatomine insects are present should consider the use of insect repellent and mosquito nets to deter insects when sleeping in compromised housing.[70,71] A traveler should also take caution when drinking unpasteurized juices or eating uncooked foods from areas with previous food borne outbreaks of *T. cruzi*.

REFERENCES

1. Chagas C. Nova tripanozomiaze humana: estudos sobre a morfolojia e o ciclo evolutivo do Schizotrypanum cruzi n. gen., n. sp., ajente etiolojico de nova entidade morbida do homem. *Mem Inst Oswaldo Cruz*. 1909;1(2):159-218. doi:10.1590/S0074-02761909000200008.
2. Requena-Méndez A, Aldasoro E, de Lazzari E, et al. Prevalence of Chagas Disease in Latin-American Migrants Living in Europe: A Systematic Review and Meta-analysis. *PLoS Negl Trop Dis*. 2015. doi:10.1371/journal.pntd.0003540.
3. Hotez PJ, Dumonteil E, Woc-Colburn L, et al. Chagas disease: "The new HIV/AIDS of the Americas." *PLoS Negl Trop Dis*. 2012;6(5):4-7. doi:10.1371/journal.pntd.0001498.
4. World Health Organisation. WHO Chagas disease (American trypanosomiasis) Factsheet. World Health Organisation. http://www.who.int/mediacentre/factsheets/fs340/en/. Published 2015.
5. Moncayo, Silveira AC. Current epidemiological trends of Chagas disease in Latin America and future challenges: Epidemiology, surveillance, and health policies. In: *American Trypanosomiasis Chagas Disease: One Hundred Years of Research: Second Edition*. ; 2017:59-88. doi:10.1016/B978-0-12-801029-7.00004-6.
6. World Health Organization. Chagas disease in Latin America: an epidemiological update based on 2010 estimates. *Wkly Epidemiol Rec*. 2015;6(90):33-44.
7. Dias JCP. Southern Cone Initiative for the elimination of domestic populations of Triatoma infestans and the interruption of transfusional Chagas disease. Historical aspects, present situation, and perspectives. *Mem Inst Oswaldo Cruz*. 2007;102(SUPPL. 1):11-18. doi:10.1590/S0074-02762007005000092.

8. Lee BY, Bacon KM, Bottazzi ME, Hotez PJ. Global economic burden of Chagas disease: A computational simulation model. *Lancet Infect Dis*. 2013;13(4):342-348. doi:10.1016/S1473-3099(13)70002-1.

9. Montgomery SP, Starr MC, Cantey PT, Edwards MS, Meymandi SK. Neglected Parasitic Infections in the United States: Chagas Disease. *Am J Trop Med Hyg*. 2014;90(5):814-818. doi:10.4269/ajtmh.13-0726.

10. Dorn PL, Perniciaro L, Yabsley MJ, et al. Autochthonous transmission of Trypanosoma cruzi, Louisiana. *Emerg Infect Dis*. 2007;13(4):13-15. doi:10.3201/eid1304.061002.

11. Garcia MN, Aguilar D, Gorchakov R, et al. Case report: Evidence of autochthonous Chagas disease in southeastern Texas. *Am J Trop Med Hyg*. 2015;92(2):325-330. doi:10.4269/ajtmh.14-0238.

12. Woody NC, Woody HB. American Trypanosomiasis (Chagas' Disease): First indigenous case in the United States. *J Am Med Assoc*. 1955;159(7):676-677.

13. Manne-Goehler J, Umeh CA, Montgomery SP, Wirtz VJ. Estimating the Burden of Chagas Disease in the United States. *PLoS Negl Trop Dis*. 2016;10(11):1-7. doi:10.1371/journal.pntd.0005033.

14. Curtis-Robles R, Zecca IB, Roman-Cruz V, et al. Trypanosoma cruzi (Agent of Chagas Disease) in sympatric human and dog populations in "colonias" of the Lower Rio Grande Valley of Texas. *Am J Trop Med Hyg*. 2017;96(4):805-814. doi:10.4269/ajtmh.16-0789.

15. Coura JR, Vĩas PA. Chagas disease: A new worldwide challenge. *Nature*. 2010;465(7301 SUPPL.). doi:10.1038/nature09221.

16. Monteith GB. CONFIRMATION OF THE PRESENCE OF TRIATOMINAE (HEMIPTERA: REDUVIIDAE) IN AUSTRALIA, WITH NOTES ON INDO-PACIFIC

SPECIES. *Aust J Entomol*. 1974. doi:10.1111/j.1440-6055.1974.tb02160.x.

17. Tartarotti E, Azeredo-Oliveira MT V, Ceron CR. Phylogenetic approach to the study of triatomines (Triatominae, Heteroptera). *Brazilian J Biol*. 2006;66(2B):703-708.

18. PattersonGeographical Distribution of Chagas Disease JS, Guhl F. Geographical Distribution of Chagas Disease. In: *American Trypanosomiasis*. ; 2010. doi:10.1016/B978-0-12-384876-5.00005-8.

19. Lent H, Wygodzinsky P. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' Disease. *Bull Am Museum Nat Hist*. 1979;163(3):123-520.

20. Gürtler RE, Cecere MC, Lauricella M a, Cardinal M V, Kitron U, Cohen JE. Domestic dogs and cats as sources of Trypanosoma cruzi infection in rural northwestern Argentina. *Parasitology*. 2007;134(Pt 1):69-82. doi:10.1017/S0031182006001259.

21. Klotz SA, Dorn PL, Klotz JH, et al. Feeding behavior of triatomines from the southwestern United States: An update on potential risk for transmission of Chagas disease. *Acta Trop*. 2009;111(2):114-118. doi:10.1016/j.actatropica.2009.03.003.

22. Curtis-Robles R, Aukland LD, Snowden KF, Hamer GL, Hamer SA. Analysis of over 1500 triatomine vectors from across the US, predominantly Texas, for Trypanosoma cruzi infection and discrete typing units. *Infect Genet Evol*. 2018;58:171-180. doi:10.1016/j.meegid.2017.12.016.

23. Kjos SA, Snowden KF, Olson JK. Biogeography and Trypanosoma cruzi infection prevalence of Chagas disease vectors in Texas, USA. *Vector-Borne Zoonotic Dis*. 2009;9(1):41-50. doi:10.1089/vbz.2008.0026.

24. Klotz SA, Dorn PL, Mosbacher M, Schmidt JO. Kissing bugs in the United States: risk for

vector-borne disease in humans. *Environ Health Insights*. 2014;8(Suppl 2):49-59. doi:10.4137/EHI.S16003.

25. Kjos SA, Marcet PL, Yabsley MJ, et al. Identification of bloodmeal sources and Trypanosoma cruzi infection in triatomine bugs (Hemiptera: Reduviidae) from residential settings in Texas, the United States. *J Med Entomol*. 2013;50(5):1126-1139.

26. Olsen PF, Shoemaker JP, Turner HF, Hays KL. Incidence of Trypanosoma Cruzi (Chagas) in Wild Vectors and Reservoirs in East-Central Alabama. *J Parasitol*. 1964;50(5):599-603. doi:10.2307/3276112.

27. Garcia MN, Woc-Colburn L, Aguilar D, Hotez PJ, Murray KO. Historical Perspectives on the Epidemiology of Human Chagas Disease in Texas and Recommendations for Enhanced Understanding of Clinical Chagas Disease in the Southern United States. *PLoS Negl Trop Dis*. 2015. doi:10.1371/journal.pntd.0003981.

28. Bradley K, Bergman D, Woods J, Crutcher J, Kirchhoff L. Prevalence of American trypanosomiasis (Chagas disease) among dogs in Oklahoma. *J Am Vet Med Assoc*. 2000;217(12):1853-1857. http://www.ncbi.nlm.nih.gov/pubmed/11132891.

29. Curtis-Robles R, Snowden KF, Dominguez B, et al. Epidemiology and molecular typing of Trypanosoma cruzi in naturally-infected hound dogs and associated triatomine vectors in Texas, USA. *PLoS Negl Trop Dis*. 2017;11(1):e0005298. doi:10.1371/journal.pntd.0005298.

30. Curtis-Robles R, Lewis BC, Hamer SA. High Trypanosoma cruzi infection prevalence associated with minimal cardiac pathology among wild carnivores in central Texas. *Int J Parasitol Parasites Wildl*. 2016;5(2):117-123. doi:10.1016/j.ijppaw.2016.04.001.

31. Pung OJ, Banks CW, Jones DN, Krissinger MW. Trypanosoma cruzi in wild raccoons,

opossums, and triatomine bugs in southeast Georgia, U.S.A. *J Parasitol*. 1995;81(2):324-326. doi:10.2307/3283947.

32. Hodo CL, Hamer SA. Toward an ecological framework for assessing reservoirs of vector-borne pathogens: wildlife reservoirs of Trypanosoma cruzi across the southern United States. *ILAR J*. 2017;58(3):379-392. doi:10.1093/ilar/ilx020.

33. Meyers AC, Meinders M, Hamer SA. Widespread Trypanosoma cruzi infection in government working dogs along the Texas-Mexico border: Discordant serology, parasite genotyping and associated vectors. *PLoS Negl Trop Dis*. 2017;11(8):1-19. doi:10.1371/journal.pntd.0005819.

34. Barr S, Schmidt S, Brown C, Klei T. Pathologic features of dogs inoculated with North American Trypanosoma cruzi isolates. *Am J Vet Res*. 1991;52(12):2033-2039.

35. Villacís AG, Arcos-Terán L, Grijalva MJ. Life cycle, feeding and defecation patterns of Rhodnius ecuadoriensis (Lent & León 1958) (Hemiptera: Reduviidae: Triatominae) under laboratory conditions. *Mem Inst Oswaldo Cruz*. 2008;103(7):690-695. http://www.ncbi.nlm.nih.gov/pubmed/19057820.

36. Zeledón R, Alvarado R, Jiron LF. Observations on the feeding and defecation patterns of three triatomine species (Hemiptera: Reduviidae). *Acta Trop*. 1977;34(1):65-77.

37. Klotz SA, Dorn PL, Klotz JH, et al. Feeding behavior of triatomines from the southwestern United States: an update on potential risk for transmission of Chagas disease. *Acta Trop*. 2009;111(2):114-118. doi:10.1016/j.actatropica.2009.03.003.

38. CDC. CDC - Chagas Disease. Cdc. http://www.cdc.gov/parasites/chagas/health_professionals/tx.html. Published 2013.

39. Brenière SF, Waleckx E, Aznar C. Other forms of transmission: Blood transfusion, organ

transplantation, laboratory accidents, oral and sexual transmission. In: *American Trypanosomiasis Chagas Disease: One Hundred Years of Research: Second Edition*. ; 2017:561-578. doi:10.1016/B978-0-12-801029-7.00025-3.

40. Dias JP, Bastos C, Araújo E, et al. Acute Chagas disease outbreak associated with oral transmission Surto de doença de Chagas aguda associada à transmissão oral. 2008;41(3):296-300.

41. Ramírez JD, Montilla M, Cucunubá ZM, Floréz AC, Zambrano P, Guhl F. Molecular epidemiology of human oral Chagas disease outbreaks in Colombia. *PLoS Negl Trop Dis*. 2013;7(2):e2041.

42. Roellig DM, Ellis AE, Yabsley MJ. Oral Transmission of Trypanosoma cruzi with Opposing Evidence for the Theory of Carnivory. *J Parasitol*. 2009;95(2):360-364. doi:10.1645/GE-1740.1.

43. Norman FF, López-Vélez R. Chagas disease and breast-feeding. *Emerg Infect Dis*. 2013;19(10):1561-1566. doi:10.3201/eid1910.130203.

44. Ribeiro M, Nitz N, Santana C, et al. Sexual transmission of Trypanosoma cruzi in murine model. *Exp Parasitol*. 2016;162:1-6. doi:10.1016/j.exppara.2015.12.017.

45. Dias JC. Cecilio Romana, Romana's sign and Chagas' disease. *Rev Soc Bras Med Trop*. 1997;30(5):407-413.

46. Sartori AMC, Ibrahim KY, Westphalen EVN, Braz LMA, Oliveira OC, Gakiya E. Manifestations of Chagas disease (American trypanosomiasis) in patients with HIV/AIDS. *Ann Trop Med Parasitol*. 2007;101(1):31-50.

47. Simões MV, Soares FA, Marin-Neto J. Severe myocarditis and esophagitis during reversible long standing Chagas' disease recrudescence in immunocompromised host. *Int*

J Cardiol. 1995;49(3):271-273. doi:10.1016/0167-5273(95)02290-D.

48. Marinho CRF, Bucci DZ, Dagli MLZ, et al. Pathology Affects Different Organs in Two Mouse Strains Chronically Infected by A Trypanosoma cruzi Clone: A Model for Genetic Studies of Chagas' Disease. *Infect Immun*. 2004. doi:10.1128/IAI.72.4.2350-2357.2004.

49. Messenger LA, Yeo M, Lewis MD, Llewellyn MS, Miles MA. Molecular genotyping of Trypanosoma cruzi for lineage assignment and population genetics. In: Peacock C, ed. *Parasite Genomics Protocols*. Vol 1201. Methods in Molecular Biology. New York, NY: Springer New York; 2015:297-337. doi:10.1007/978-1-4939-1438-8.

50. Miles M, Póvoa M, Prata A, Cedillos R, De Souza A, Macedo V. Do radically dissimilar Trypanosoma cruzi strains (zymodemes) cause Venezuelan and Brazilian forms of Chagas' disease? *Lancet*. 1981;(June 20):1338-1340.

51. BUSTAMANTE JM, RIVAROLA HW, FERNÁNDEZ AR, et al. Indeterminate Chagas' disease: Trypanosoma cruzi strain and re-infection are factors involved in the progression of cardiopathy. *Clin Sci*. 2003. doi:10.1042/CS20020245.

52. Meymandi S, Hernandez S, Park S, Sanchez DR, Forsyth C. Treatment of Chagas Disease in the United States. *Neglected Trop Dis*. 2018;10(3):373–388. doi:10.1007/s40506-018-0170-z.

53. World Health Organization. *CONTROL OF CHAGAS DISEASE: Second Report of the WHO Expert Committee World Health Organization*. Vol 905.; 2002.

54. Laguens RP, Cossio PM, Diez C, et al. Immunopathologic and morphologic studies of skeletal muscle in Chagas' disease. *Am J Pathol*. 1975;80(1):153.

55. Traynor K. Benznidazole approved for Chagas disease in children. *Am J Heal Pharm*. 2017;74(19):1519-1519. doi:10.2146/news170065.

56. Urbina JA. Chemotherapy of Chagas' disease: The how and the why. *J Mol Med*. 1999;77(3):332-338. doi:10.1007/s001090050359.

57. Oliveira I, Torrico F, Muoz J, Gascon J. Congenital transmission of Chagas disease: A clinical approach. *Expert Rev Anti Infect Ther*. 2010;8(8):945-956. doi:10.1586/eri.10.74.

58. Viotti R, Vigliano C, Lococo B, et al. Side effects of benznidazole as treatment in chronic Chagas disease: Fears and realities. *Expert Rev Anti Infect Ther*. 2009;7(2):157-163. doi:10.1586/14787210.7.2.157.

59. Marin-Neto JA, Rassi A, Morillo CA, et al. Rationale and design of a randomized placebo-controlled trial assessing the effects of etiologic treatment in Chagas' cardiomyopathy: The BENznidazole Evaluation For Interrupting Trypanosomiasis (BENEFIT). *Am Heart J*. 2008. doi:10.1016/j.ahj.2008.04.001.

60. Rassi A, Marin-Neto JA, Rassi A. Chronic chagas cardiomyopathy: A review of the main pathogenic mechanisms and the efficacy of aetiological treatment following the BENznidazole evaluation for interrupting trypanosomiasis (BENEFIT) trial. *Mem Inst Oswaldo Cruz*. 2017. doi:10.1590/0074-02760160334.

61. Gurtler R, Kitron U, Cecere M, Segura E, Cohen J. Sustainable vector control and management of Chagas disease in the Gran Chaco, Argentina. *Proc Natl Acad Sci U S A*. 2007;104(41):16194-16199. doi:10.1073/pnas.0700863104.

62. Lucero DE, Morrissey L a, Rizzo DM, et al. Ecohealth interventions limit triatomine reinfestation following insecticide spraying in La Brea, Guatemala. *Am J Trop Med Hyg*. 2013;88(4):630-637. doi:10.4269/ajtmh.12-0448.

63. Rojas De Arias A, Ferro EA, Ferreira ME, Simancas LC. Chagas disease vector control through different intervention modalities in endemic localities of Paraguay. *Bull World*

Health Organ. 1999.

64. Klotz SA, Shirazi FM, Boesen K, et al. Kissing Bug (Triatoma spp.) Intrusion into Homes: Troublesome Bites and Domiciliation. *Environ Health Insights*. 2016;10:45-49. doi:10.4137/EHI.S32834.

65. Castro MCM, Barrett T V., Santos WS, Abad-Franch F, Rafael JA. Attraction of Chagas disease vectors (Triatominae) to artificial light sources in the canopy of primary Amazon rainforest. *Mem Inst Oswaldo Cruz*. 2010;105(8):1061-1064. doi:10.1590/S0074-02762010000800019.

66. Walter A, Lozano-Kasten F, Bosseno MF, et al. Peridomicilary habitat and risk factors for Triatoma infestation in a rural community of the Mexican occident. *Am J Trop Med Hyg*. 2007;76(3):508-515. doi:76/3/508 [pii].

67. Rojas-de-Arias A. Chagas disease prevention through improved housing using an ecosystem approach to health. *Cad Saude Publica*. 2001;17:89–97.

68. I. A, M.T. C, M.R. B, et al. Congenital Chagas disease: the importance of screening before the delivery. Our experience in a regional hospital in Catalonia. *Trop Med Int Heal*. 2011.

69. Neto EC, Rubin R, Schulte J, Giugliani R. Newborn screening for congenital infectious diseases. *Emerg Infect Dis*. 2004. doi:10.3201/eid1006.030830.

70. Zamora D, Klotz SA, Meister EA, Schmidt JO. Repellency of the Components of the Essential Oil, Citronella, to Triatoma rubida, Triatoma protracta, and Triatoma recurva (Hemiptera: Reduviidae: Triatominae). *J Med Entomol*. 2015;52(4):719-721. doi:10.1093/jme/tjv039.

71. Levy MZ, Quíspe-Machaca VR, Ylla-Velasquez JL, et al. Impregnated netting slows infestation by Triatoma infestans. *Am J Trop Med Hyg*. 2008;79(4):528-534.

CHAPTER II

TRYPANOSOMA CRUZI (AGENT OF CHAGAS DISEASE) IN SYMPATRIC HUMAN AND DOG POPULATIONS IN "COLONIAS" OF THE LOWER RIO GRANDE VALLEY OF TEXAS*

INTRODUCTION

The vector-borne parasite *Trypanosoma cruzi* causes Chagas disease in humans and dogs throughout the Americas. An estimated 6 million people are infected throughout Latin America[1]; and estimates of infected immigrants living in the United States range from approximately 72,000 to over 300,000.[2,3] Locally acquired human cases have been recognized in the southern United States, where Texas has documented transmission.[4–6] Vector- borne transmission is via infected triatomine "kissing bug" insects found throughout the Americas, including the southern United States.[7,8] The parasite may also be transmitted congenitally, via blood transfusion or organ transplant, and through ingestion of infected bugs or contaminated foods.[8] Infected persons and dogs may develop acute nonspecific disease, followed by a prolonged asymptomatic period; chronic disease is characterized by parasite damage to tissues, including the heart.[1] In some cases, mortality has been documented in canines during the acute phase.[9,10] No human or canine vaccine exists, and anti- parasitic treatment options are limited. *Trypanosoma cruzi* infection in canines is well described in the southern United States,[10–14] though the degree to which canine infection reflects human disease risk in the United States is unknown.

*Reprinted from (Curtis-Robles & Zecca et al., 2017, https://doi.org/10.4269/ajtmh.16-0789) Open Access Attribution 4.0 International (CC BY 4.0) With minor grammatical changes and the addition of APPENDIX I

Studies throughout the Americas have shown dogs to be important reservoir hosts for, and potential sentinels of, *T. cruzi*.[15] Dogs in a household have been shown to be a risk factor for vector presence in houses,[16–18] and their presence is associated with increased odds of infected bugs and people within households in South American studies.[17,19,20] However, canine infection has been shown to vary widely across studies, likely influenced by local transmission dynamics (sylvatic versus domestic or peridomestic), circulating *T. cruzi* strain types, recent vector control initiatives, and diagnostic methods and reporting.[15] Canines have been proposed as sentinels for *T. cruzi* infection in humans,[11,21,22] although the degree to which canine infection is indicative of human risk is dependent upon consideration of local transmission dynamics.[15]

Along the United States–Mexico border, approximately 1.7 million individuals inhabit "colonias"—economically distressed and unincorporated border communities with inadequate sewer, water, and/or electric services.[23] Although the term "colonia" most directly translates to "community" or "neighborhood" in Spanish throughout many parts of Latin America, in the United States the term is used most specifically to describe impoverished rural settlements along the United States–Mexico border region (see[23–25] for more detailed information regarding colonias). In Texas, there are an estimated 1,800–2,300 designated colonias inhabited by 400,000–500,000 predominantly Hispanic individuals.[24] The actual number of colonias and their population size may be greater due to unrecorded colonias and undocumented or transient individuals. Such Hispanic and Latino populations in the United States may be at higher risk for Chagas disease if they have emigrated from countries with high disease burdens, given that the vast majority of individuals in the United States reported to have Chagas disease were born in or

have travel histories to endemic regions in Latin America.[2] Health disparities related to limited health education, poor access to health care, low incomes, and poor housing structure may be important risk factors for locally acquired infections. We suggest that people living in colonias may be at high risk for vector-borne infections because substandard housing surrounded by wild habitats may be conducive to vector colonization of homes. Further, because many dogs in colonias typically live outdoors and roam freely, these dogs may be at increased risk of exposure to kissing bugs and infected wildlife that may serve as sources of infective stages of *T. cruzi*.[26–29] Given these risk factors, the objective of this study was to determine *T. cruzi* infection prevalence in sympatric human and dog populations of rural colonias in south Texas.

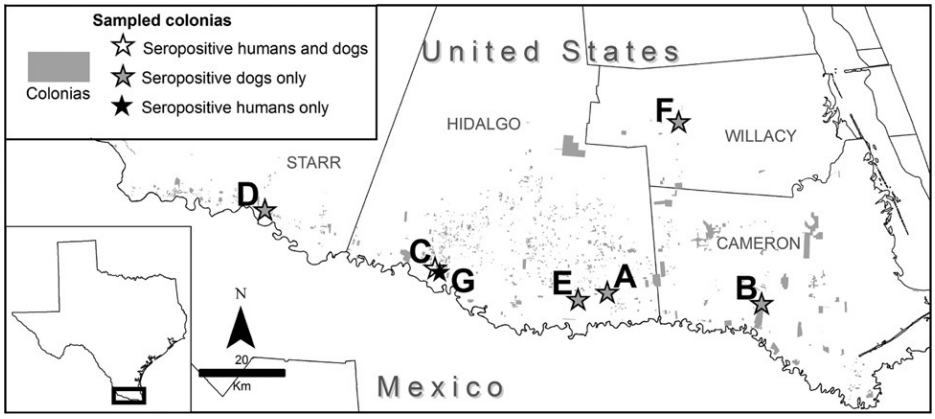

Figure 4. Colonias in south Texas. Seven colonias in the four south Texas counties of the Rio Grande Valley were sampled; seropositive individuals are defined as those that were positive on at least two tests. One additional human (in Colonia B) was seropositive on only one test.

MATERIALS AND METHODS

Humans and dogs in south Texas colonias (Figure 4) were sampled from July to November 2015, coincident with seasonal triatomine activity across Texas.[7] Colonias were selected to represent the lower Rio Grande Valley (Hidalgo, Cameron, Starr, and Willacy counties) with selection

based, in some cases, on preexisting relationships with the project promotora (community health worker) and, in one case (Colonia B), geographic location near previous report of Chagas disease in dogs.[12] All of the colonias sampled were located in border counties or in a county adjacent to a border county. The total number of households per sampled colonia ranged from 30 to 600 and approximately 5–25% of the households were sampled (5% from largest colonia and 25% from smallest colonia). The majority of the sampled colonias were surrounded by agricultural fields, undeveloped land, or natural areas. Most of the sampled colonias had unpaved or damaged paved roads. Housing within each colonia varied from severely dilapidated mobile homes to new brick homes. The most commonly observed housing consisted of mobile homes with poorly constructed add-ons or partially completed homes in which the construction appeared to have ceased. Electricity, running water, and sewage systems were observed in the majority of the sampled colonia households, although some homes operated under unreliable services due to unregulated electricity lines, water pipes, and sewage systems.

Study documents including consent forms and outreach materials were available to participants in English and Spanish. To facilitate study enrollment, the promotora alerted each community days prior to sampling and provided outreach materials about kissing bugs and Chagas disease to 10–20 households in each colonia. Colonia members who received information were encouraged to invite other members of their colonia. Sampling of humans (age $\geq$ 4 years) and dogs (age $\geq$ 3 months) occurred at a central location in each colonia; human-only sampling occurred in one colonia (Colonia G) that prohibited canines. Children under the age of 4 years were not sampled because our team did not perform pediatric evaluations that would have been required prior to blood draws. After informed consent was obtained from adult participants and from parents or legal guardians of minors, 2–5 mL of blood was obtained from participating individuals and their

canines. Rabies vaccination of dogs after blood draws was provided as an incentive for owners to bring their dogs for sampling. A brief survey of each human participant included sociodemographic questions and details of travel history. Participants were shown pictures of kissing bugs and resin-encapsulated kissing bug specimens and asked to indicate if and where they had previously seen kissing bug vectors. Kissing bugs found by participants during the study were accepted for identification and *T. cruzi* testing. Human and canine sampling and survey protocols were approved by Texas A&M University Institutional Review Board and Institutional Animal Care and Use Committee, respectively.

Human and canine serum or plasma samples were initially tested for anti-*T. cruzi* antibodies using Chagas Stat- Pak (Chembio Diagnostic Systems, Inc., Medford, NY). This rapid immunochromatographic test has shown high sensitivity (> 95%) and specificity (> 95%) in human samples, compared with other serological techniques[30–35]; however, others have found considerable variation in and lower sensitivity (26.6–87.5%) depending upon geographic region.[36] Chagas Stat-Pak has been used previously for research in dogs[11,13,37]; one study calculated sensitivity and specificity of 100% (however, high values may have been the result of not counting faint bands as positive).[13] All positive samples, and a subset of negative dog samples, were then tested with Chagas Detect Plus Rapid Test (InBios International, Inc., Seattle, WA), a rapid immunochromatographic test based on specific recombinant antigens designed for use in humans[38] (showing 96–99% sensitivity and 97–99% specificity[39]). Both the Chagas Stat-Pak and InBios Chagas Detect Plus Rapid tests have integrated test controls; in all cases, "control" bands appeared as expected. When sample volumes allowed, human samples that initially tested positive were tested for IgG antibodies at Mayo Medical Laboratories (Rochester,

MN) using Hemagen Chagas ELISA kit (Hemagen Diagnostics, Inc., Columbia, MD), and canine samples that initially tested positive were tested for IgG antibodies using indirect fluorescent antibody (IFA) testing (Texas Veterinary Medical Diagnostic Laboratory, College Station, TX). As per Texas Veterinary Medical Diagnostic Laboratory reporting standard, samples positive at dilutions of 1:20 or greater were considered IFA-positive. The IFA test is currently the most widely available test for diagnosis of canine anti-*T. cruzi* antibodies in the southern United States. Serological tests were often performed after freezing and thawing of serum with periods of storage that exceeded 1 month. Considering potential variability in detection sensitivity and specificity, we defined as positive only those individuals that were positive on two or more independent tests. We do, however, also present the data for those individuals that reacted on only a single test because the true infection status of individuals with discordant test results is currently unknown.

To directly detect *T. cruzi*, DNA extracted from the buffy coat (E.Z.N.A. kit; Omega Bio-Tek, Norcross, GA) was subjected to quantitative polymerase chain reaction (PCR) to amplify nuclear T. *cruzi* DNA[40,41] using a Stratagene MxPro3000 instrument (Agilent Technologies, Santa Clara, CA) and previously described thermocycling parameters,[40] except with a 3-minute initial denaturation. Reactions contained 5 μL of DNA, primers at a final concentration of 0.75 μM each, 0.25 μM of probe, and iTaq Universal Probes Supermix (BioRad Laboratories, Hercules, CA), in a total volume of 20 μL. Successful amplification was assured by visually checking machine-calculated thresholds and reaction curves. A Ct value less than 31 was positive, based on internal laboratory validations using serial dilutions of *T. cruzi* to calculate limit of detection and PCR efficiency for this assay. We used a multiplex probe-based assay amplifying the spliced leader intergenic region (SL-IR) to determine *T. cruzi* discrete typing units (DTUs).[42] Assays

were run with a 20-μL reaction volume using the Multiplex PCR Kit (Qiagen, Valencia, CA) on a BioRad CFX96 following published protocol.[42] DNA-negative water controls and positive controls of DNA extracted from Sylvio X10 CL4 (ATCC 50800, American Type Culture Collection, Manassas, VA) and *T. cruzi*-infected *Triatoma sanguisuga* from Texas were included in all PCR batches.

Kissing bug specimens were identified morphologically to species,[43] sexed, and dissected. DNA from bug hindguts was extracted and subjected to quantitative PCR[40] to determine infection status; parasite DTU was ascertained as described above.[42] To determine blood meal source, bug hindgut DNA was subjected to PCR amplifications targeting cytochrome b DNA.[44–47] We used an iterative process in which extracted DNA was subjected to multiple separate PCRs for the same or different regions of cytochrome b for three reasons. First, given degraded DNA from these insects that were collected and stored by colonia residents, some attempts to amplify were negative even when residual blood traces were observed in the hindgut, and the overall chance of assigning a blood meal host was greater with multiple attempts. Second, rerunning all samples was especially important when sequences comprised human DNA. Although we decontaminated the external surface of insects prior to dissection, we are aware that contamination may occur and human DNA could serve as a likely source; accordingly, rerunning PCR and resequencing across multiple genetic regions afforded an independent opportunity to confirm the presence of human DNA. Finally, the iterative process afforded the opportunity to generate different amplicons and sequences representing different host species from the same insects that could be interpreted as mixed blood meals from feeding on multiple host species. PCR using the "herp" primer set[44,45] included 3 μL template DNA, primers at final concentrations of 0.66 μM each, and FailSafe PCR

Enzyme Mix with PreMix E (Epicentre, Madison, WI) in a final reaction volume of 50 μL using previously described cycling conditions.[44] PCR using the "BM" primer set[45,47,48] included 1.5 μL template DNA, primers at final concentrations of 0.66 μM each, and FailSafe PCR Enzyme Mix with PreMix E (Epicentre) in a final reaction volume of 15 μL using previously described cycling conditions.[47] PCR using the "mammalian a" primer set[45–47] included 1.5 μL template DNA, primers at final concentrations of 0.66 μM each, and FailSafe PCR Enzyme Mix with PreMix E (Epicentre) in a final reaction volume of 15 μL using previously described cycling conditions.[46,47] DNA-negative water controls and positive controls of DNA extracted from white-tailed deer (*Odocoileus virginianus*), opossum (*Didelphis virginiana*), or cynomolgus macaque (*Macaca fascicularis*) were included in all PCR batches. Amplicons were visualized on 1.5% agarose gels with ethidium bromide, and amplicons of expected product sizes were sequenced using Sanger sequencing (Eton Bioscience Inc., San Diego, CA) in both directions. Forward and reverse strands were aligned and the consensus region was compared with published sequences using Basic Local Alignment Search Tool (National Center for Biotechnology Information, U.S. National Library of Medicine).

To evaluate the relationship between putative risk factors and canine seroprevalence, bivariable analysis using χ^2 or Fisher's exact test was used. Variables were colonia (A–F), canine age (< 2 years, ≥ 2 years), sex, and breed group (per American Kennel Club: herding, sporting, terrier, toy, and "other"; "other" was comprised of breed groups with relatively small sample sizes including hound, mix, nonsporting, and working breeds). Logistic regression models were built using Program R[49] using the lme4 package to further investigate risk factors with $P < 0.25$ in the initial screening or those with justification for inclusion based on published data. Odds ratios and

95% confidence intervals were calculated. Descriptive statistics for human data were calculated, but the low number of seropositive humans precluded further analysis.

RESULTS

We collected samples from 233 humans and 209 dogs from 143 households, of which 77 households (53.8%) had both humans and dogs contribute samples to the study. Of human participants, 146 were female (62.7%), and 87 were male (37.3%). There were 81 children (4–17 years of age) sampled (34.8% of total). Mean age of females was 35.4 years (standard deviation [SD]: 19.7 years; range: 4– 83 years), and of males was 27.0 years (SD: 22.1 years; range: 4–80 years). Most participants surveyed (78.0%) spoke only Spanish or "more Spanish than English" at home. A total of 44 (18.9%) individuals confirmed having seen kissing bugs. Bug sightings were mainly stated to be in Texas (84.1%) with the remainder seen in Mexico (15.9%). Of Texas sightings, 62.2% occurred inside the respondent's home or outdoors in the colonia. The remaining sightings occurred in other towns, friends' homes, ranches, and/or workplace environments.

Anti-*T. cruzi* antibodies were present in three of 233 (1.3%) human blood samples as evidenced by reaction in ≥ 2 independent tests, and one additional human was positive on a single test only (Tables 1 and 2). Two positive individuals—a 68-year-old man (GH03) and a 40-year-old woman (GH16)—were from the same colonia (Colonia G). The other positive individual was a 13-year-old girl from Colonia C (CH29; Table 2). A 13-year-old boy from Colonia B (BH54) was associated with a single positive test result. Epidemiological follow-up revealed the two positive adults were born in Mexico, and the positive child was born in

Texas to a mother that was determined to be seronegative in our study. All three positive individuals reported travel to Mexico ranging from < 1-week biannual trips to 8-week trips every 5 years. The child associated with a single positive test result (BH54) reported no travel outside south Texas and was born to a mother that was determined to be seronegative in our study (Table 2). Additionally, the 13-year old child of GH16 was determined to be seronegative in our study. Blood samples from 186 humans had sufficient volume for DNA extraction and attempted PCR amplification; none had evidence of *T. cruzi* DNA.

TABLE 1

Human *Trypanosoma cruzi* antibody testing results (*N* = 233) in the colonias of south Texas, 2015

Colonia	County	Month sampled	No. tested	No. seropositive	Seroprevalence (%)
A	Hidalgo	July	11	0	0
B	Cameron	July	54	0*	0
C	Hidalgo	July	28	1	3.6
D	Starr	November	35	0	0
E	Hidalgo	July	45	0	0
F	Willacy	November	44	0	0
G	Hidalgo	July	16	2	12.5
Total			233	3	1.3

Seroprevalence estimates are based on positivity based on two or more independent test results.
*One individual was positive on a single test only and therefore did not meet the positivity criterion for the study.

TABLE 2

Details from surveys of four individuals associated with at least one *Trypanosoma cruzi* positive serological test result, Texas, 2015

	GH03	GH16	CH29	BH54
Serologic test results (Chagas Stat-Pak/Chagas Detect/Hemagen)	+/+/+	+/+/–	+/+/not run	+/–/–
Colonia, county	G, Hidalgo	G, Hidalgo	C, Hidalgo	B, Cameron
Sex	Male	Female	Female	Male
Ethnicity	Hispanic	Hispanic	Hispanic	Hispanic
Age, years	68	40	13	13
Occupation	Agriculture and oil industry	Homemaker	Student	Student
Dogs included in study	No	No	No	1 seropositive dog and 1 seronegative dog
Kissing bug sighting in Texas	No	No	Yes	No
Birthplace	Mexico	Mexico	United States	United States
Time lived in United States	20 years	5 years	13 years	13 years
Time lived in present colonia	1 year	1 year	13 years	1 year
Most recent prior living location	Rio Grande Valley	Mission, TX	NA	Brownsville, TX
Travel within Texas or the United States	Yes (Louisiana—10 years ago)	No	No	No
Travel to other countries	Yes (Mexico)	Yes (Mexico)	Yes (Mexico)	No
Duration of travel	8 weeks	1–2 weeks	< 1 week	NA
Frequency of travel to Mexico	Every 5 years	Every 5 years	Twice per year	NA
Seasonality of travel to Mexico	Summer	Summer	Summer	NA
Maternal birthplace	Mexico	Mexico	Mexico	Mexico
Serostatus of mother	Unknown	Unknown	Seronegative	Seronegative
History of blood transfusion or organ transplant	No	No	No	No
Medical history involving heart or gastrointestinal disease	Hypertension	No	No	No

NA = not applicable.

Of 209 dogs, 96 were female (45.9%) and 113 were male (54.1%). The majority (128 dogs, 61.2%) were small-breed dogs, weighing less than 9 kg. Dogs had a mean age of 2.8 years (SD: 2.53 years; range: 3 months to 13 years). A diversity of breed groups and mixes were represented, with the largest representation comprised of toy breeds (83 dogs, 39.7% of total). In particular, Chihuahua and Chihuahua- mix dogs represented 34.0% of the total.

Anti-*T. cruzi* antibodies were present in 41 of 209 (19.6%) canine blood samples based on positivity on ≥ 2 tests. Seropositive dogs were found in all six colonias with dogs (Table 3). Seven seropositive dogs were in single-dog households; 16 seropositive dogs were the only seropositive dog in a household from which multiple dogs were tested, and 18 seropositive dogs were from eight multidog households having two or three seropositive dogs. One seropositive dog lived in the same house as the human (BH54) that was positive on only one serologic assay. In bivariable analyses, only the relationship between colonia and seropositivity was associated with a P value below the cutoff (within-colonia seroprevalence ranged from 9.4% to 31.8%, P = 0.130, Fisher's exact test), but all four putative risk factors were retained in the regression model based on previous findings.[10,50,51] A logistic regression model did not reveal differences in the odds of canine seropositivity by sex, age, or breed group (Table 3). Among the colonias, the odds of seropositivity were significantly lower among dogs residing in the Colonia F relative to dogs in the referent (OR = 0.191, 95% CI = 0.050–0.688, P = 0.012; Table 3).

Of 157 dogs that tested negative on the Chagas Stat- Pak, 11 were randomly selected and subjected to additional testing, of which a single dog (9.1%) was positive on IFA (at a 1:20 dilution) but negative on Chagas Detect Plus Rapid test. Applying this prevalence of potentially positive samples to the population of Chagas Stat-Pak-negative dogs, we extrapolate 14

additional dogs might have been seropositive. When summing the 41 dogs positive on at least 2 tests, the 11 dogs positive on only a single test, and this estimate of potential false negatives, a more liberal estimate of canine seroprevalence is 66 of 209 dogs (31.6%).

TABLE 3

Logistic regression model output to identify signalment predictors of *Trypanosoma cruzi* seropositivity among 209 dogs in the colonias of south Texas, 2015

Demographic factor	No. tested	No. seropositive	Seroprevalence (%)	Odds ratio	95% confidence interval	*P* value
Colonia						
A	22	7	31.8		Referent	
B	54	12	22.2	0.592	0.180–2.007	0.390
C	14	4	28.6	0.945	0.192–4.285	0.942
D	32	7	21.9	0.510	0.135–1.864	0.304
E	23	5	21.7	0.547	0.122–2.279	0.413
F	64	6	9.4	0.191	0.050–0.688	**0.012**
Age						
< 2 years	93	18	19.4		Referent	
≥ 2 years	116	23	19.8	1.038	0.511–2.110	0.917
Sex						
Female	96	19	19.8		Referent	
Male	113	22	19.5	1.029	0.508–2.086	0.936
Breed group						
Herding	30	7	23.3		Referent	
Sporting	19	3	15.8	0.661	0.145–3.017	0.593
Terrier	39	6	15.4	0.601	0.177–2.044	0.415
Toy	83	17	20.7	0.884	0.319–2.452	0.813
Other	38	8	21.1	0.918	0.284–2.970	0.887
Total	209	41	19.6*			

*This estimate increases to 31.6% when including extrapolated potential false negatives.

Of 184 canines with sufficient blood sample for DNA extraction and PCR amplification, five dogs (2.7%) had evidence of T. *cruzi* DNA. All five also reacted on serological assays, with four positives on Chagas Stat-Pak plus at least one other assay, and one dog positive on Chagas Stat-Pak but negative on IFA and Chagas Detect Plus Rapid assays. Three PCR-positive dogs were from Colonia A (N = 20), including two from the same household, and the remaining two were from Colonia B (N = 54) and D (N = 27).

No PCR-positive dogs were detected in colonias C (N = 13), E(N=23), or F(N=47). Four of the five dogs were sampled in July and one was sampled in November. The SL-IR PCR allowed for ascertaining the parasite DTU in four of these dogs; all were infected with TcI.

Three kissing bugs were collected from around homes in colonias during the study. COL1 was an adult female *Triatoma gerstaeckeri*. The submitter of COL1 indicated that the insect was feeding on his arm and that he regularly found kissing bugs around his raised wooden home. COL1 was positive for *T. cruzi* DTU TcI. When sequencing the amplicons from the "BM" primer set, a consensus sequence of 220 base pairs (bp) most closely matched (100% identity) human sequences. On the second independent attempt using the same primer set, a consensus sequence of 232 bp most closely matched (99% identity) raccoon (*Procyon lotor*). Amplification of this bug sample was attempted twice using the "herp" primer set and once using the "mammalian a" primer set, but these attempts were unsuccessful. COL2 was an adult female *T. gerstaeckeri* found by a colonia resident who reported regularly seeing and crushing triatomines both inside and outside of her home. COL2 was negative for *T. cruzi*. When sequencing amplicons from the "BM" primer set, a consensus sequence of 218 bp was identical to human sequences (100% identity). On the second independent attempt using the same primer set, a consensus sequence of 245 bp was again identical to human (100% identity). Additional attempts, twice using the "herp" primer set and once using the "mammalian a" primer set, were unsuccessful. Finally, COL3 was a nymph found feeding on a dog. COL3 was negative for *T. cruzi*. When sequencing amplicons from the "herp" primer set, a consensus sequence of 82 bp was identical to *Canis lupus* (100% identity). Although the bidirectionally confirmed consensus sequence was only 82 bp, each of the individual forward and reverse sequences were longer (> 220 bp), and each individually also matched *C. lupus* with 100% identity. Additional attempts, once using the "herp" primer set, once using the "mammalian a" primer set, and twice using the "BM" primer set, were unsuccessful.

DISCUSSION

Humans and dogs from seven colonias in south Texas were tested for anti-*T. cruzi* antibodies and *T. cruzi* DNA. We detected 1.3% seroprevalence in 233 humans, and 19.6% seroprevalence in 209 cohabiting canines of diverse breeds, although adjustment for potential false negatives suggests canine seroprevalence may approach 31.6%. Given that approximately 500,000 humans live in Texas colonias,[24] extrapolation of our study findings suggest 6,500–8,500 infected people may reside in Texas colonias. No human samples had evidence of *T. cruzi* DNA, whereas five dogs (2.7% of 184 tested) also had evidence of *T. cruzi* DNA in their blood, likely indicating potential parasitemia. Further, adult and nymph triatomine vectors, including an infected insect and insects with evidence of having fed on humans, dog, and raccoon, were collected in the colonias. Cumulatively, these results highlight active transmission cycles of *T. cruzi* in south Texas colonias.

In contrast to the many studies testing populations throughout Latin America for *T. cruzi* infection, studies in the United States are limited, and Texas is one of few U.S. states with history of such studies. A study in the 1960s found 1.4% seroprevalence in 500 children from south central Texas.[52] A 1970s study of people along the south Texas border area showed 0.8% seroprevalence.[29] More recently, a study from 2008 to 2012 found a seroprevalence of 0.015% in blood donors.[53] In the colonias, the seroprevalence we detected (1.3%) is two orders of magnitude higher than the recent finding in Texas blood donors, likely reflective of sociodemographic and health status differences between colonia populations and the general blood donor population.

Although autochthonous infection is possible in all cases of humans associated with positive test results, it may be more likely that infections were acquired in Mexico for at least three of these four individuals, where there is an estimated infection prevalence of 0.78%.[1] The two seropositive adults were born in Mexico and moved to the United States in adulthood, with sporadic, short-duration visits to Mexico. The seropositive child was born in Texas and had visited Mexico for short durations (< 1 week) approximately twice per year. Although *T. cruzi* transmission could have occurred during such visits, vector-borne transmission of *T. cruzi* is inefficient—one mathematical model indicates 900–4,000 contacts with infected bugs are necessary for vector-borne parasite transmission.[54] On the other hand, there is at least one documented case of infection in a child in a house with very low vector presence.[55] The child (BH54) with a positive result on only a single test was born in Texas, with no travel history outside the Rio Grande Valley, and his mother was seronegative, suggesting a potential autochthonous infection, although further testing would be needed to confirm infection.

Although sampling was demographically biased, with mainly mothers and children sampled since men were usually working away from the home, women in Latino communities traditionally play a major role in healthcare decision making and accessing health services[14] and therefore were a priority in our outreach efforts. A study of Latino immigrants in the state of Georgia described minimal Chagas awareness, education, screening, and treatment in participants due to cultural and language barriers, economic limitations, fear of deportation, and cultural preference for traditional medicines.[56] Colonia residents are similarly likely to face such factors inhibiting Chagas disease awareness, in addition to additional decidedly unique characteristics of colonias (see [24,57]) that may further limit Chagas disease awareness, including

poverty, poor infrastructure, and lack of access to health care and education. Others have noted that colonia residents, known to be primarily Spanish speaking individuals, are more likely to have poorer physical health than the general U.S. population.[58] A prior serologic study of Chagas disease in the general Texan population in the 1960s specifically noted that houses with wood frame construction raised off the ground were conducive to close proximity of infected wildlife[52]; construction of many houses in colonias we sampled met this description, with dogs commonly observed to rest or sleep under the houses (Figure 5).

Our finding of 19.6% seroprevalence in canines is higher than previous studies in south Texas, which showed 6.7–8.8% seroprevalence in stray or shelter dogs.[11,12,29] Canine infection has been shown to vary widely across studies throughout the Americas, likely influenced by local transmission dynamics (sylvatic versus domestic or peridomestic), circulating *T. cruzi* strain types, recent vector control initiatives, and diagnostic methods and reporting.[15] Dog presence in households has been shown to be a risk factor for vector presence,[16–18] and is associated with increased odds of infected bugs and people within households in South American studies.[17,19,20,59] Canines have been proposed as sentinels for *T. cruzi* infection in humans[11,15,21,22]; however, the degree to which canine infection is indicative of human risk is dependent upon consideration of local transmission dynamics.[15] Despite evidence of widespread canine exposure,[10] the degree to which infected dogs reflect a risk of human disease in the very different ecological settings across south Texas and the United States is largely unknown. The risk of vector-borne transmission of *T. cruzi* in humans can increase when animals live in or around households,[16–19] which is the case for most colonia residents. However, the three seropositive humans in our study resided in a colonia that did not allow dog ownership or did not

report owning a dog; in contrast, a seropositive canine resided on the property of the child associated with a single positive test result (BH54), indicating the surrounding environment supported potential autochthonous infection.

We found 2.7% of canines had *T. cruzi* DNA in their blood. Although molecular methods alone do not conclusively confirm parasitemia, these observations may indicate infectious levels of circulating parasites.[60] Only DTU TcI was found in PCR-positive dogs, which contrasts limited prior findings of mainly TcIV in U.S. dogs.[61] The few U.S. human infections for which parasite DTU has been determined have revealed exclusively TcI.[61]

Due to financial limitations and cultural factors, dog ownership in colonias is typically regarded differently than in the general U.S. population. Dog owners who consider their dogs as "family" are more likely to provide regular care to their animals, and in comparison to the general population, low-income Hispanics—who compose the majority of colonia populations are less likely to consider their dogs as "family."[62] Canines in colonias are often kept tied or loose outdoors, resulting in stray dog packs that may cause public health hazards.[63] Weak infrastructure in colonias further limits county government efforts of stray animal control.[23] The outdoor-stray lifestyle of colonia dogs is likely to increase their risk of exposure to vectors and sylvatic reservoirs, thus increasing *T. cruzi* transmission risk. Though travel histories of dogs were not known, based on the subset of seropositive dogs with owners who completed a survey (N = 31), the ages of nearly all (93.5%) the dogs were less than the period of time their owners had lived in the colonias or immediate area, suggesting locally acquired infections.

The lack of age, sex, and breed predispositions for canine Chagas seropositivity in our data suggests that all dogs that share environments with infected bugs are at risk. When compared with dogs in Colonia A (the model referent), dogs in Colonia F had significantly lower odds of being seropositive. The county of Colonia F has a lower percentage of Hispanics and is more rural than the counties of the other sampled colonias,[64] congruent with a prior study found lower seroprevalence in rural dogs as compared with urban dogs.[65]

Incongruences across different serological tests are a challenge in Chagas disease diagnostics,[32,66] and findings in this study are no exception. Differences in test design and interpretation, local circulating *T. cruzi* strains, antigens used, and sample integrity (which may be compromised in repeated thaw/freeze cycles) have been suggested as potentially affecting classification of samples as positive versus negative.[36,67] An important consideration is that the low seroprevalence in sampled humans limits the positive predictive value of the diagnostic tests. To reduce the number of potential false positives in this study, we used the criteria of positive results on at least two independent tests to indicate a positive individual. Additionally, although population-level epidemiological research may proceed with presentation of ranges of estimated seroprevalence based on different diagnostics, individual-level diagnosis and prognosis suffer from lack of clear understanding of what positivity on only one test signifies. Individuals in this study who were positive on at least one serological test were given contact information of local medical providers able to assist with further testing and treatment options.

Triatomines submitted by participants during the study included a *T. cruzi*-infected *T. gerstaeckeri* and evidence of the triatomines having fed on human, dog, and raccoon; these findings underscore active transmission risk among humans, dogs, and wildlife within colonias.

We found success in assigning blood meal hosts using an iterative process in which each insect DNA extract was subjected to multiple PCRs; this approach afforded confidence through being able to assess reproducibility of results. In addition to evidence of feeding on a dog in one bug and a human in another bug, DNA from a third insect generated both human and raccoon sequences. Given this insect fed a minimum of six times (at least once per nymphal instar plus adult stage), but likely many more times, evidence of mixed hosts in the hindgut is biologically reasonable and in this case underscores transmission risk between wildlife and humans.

Using a One Health framework, our observations of infected dogs, humans, and triatomines within shared households highlight the border colonias as at-risk communities for Chagas disease. Education and outreach campaigns with associated vector control measures should appropriately focus on these communities with high priority.

Please see Appendix I for additional information.

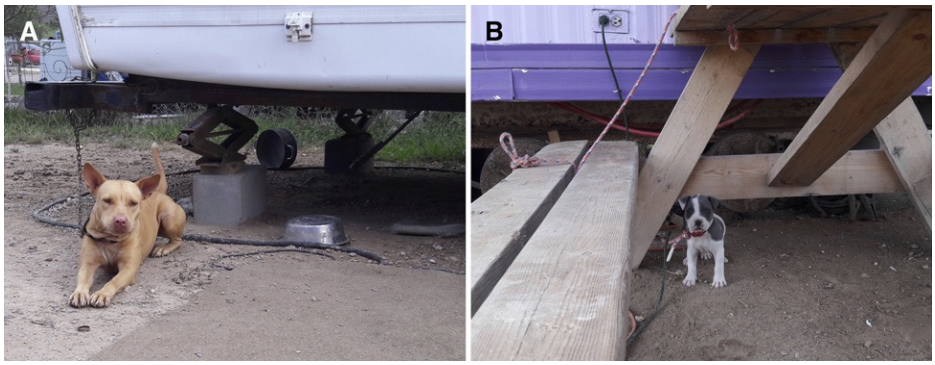

Figure 5. (A) Adult and (B) puppy pitbull mix dogs under homes in colonias, Rio Grande Valley of Texas, 2015. Many dogs in colonias are kept outside, where they rest and sleep under the raised houses.

REFERENCES

1. World Health Organization, 2015. Chagas disease in Latin America: an epidemiological update based on 2010 estimates. Wkly Epidemiol Rec 6: 33–34.

2. Bern C, Montgomery SP, 2009. An estimate of the burden of Chagas disease in the United States. Clin Infect Dis 49: e52 – e54.

3. Schmunis GA, 2007. Epidemiology of Chagas disease in non- endemic countries: the role of international migration. Mem Inst Oswaldo Cruz 102 (Suppl): 75–85.

4. Hanford EJ, Zhan FB, Lu Y, Giordano A, 2007. Chagas disease in Texas: recognizing the significance and implications of evidence in the literature. Soc Sci Med 65: 60–79.

5. Garcia MN, Aguilar D, Gorchakov R, Rossmann SN, Montgomery SP, Rivera H, WocColburn L, Hotez PJ, Murray KO, 2015. Case report: evidence of autochthonous Chagas disease in southeastern Texas. Am J Trop Med Hyg 92: 325 – 330.

6. Cantey PT, Stramer SL, Townsend RL, Kamel H, Ofafa K, Todd CW, Currier M, Hand S, Varnado W, Dotson E, Hall C, Jett PL, Montgomery SP, 2012. The United States Trypanosoma cruzi infection study: evidence for vector-borne transmission of the parasite that causes Chagas disease among United States blood donors. Transfusion 52: 1922–1930.

7. Curtis-Robles R, Wozniak EJ, Auckland LD, Hamer GL, Hamer SA, 2015. Combining public health education and disease ecology research: using citizen science to assess Chagas disease entomological risk in Texas. PLoS Negl Trop Dis 9: 1–12.

8. BernC,KjosS,YabsleyMJ,MontgomerySP,2011.Trypanosoma cruzi and Chagas' disease in the United States. Clin Microbiol Rev 24: 655–681.

9. Barr SC, Gossett KA, Klei TR, 1991. Clinical, clinicopathologic, and parasitologic observations of trypanosomiasis in dogs infected with North American Trypanosoma cruzi isolates. Am J Vet Res 52: 954–960.

10. Kjos S, Snowden K, Craig T, Lewis B, Ronald N, Olson J, 2008. Distribution and characterization of canine Chagas disease in Texas. Vet Parasitol 152: 249–256.

11. Tenney TD, Curtis-Robles R, Snowden KF, Hamer SA, 2014. Shelter dogs as sentinels for Trypanosoma cruzi transmission across Texas. Emerg Infect Dis 20: 1323–1326.

12. Beard CB, Pye G, Steurer FJ, Rodriguez R, Campman R, Peterson AT, Ramsey J, Wirtz RA, Robinson LE, 2003. Chagas disease in a domestic transmission cycle, southern Texas, USA. Emerg Infect Dis 9: 103–105.

13. Nieto PD, Boughton R, Dorn PL, Steurer F, Raychaudhuri S, Estandiari J, Goncalves E, Diaz J, Malone JB, 2009. Comparison of two immunochromatographic assays and the indirect immunofluorescence antibody test for diagnosis of Trypanosoma cruzi infection in dogs in south central Louisiana. Vet Parasitol 165: 241–247.

14. Barr SC, Van Beek O, Carlisle-Nowak MS, Lopez JW, Kirchoff LV, Allison N, Zajac A, de Lahunta A, Schlafer DH, Crandall WT, 1995. Trypanosoma cruzi infection in Walker hounds from Virginia. Am J Vet Res 56: 1037–1044.

15. Gürtler RE, Cardinal MV, 2015. Reservoir host competence and the role of domestic and commensal hosts in the transmis- sion of Trypanosoma cruzi. Acta Trop 151: 32–50.

16. Bustamante DM, De Urioste Stone SM, Juárez JG, Pennington PM, 2014. Ecological, social and biological risk factors for continued Trypanosoma cruzi transmission by Triatoma dimidiata in Guatemala. PLoS One 9: 343–352.

17. Gürtler RE, Cecere MC, Rubel DN, Schweigmann NJ, 1992. Determinants of the domiciliary density of Triatoma infestans, vector of Chagas disease. Med Vet Entomol 6: 75–83.

18. Dumonteil E, Nouvellet P, Rosecrans K, Ramirez-Sierra MJ, Gamboa-León R, Cruz-Chan V, Rosado-Vallado M, Gourbière S, 2013. Eco-bio-social determinants for house infestation by non-domiciliated Triatoma dimidiata in the Yucatan Peninsula, Mexico. PLoS Negl Trop Dis 7: 1–9.

19. Gürtler R, Cécere M, Rubel D, Petersen R, Schweigmann N, Lauricella M, Bujas M, 1991. Chagas disease in northwest Argentina: infected dogs as a risk factor for the domestic transmission of Trypanosoma cruzi. Trans R Soc Trop Med Hyg 85: 741–745.

20. Mott KE, Mota EA, Sherlock I, Hoff R, Muniz TM, Oliveira TS, Draper CC, Lehman JS, Morrow RH, de Oliveira TS, 1978. Trypanosoma cruzi infection in dogs and cats and household seroreactivity to T. cruzi in a rural community in northeast Brazil. Am J Trop Med Hyg 27: 1123–1127.

21. Castañera MB, Lauricella MA, Chuit R, Gürtler RE, 1998. Evaluation of dogs as sentinels of the transmission of Trypanosoma cruzi in a rural area of northwestern Argentina. Ann Trop Med Parasitol 92: 671–683.

22. Castillo-Neyra R, Chou Chu L, Quispe-Machaca V, Ancca- Juarez J, Malaga Chavez FS, Bastos Mazuelos M, Naquira C, Bern C, Gilman RH, Levy MZ, 2015. The potential of canine sentinels for reemerging Trypanosoma cruzi transmission. Prev Vet Med 120: 349–356.

23. Housing Assistance Council, 2013. Housing in the Border Colonias. Washington, DC: Housing Assistance Council, 1–11.

24. Barton J, Perimeter ER, Marquez RR, 2015. Las Colonias in the 21st Century: Progress along the Texas-Mexico Border. Dallas, TX: Federal Reserve Bank of Dallas, 1–33.

25. Núñez-Mchiri GG, 2009. The political ecology of the colonias on the U.S.-Mexico border: human-environmental challenges and community responses in southern New Mexico. South Rural Sociol 24: 67–91.

26. Rocha FL, Roque ALR, de Lima JS, Cheida CC, Lemos FG, de Azevedo FC, Arrais RC, Bilac D, Herrera HM, Mourão G, Jansen AM, 2013. Trypanosoma cruzi infection in neotropical wild carnivores (Mammalia: Carnivora): at the top of the T. cruzi transmission chain. PLoS One 8: e67463.

27. Thomas ME, Rasweiler JJ IV, D'Alessandro A, 2007. Experimental transmission of the parasitic flagellates Trypanosoma cruzi and Trypanosoma rangeli between triatomine bugs or mice and captive neotropical bats. Mem Inst Oswaldo Cruz 102: 559–565.

28. Curtis-Robles R, Lewis BC, Hamer SA, 2016. High Trypanosoma cruzi infection prevalence associated with minimal cardiac pathology among wild carnivores in central Texas. Int J Parasitol Parasites Wildl 5: 117–123.

29. Burkholder JE, Allison TC, Kelly VP, 1980. Trypanosoma cruzi (Chagas) (Protozoa: Kinetoplastida) in invertebrate, reservoir, and human hosts of the Lower Rio Grande Valley of Texas. J Parasitol 66: 305–311.

30. Luquetti AO, Ponce C, Ponce E, Esfandiari J, Schijman A, Revollo S, Añez N, Zingales B, Ramgel-Aldao R, Gonzalez A, Levin MJ, Umezawa ES, Franco da Silveira J, 2003. Chagas' disease diagnosis: a multicentric evaluation of Chagas Stat- Pak, a rapid immunochromatographic assay with recombinant proteins of Trypanosoma cruzi. Diagn Microbiol Infect Dis 46: 265–271.

31. Ponce C, Ponce E, Vinelli E, De Aguilar V, Gonzalez A, Rangel-aldao R, Levin MJ, Umezawa ES, Luquetti AO, Montoya A, Zingales B, Esfandiari J, 2005. Validation of a rapid and reliable test for diagnosis of Chagas' disease by detection of Trypanosoma cruzi-specific antibodies in blood of donors and patients in Central America. J Clin Microbiol 43: 5065–5068.

32. Sánchez-Camargo CL, Albajar-Viñas P, Wilkins PP, Nieto J, Leiby DA, Paris L, Scollo K, Flórez C, Guzmán-Bracho C, Luquetti AO, Calvo N, Tadokoro K, Saez-Alquezar A, Palma PP, Martin M, Flevaud L, 2014. Comparative evaluation of 11 commercialized rapid diagnostic tests for detecting Trypanosoma cruzi antibodies in serum banks in areas of endemicity and nonendemicity. J Clin Microbiol 52: 2506–2512.

33. Chappuis F, Mauris A, Holst M, Albajar-Vinas P, Jannin J, Luquetti AO, Jackson Y, 2010. Validation of a rapid immuno- chromatographic assay for diagnosis of Trypanosoma cruzi infection among Latin-American migrants in Geneva, Switzerland. J Clin Microbiol 48: 2948–2952.

34. Barfield CA, Barney RS, Crudder CH, Wilmoth JL, Stevens DS, Mora-Garcia S, Yanovsky MJ, Weigl BH, Yanovsky J, 2011. A highly sensitive rapid diagnostic test for Chagas disease that utilizes a recombinant Trypanosoma cruzi antigen. IEEE Trans Biomed Eng 58: 814–817.

35. Chippaux JP, Santalla JA, Postigo JR, Romero M, Salas Clavijo NA, Schneider D, Brutus L, 2009. Sensitivity and specificity of Chagas Stat-Pak test in Bolivia. Trop Med Int Health 14: 732 – 735.

36. Verani JR, Seitz A, Gilman RH, LaFuente C, Galdos-Cardenas G, Kawai V, de LaFuente E, Ferrufino L, Bowman NM, Pinedo-Cancino V, Levy MZ, Steurer F, Todd CW, Kirchhoff LV, Cabrera L, Verastegui M, Bern C, 2009. Geographic variation in the sensitivity of recombinant antigen-based rapid tests for chronic Trypanosoma cruzi infection. Am J Trop Med Hyg 80: 410–415.

37. Barbabosa-Pliego A, Díaz-Albiter HM, Ochoa-García L, Aparicio-Burgos E, López-Heydeck SM, Velásquez-Ordoñez V, Fajardo-Muñoz RC, Díaz-González S, De Oca-Jimenez RM, Barbosa-Mireles M, Guzmán-Bracho C, Estrada-Franco JG, Garg NJ, Vázquez-Chagoyán JC, 2009. Trypanosoma cruzi circulating in the southern region of the State of Mexico (Zumpahuacan) are pathogenic: a dog model. Am J Trop Med Hyg 81: 390–395.

38. Houghton RL, Stevens YY, Hjerrild K, Guderian J, Okamoto M, Kabir M, Reed SG, Leiby DA, Morrow WJW, Lorca M, Raychaudhuri S, 2009. Lateral flow immunoassay for diagnosis of Trypanosoma cruzi infection with high correlation to the radioimmunoprecipitation assay. Clin Vaccine Immunol 16: 515–520.

39. Shah V, Ferrufino L, Gilman RH, Ramirez M, Saenza E, Malaga E, Sanchez G, Okamoto EE, Sherbuck JE, Clark EH, Galdos- Cardenas G, Bozo R, Flores-Franco JL, Colanzi R, Verastegui M, Bern C, 2014. Field evaluation of the InBios Chagas detect plus rapid test in serum and whole-blood specimens in Bolivia. Clin Vaccine Immunol 21: 1645–1649.

40. Duffy T, Cura CI, Ramirez JC, Abate T, Cayo NM, Parrado R, Bello ZD, Velazquez E, Muñoz-Calderon A, Juiz NA, Basile J, Garcia L, Riarte A, Nasser JR, Ocampo SB, Yadon ZE, Torrico F, de Noya BA, Ribeiro I, Schijman AG, 2013. Analyti- cal performance of a multiplex real-time PCR assay using TaqMan probes for quantification of Trypanosoma cruzi satellite DNA in blood samples. PLoS Negl Trop Dis 7: e2000.

41. Piron M, Fisa R, Casamitjana N, López-Chejade P, Puig L, Vergés M, Gascón J, Gómez i Prat J, Portús M, Sauleda S, 2007. Development of a real-time PCR assay for Trypanosoma cruzi detection in blood samples. Acta Trop 103: 195–200.

42. Cura CI, Duffy T, Lucero RH, Bisio M, Péneau J, Jimenez- Coello M, Calabuig E, Gimenez MJ, Valencia Ayala E, Kjos SA, Santalla J, Mahaney SM, Cayo NM, Nagel C, Barcán L, Málaga Machaca ES, Acosta Viana KY, Brutus L, Ocampo SB, Aznar C, Cuba Cuba CA, Gürtler RE, Ramsey JM, Ribeiro I, VandeBerg JL, Yadon ZE, Osuna A, Schijman AG, 2015. Multiplex real-

time PCR assay using TaqMan probes for the identification of Trypanosoma cruzi DTUs in biological and clinical samples. PLoS Negl Trop Dis 9: e0003765.

43. Lent H, Wygodzinsky P, 1979. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. Bull Am Mus Nat Hist 163: 123–520.

44. Cupp EW, Zhang D, Yue X, Cupp MS, Guyer C, Sprenger TR, Unnasch TR, 2004. Identification of reptilian and amphibian blood meals from mosquitoes in an eastern equine encephalomyelitis virus focus in central Alabama. Am J Trop Med Hyg 71: 272–276.

45. Hamer GL, Kitron UD, Goldberg TL, Brawn JD, Loss SR, Ruiz MO, Hayes DB, Walker ED, 2009. Host selection by Culex pipiens mosquitoes and West Nile virus amplification. Am J Trop Med Hyg 80: 268–278.

46. Molaei G, Andreadis TG, Armstrong PM, Anderson JF, Vossbrinck CR, 2006. Host feeding patterns of Culex mosquitoes and West Nile virus transmission, northeastern United States. Emerg Infect Dis 12: 468–474.

47. Kjos SA, Marcet PL, Yabsley MJ, Kitron U, Snowden KF, Logan KS, Barnes JC, Dotson EM, 2013. Identification of bloodmeal sources and Trypanosoma cruzi infection in triatomine bugs (Hemiptera: Reduviidae) from residential settings in Texas, the United States. J Med Entomol 50: 1126 – 1139.

48. Boakye D, Tang J, Truc P, Merriweather A, Unnasch T, 1999. Identification of bloodmeals in haematophagous Diptera by cytochrome B heteroduplex analysis. Med Vet Entomol 13: 282 – 287.

49. R Development Core Team, 2008. R: A Language and Environment for Statistical Computing. Available at: https:// r-project.org. Accessed June 21, 2016.

50. Gürtler RE, Lauricela M, Solarz ND, Bujas MA, Wisnivesky-Colli C, 1986. Dynamics of transmission of Trypanosoma cruzi in a rural area of Argentina. I—The dog reservoir: an epidemiological profile. Rev Inst Med Trop Sao Paulo 28: 4–11.

51. Cardinal M, Lauricella M, Marcet P, Orozco M, Kitron U, Gürtler R, 2007. Impact of community-based vector control on house infestation and Trypanosoma cruzi infection in Triatoma infestans, dogs and cats in the Argentine Chaco. Acta Trop 103: 201–211.

52. Woody NC, DeDianous N, Woody HB, 1961. American trypanosomiasis II. Current serologic studies in Chagas' disease. J Pediatr 58: 738–745.

53. Garcia MN, Woc-Colburn L, Rossmann SN, Townsend RL, Stramer SL, Bravo M, Kamel H, Beddard R, Townsend M, Oldham R, Bottazzi ME, Hotez PJ, Murray KO, 2014. Trypanosoma cruzi screening in Texas blood donors, 2008–2012. Epidemiol Infect 1–4.

54. Nouvellet P, Dumonteil E, Gourbière S, 2013. The improbable transmission of Trypanosoma cruzi to human: the missing link in the dynamics and control of Chagas disease. PLoS Negl Trop Dis 7: e2505.

55. Gürtler RE, Cecere MC, Lauricella MA, Petersen RM, Chuit R, Segura EL, Cohen JE, 2005. Incidence of Trypanosoma cruzi infection among children following domestic reinfestation after insecticide spraying in rural northwestern Argentina. Am J Trop Med Hyg 73: 95–103.

56. Minneman RM, Hennink MM, Nicholls A, Salek SS, Palomeque FS, Khawja A, Albor LC, Pennock CC, Leon JS, 2012. Barriers to testing and treatment for Chagas disease among Latino immigrants in Georgia. J Parasitol Res 2012: 295034.

57. Umstattd Meyer MR, Sharkey JR, Patterson MS, Dean WR, 2013. Understanding contextual barriers, supports, and opportunities for physical activity among Mexican-origin children in Texas border colonias: a descriptive study. BMC Public Health 13: 1–15.

58. Mier N, Ory MG, Zhan D, Conkling M, Sharkey JR, Burdine JN, 2008. Health-related quality of life among Mexican Americans living in colonias at the Texas-Mexico border. Soc Sci Med 66: 1760–1771.

59. Estrada-Franco JG, Bhatia V, Diaz-Albiter H, Ochoa-Garcia L, Barbabosa A, Vazquez-Chagoyan JC, Martinez-Perez MA, Guzman-Bracho C, Garg N, 2006. Human Trypanosoma cruzi infection and seropositivity in dogs, Mexico. Emerg Infect Dis 12: 624–630.

60. Enriquez GF, Bua J, Orozco MM, Wirth S, Schijman AG, Gürtler RE, Cardinal MV, 2014. High levels of Trypanosoma cruzi DNA determined by qPCR and infectiousness to Triatoma infestans support dogs and cats are major sources of parasites for domestic transmission. Infect Genet Evol 25: 36–43.

61. Roellig DM, Brown EL, Barnabé C, Tibayrenc M, Steurer FJ, Yabsley MJ, 2008. Molecular typing of Trypanosoma cruzi isolates, United States. Emerg Infect Dis 14: 1123–1125.

62. American Veterinary Medical Association, 2007. U.S. Pet Ownership and Demographics Sourcebook. Schaumburg, IL: American Veterinary Medical Association.

63. Poss JE, Bader JO, 2007. Attitudes toward companion animals among Hispanic residents of a Texas border community. J Appl Anim Welf Sci 10: 243–253.

64. U.S. Census Bureau, 2014. State and County Quickfacts: Hidalgo County, Cameron County, Starr County, Willacy County, TX. Available at: http://quickfacts.census.gov. Accessed September 14, 2016.

65. Jimenez-Coello M, Poot-Cob M, Ortega-Pacheco A, Guzman- Marin E, Ramos-Ligonio A, Sauri-Arceo CH, Acosta-Viana

KY, 2008. American trypanosomiasis in dogs from an urban and rural area of Yucatan, Mexico. Vector Borne Zoonotic Dis 8: 755 – 761.

66. Afonso AM, Ebell MH, Tarleton RL, 2012. A systematic review of high quality diagnostic tests for Chagas disease. PLoS Negl Trop Dis 6: e1881.

67. Tarleton RL, Gurtler RE, Urbina JA, Ramsey J, Viotti R, 2014. Chagas disease and the London declaration on neglected tropical diseases. PLoS Negl Trop Dis 8: 8–13.

APPENDIX I

New blood samples from 4 serologically reactive individuals (including 3 we classified as positive for research purposes; see Table 2 of original article) were obtained approximately one year after the original study, in order to provide data potentially useful for medical providers to determine if treatment was warranted. The blood samples were sent to the CDC for additional serologic testing where the Weiner EIA and, for two samples, TESA-blot, were performed. Positive results were obtained for one sample (individual GH03 from Table 2 of original study). If positivity on the tests run at CDC had been the criterion used to define seroprevalence in our epidemiological study, then the apparent seroprevalence among the collected samples would be 1/233 (0.4%; 95% CI 0.08-2.4%) in contrast to the 3/233 (1.3%; 95% CI 0.4-3.7%) we reported in the original publication. We present these additional data points to further highlight the challenges of discordant test results in Chagas serological testing.

CHAPTER III

EXPANDED *TRYPANOSOMA CRUZI* SURVEILLANCE AMONG DOGS LIVING IN UNDERSERVED COMMUNITIES (COLONIAS) ALONG THE US-MEXICO BORDER

INTRODUCTION

Colonias are underserved and typically impoverished communities along the United States-Mexico border. Colonias consist of mostly Hispanic populations living in substandard conditions are some of the most medically neglected communities in the United States.[1] Long term residence in a colonia has been linked to decreased physical and mental health due to lack of resources.[1] Though the aid of community health workers, called promotoras, health professionals have been able to infiltrate communities and provide aid using culturally competent information and education.[2] Colonias continue to be underserved and the lack of improvement in social, political, and environmental factors in the region increase the risk of multiple infectious diseases, including Chagas disease.[3]

Chagas disease is caused by a single celled parasite that is mainly transmitted by a vector commonly known as a triatomine or kissing bug. The parasite affects around 8 million people worldwide and is more common in Latin American countries.[4] However, autochthonous and imported cases in the United States have been reported with associated pathology which can include cardiac abnormalities and death.[5,6] Triatomines are found in many parts of the southern United States and are in south Texas.[7] Through our citizen science program, we engage communities, including colonias, to collect the vectors so we can study vector abundance and

assess infection prevalence.[8] The citizen science program allows us to observe fluctuation in vector communities that should relate to canine infection and also human risk.[9] Furthermore, instilling surveillance programs into colonias may be beneficial to closely monitor infectious diseases in at risk communities.[10,11]

After an initial study to show dogs and humans of colonias are at risk[12], we continuously engaged communities and surveilled dogs in colonias of the Rio Grande Valley of Texas to examine temporal and geographic patterns of infection of *Trypanosoma cruzi.* Assessing dogs in these border communities will allow us to collect additional prevalence data, observe disease patterns, maintain community trust, and help us understand the role dogs play in the dynamics of the transmission cycle.

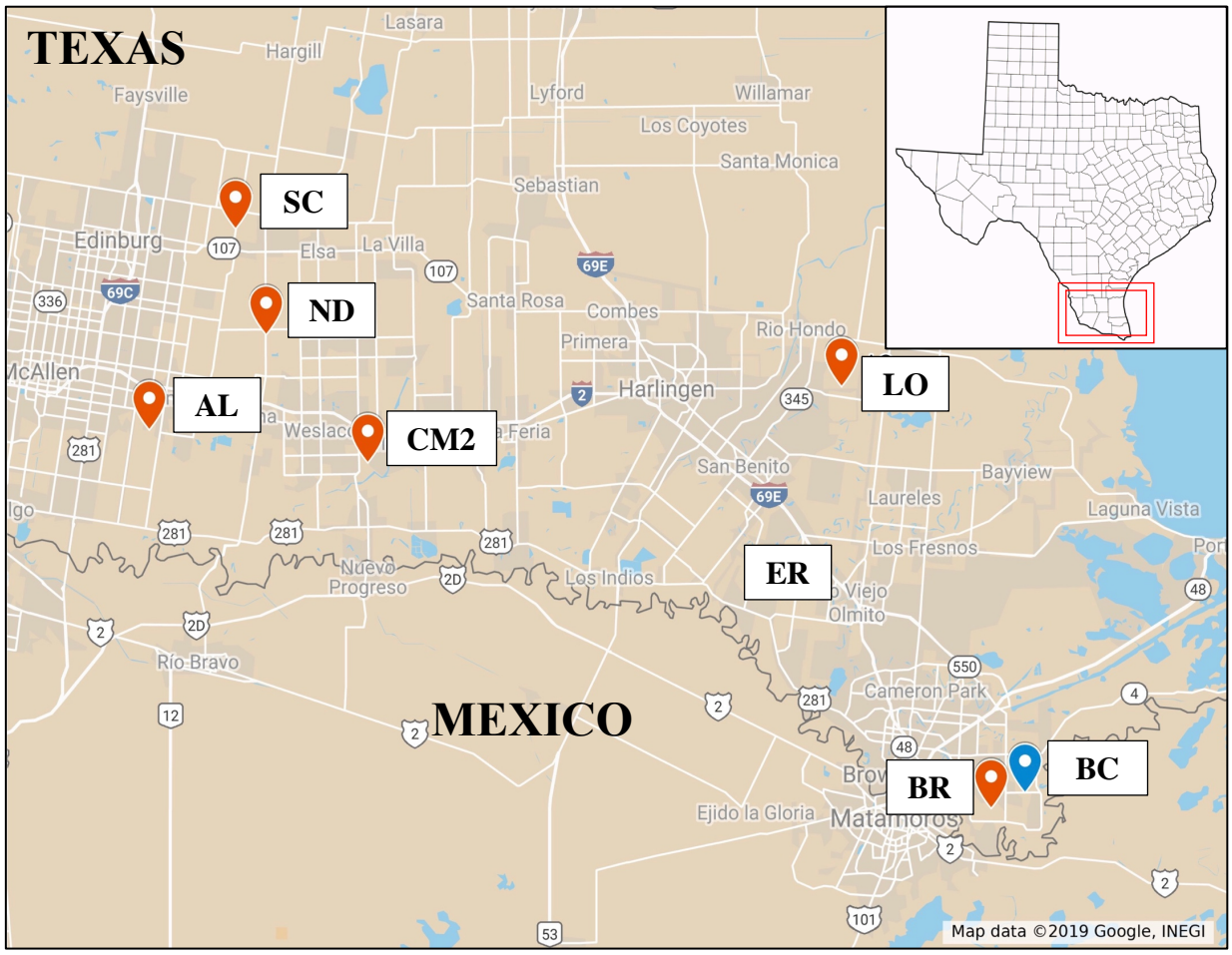

Figure 6. Locations of the eight sampled colonias located in the Rio Grande Valley, Texas along the US-Mexico border. Blue maker shows colonia BC that was sampled once in 2018 (BC) and again in 2019 .

MATERIALS & METHODS

In one-two seasons per year (summer, fall, or winter) of 2016-2019, we visited eight South Texas Colonias (ND, LO, ER, CM2, SC, AL, BR, BC; Figure 6), different to those previously sampled, to collect blood samples from canines (n=340). Colonia BC was visited in the summer of 2018 and again in the winter of 2019 whereas all other colonias were sampled at in a single season.

Following informed consent by dog owners, from dogs over 5 lbs, approximately 4mL of blood was collected using jugular or cephalic venipuncture and split into an EDTA tube and a serum collection tube. Samples were stored on ice in the field. Less blood (0.5-2mL) was taken from small dogs that appeared to be under 5lbs. Age (Puppies: <1 year, Young Adults:1-2 years, Adults:3-6 years, Seniors:7+ years), breed, and sex of sampled canines was collected. Blood was collected from dogs as young as six weeks. A rabies vaccination was administered to all dogs three months and older as an incentive for participation and as a public health protective measure. Canine sampling and vaccination protocols were approved by the Institutional Committee on Animal Use and Care at Texas A&M Univesrsity (AUP# 20180460).

In the laboratory, blood tubes were centrifuged and serum and buffy coat aliquots were processed further as follows. Canine serum samples were screened for *T. cruzi* antibodies using three independent serologic assays, including two rapid immunochromatographic tests (Chagas StatPAK (Chembio Diagnostic Systems, Inc., Medford, NY) & Chagas Detect Plus-InBios (InBios International, Inc., Seattle, WA) and the *Trypanosoma cruzi* indirect fluorescent antibody (IFA) (Texas Veterinary Medical Diagnostic Laboratory, College Station, TX). Both of the rapid tests were designed for human use and have been used in previous canine studies [13,14]All dogs were screened initially on the Chagas StatPAK. Any dog with a reactive Chagas StatPAK and 10% of the non-reactive Chagas StatPAK were next tested on the Chagas Detect Plus-InBios. In the summer of 2016 and winter of 2019, any dog reactive on at least one rapid test were then tested using the IFA. In fall 2016 and summer 2017, a stratified random sampling of dogs that showed reactivity on one or both rapid tests were tested using the IFA. Canines were considered seropositive only if they were positive two or more serological tests.

DNA was extracted from 250-350µl of buffy coats using the E.Z.N.A. kit (Omega Bio- Tek, Norcross, GA.) from all samples except samples from winter 2019. The samples were initially screened using the Cruzi 1/2/3 qPCR determined by amplification of a 166-bp region of repetitive nuclear satellite DNA to detect *T. cruzi* DNA.[12,15,16] Samples with a Ct less than 40 were suspect positive.[16] All suspect-positives were then subjected to discrete typing unit (DTU) determination using a multiplex qPCR assay amplifying the spliced leader intergenic region (SL-IR).[15] If negative on the DTU assay, the samples were subjected to additional PCR testing using *T. cruzi* 121/122 primers to amplify a 330bp region of kinetoplast DNA.[17–19] Samples were categorized as PCR-positive if they were positive on the screening qPCR and a subsequent test.

Statistical analysis was performed in RStudio version 1.1.423.[20] Bivaritate analysis using χ^2 or Fisher's exact test was used to evaluate the relationship between assumed risk factors and canine serostatus. Variables were colonia, season, age group, breed, and sex of the dogs. Generalized linear models were used to further investigate risk factors with P value ≤ 0.25 in the bivariate analysis or those with sufficient justification for inclusion, followed by calculating odds ratios and 95% confidence intervals.

RESULTS

A total of 340 dogs were sampled from 8 colonias during 9 sampling trips from 2016-2019.
A total of 148 (43.5%) dogs were female and 192 (56.5%) were male. The age breakdown is as follows: Puppies: <1 year (24.1%, n=82), Young Adults:1-2 years (33.5%, n=114), Adults:3-6 years (35.0%, n=119), and Seniors:7+ years (7.4%, n=25). Due to the preponderance of unknown

or mixed breed dogs in the dataset, breed was not analyzed further. Serological testing identified 110 of 340 (32.4%) seropositive dogs on the basis of a positive test result in at least two independent antibody detection platforms. All colonias had positive dogs, with seroprevalence among dogs across the 8 colonias ranging from 20.0-55.9% (Table 4). A total of 10 (3.3%) dogs of 300 were PCR positive; 9 (90%) of the PCR-positive dogs were also seropositive. There were seven dogs seropositive on all three serological assays and of those five had a PCR positive blood clot. The DTU of 6 of the 10 PCR-positive dogs was determined, and in all cases, it was TcI.

There was geographic variation in seroprevalence, in which, the odds of seropositivity among dogs in colonias ER (OR=5.06, CI= 2.01-13.46, P-value=0.001), CM (in 2019 only) (OR=3.75, CI=1.45-10.10, P-value=0.007), and SC (OR=3.23, CI= 1.31-8.33, P-value=0.01), were 3-5 times greater than dogs in the referent colonia ND. Seroprevalence varied among age groups: Puppies: <1 year (20.7%, n=17), Young Adults:1-2 years (28.9%, n=33), Adults:3-6 years (40.3%, n=48), and Seniors:7+ years (48%, n=12). Adult dogs (OR=2.60, CI= 1.35-5.17, P-value=0.005) and senior dogs (OR=3.88, CI= 1.46-10.50, P-value=0.007) were approximately three to four times more likely to be seropositive than dogs below the age of 2 years when season and sex were included in the model as fixed effects. Females (38.5%, n=57) were approximately twice as likely (OR=1.71, CI= 1.07-2.79, P-value=0.03) to be seropositive than males (seroprevalence: 27.6%, n=53). Dogs sampled in the fall were approximately 2.5 times more likely to be seropositive (OR=2.42, CI= 1.08-5.87, P-value=0.04) when compared to dogs sampled in the summer and winter, but only significant when colonia was excluded from the model as a fixed effect.

Table 4. Seroprevalence, PCR Status, and Demographics of Dogs from RGV Colonias (2016-2019)

Colonia	Year	Seroprevalence % (sample size)	PCR + Dog(s)	Season	Sex		Puppy <1 year	Young Adult: 1-2 years	Adult: 3-6 years	Senior: 7+ years
					F	M				
ND	2016	20.0% (55)	1	Summer	26	29	17	19	18	1
LO	2016	26.7% (30)	3	Summer	14	16	2	13	9	6
ER	2016	55.9% (34)	2	Fall	16	18	11	8	13	2
CM2	2016	48.4% (31)	1	Fall	15	16	9	11	9	2
AL	2017	30.0% (20)	0	Fall	9	11	1	10	6	3
BR	2017	26.1% (23)	1	Fall	10	13	6	6	10	1
SC	2017	44.7% (38)	0	Fall	12	26	6	16	14	2
BC	2018	27.5% (69)	2	Summer	32	37	15	20	29	5
BC	2019	22.5% (40)	-	Winter	14	26	15	11	11	3
Total		32.4% (340)	10		148	192	82	114	119	25

DISCUSSION

Dogs across all sampled colonias showed exposure to *T. cruzi*. Our prior canine research in the RGV using the same diagnostic and interpretive criteria showed the highest prevalence in a colonia was 31.8% which is lower than the average of this study (32.4%). The highest prevalence observed in this study was 55.9% and the subsequent highest seroprevalences were from a colonias sampled in the fall, which is likely due to the time needed to develop antibodies following exposure during the peak summer activity period of vectors in the region.[7] The prevalence of PCR-positive dogs (3.5%) was much less than the prevalence of seropositive dogs (35.5%), an expected finding given the dynamics of the parasite in infected hosts. PCR-positivity may signal acute infections or dogs that are more likely to be infectious to vectors. Combined with prior evidence of triatomines from these regions feeding on dogs and infiltrating human dwellings, dogs may serve as reservoirs and play an important role in the transmission cycle of *T. cruzi*.[12]

As in other studies[21], we found that older dogs were more likely to be seropositive, with the odds of seropositivity in adult and senior dogs three to four time greater than those of dogs under two. Approximately 20.7% of dogs under one year of age were seropositive and one was PCR positive- given the unlikely travel history in young dogs, these data likely signal recent autochthonous infection.

The dogs with a determined DTU in this study were exclusively infected with TcI; this DTU has previously been associated with human disease in the US.[22] Correspondingly, feral cats and opossums of the region were also exclusively infected with TcI. Additionally, kissing bugs, primarily *Triatoma gerstaeckeri*, found in the RGV region are more likely to be infected with TcI even though TcIV is present in the area.[7,23]

The diagnosis of *T. cruzi* infections in humans is a major barrier in the human medical field [24,25] and unfortunately the lack of gold standard approaches in veterinary diagnostics presents similar challenges. Although the rapid assays used in this study have been used in dog and other animal research[12,13,26], there are no data available to assess their sensitivity or specificity. By requiring seroreactivity on at least two independent tests to consider a dog seropositive, we attempted to parallel the human diagnostic realm in which the World Health Organization recommends the use of multiple testing platforms.[27] Nonetheless, the potential for false negatives within the conservative interpretation criteria we applied is possible. One of the PCR positive dogs was seronegative; if the PCR positivity of blood reflected parasitemia after an acute infection, then one interpretation of this result is that serological tests are not effective at detecting acute infection in dogs. Serological and PCR testing of blood in parallel would be an ideal diagnostic

approach but PCR is not a routinely-offered diagnostic test and low levels of circulating parasite may not be detectible.

Domestic dogs living in colonias may serve as reservoirs to infect vectors in proximity to colonia residents, potentially increasing the risk of vector-borne transmission. Under a One Health framework, the identification of local reservoirs is a key component in developing public health interventions.

REFERENCES

1. Mier N, Ory MG, Zhan D, Conkling M, Sharkey JR, Burdine JN. Health-related quality of life among Mexican Americans living in colonias at the Texas-Mexico border. *Soc Sci Med*. 2008;66(8):1760-1771. doi:10.1016/j.socscimed.2007.12.017
2. Olney CA, Warner DG, Reyna G, Wood FB, Siegel ER. MedlinePlus and the challenge of low health literacy: findings from the Colonias project. *J Med Libr Assoc*. 2007.
3. Hotez PJ. The rise of neglected tropical diseases in the "new Texas." *PLoS Negl Trop Dis*. 2018. doi:10.1371/journal.pntd.0005581
4. Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United States. *Clin Infect Dis*. 2009;49(5):e52-4. doi:10.1086/605091
5. Garcia MN, Woc-Colburn L, Aguilar D, Hotez PJ, Murray KO. Historical Perspectives on the Epidemiology of Human Chagas Disease in Texas and Recommendations for Enhanced Understanding of Clinical Chagas Disease in the Southern United States. *PLoS Negl Trop Dis*. 2015. doi:10.1371/journal.pntd.0003981
6. Garcia MN, Murray KO, Hotez PJ, et al. Development of Chagas cardiac manifestations among Texas blood donors. *Am J Cardiol*. 2015;115(1):113-117. doi:10.1016/j.amjcard.2014.09.050
7. Curtis-Robles R, Aukland LD, Snowden KF, Hamer GL, Hamer SA. Analysis of over 1500 triatomine vectors from across the US, predominantly Texas, for Trypanosoma cruzi infection and discrete typing units. *Infect Genet Evol*. 2018;58:171-180. doi:10.1016/j.meegid.2017.12.016
8. Hamer SA, Curtis-Robles R, Hamer GL. Contributions of citizen scientists to arthropod vector data in the age of digital epidemiology. *Curr Opin Insect Sci*. 2018;28:98-104.

doi:10.1016/j.cois.2018.05.005

9. Curtis-Robles R, Lane S, Levy M, Hamer S, Hamer G. Bionomics and spatial distribution of Triatomine vectors of Trypanosoma cruzi in Texas and other southern states, USA. *Am J Trop Med Hyg*. 2017;In press.

10. Weinberg M, Waterman S, Lucas C, et al. The U.S.-Mexico Border Infectious Disease Surveillance Project: Establishing Binational Border Surveillance. *Emerg Infect Dis*. 2003;9(1):97-102.

11. Chuit R, Paulone I, Wisnivesky-Colli C, et al. Result of a first step toward community-based surveillance of transmission of Chagas' disease with appropriate technology in rural areas. *Am J Trop Med Hyg*. 1992;46(4):444-450. http://www.ncbi.nlm.nih.gov/pubmed/1575292.

12. Curtis-Robles R, Zecca IB, Roman-Cruz V, et al. Trypanosoma cruzi (Agent of Chagas Disease) in sympatric human and dog populations in "colonias" of the Lower Rio Grande Valley of Texas. *Am J Trop Med Hyg*. 2017;96(4):805-814. doi:10.4269/ajtmh.16-0789

13. Nieto PDPD, Boughton R, Dorn PL, et al. Comparison of two immunochromatographic assays and the indirect immunoflourscence antibody test for diagnosis of Trypanosoma cruzi infection in dogs in south central Louisiana. *Vet Parasitol*. 2009;165(3-4):241-247. doi:10.1016/j.vetpar.2009.07.010

14. Tenney TD, Curtis-Robles R, Snowden KF, Hamer SA. Shelter dogs as sentinels for Trypanosoma cruzi transmission across Texas. *Emerg Infect Dis*. 2014;20(8):1323-1326. doi:10.3201/eid2008.131843

15. Cura CI, Duffy T, Lucero RH, et al. Multiplex real-time PCR assay using TaqMan probes for the identification of Trypanosoma cruzi DTUs in biological and clinical samples. *PLoS*

Negl Trop Dis. 2015;9(5):e0003765. doi:10.1371/journal.pntd.0003765

16. Duffy T, Cura CI, Ramirez JC, et al. Analytical performance of a multiplex Real-Time PCR assay using TaqMan probes for quantification of Trypanosoma cruzi satellite DNA in blood samples. *PLoS Negl Trop Dis*. 2013;7(1):e2000. doi:10.1371/journal.pntd.0002000

17. Wincker P, Britto C, Pereira JB, Cardoso MA, Oelemann W, Morel CM. Use of a simplified polymerase chain reaction procedure to detect Trypanosoma cruzi in blood samples from chronic chagasic patients in a rural endemic area. *Am J Trop Med Hyg*. 1994;51(6):771-777.

18. Virreira M, Torrico F, Truyens C, et al. Comparison of polymerase chain reaction methods for reliable and easy detection of congenital Trypanosoma cruzi infection. *Am J Trop Med Hyg*. 2003;68(5):574-582. http://www.ncbi.nlm.nih.gov/pubmed/12812349.

19. Curtis-Robles R, Lewis BC, Hamer SA. High Trypanosoma cruzi infection prevalence associated with minimal cardiac pathology among wild carnivores in central Texas. *Int J Parasitol Parasites Wildl*. 2016;5(2):117-123. doi:10.1016/j.ijppaw.2016.04.001

20. R Development Core Team. R: A language and environment for statistical computing. 2008. https://www.r-project.org.

21. Castañera MB, Lauricella MA, Chuit R, Gürtler RE. Evaluation of dogs as sentinels of the transmission of Trypanosoma cruzi in a rural area of north-western Argentina. *Ann Trop Med Parasitol*. 1998;92(6):671-683. http://www.ncbi.nlm.nih.gov/pubmed/9924546.

22. Garcia MN, Burroughs H, Gorchakov R, et al. Molecular identification and genotyping of Trypanosoma cruzi DNA in autochthonous Chagas disease patients from Texas, USA. *Infect Genet Evol*. 2017;49:151-156. doi:10.1016/j.meegid.2017.01.016

23. Curtis-Robles R, Hamer SA, Lane S, Levy MZ, Hamer GL. Bionomics and spatial distribution of triatomine vectors of Trypanosoma cruzi in Texas and other southern states, USA. *Am J Trop Med Hyg*. 2018;98(1):113-121. doi:10.4269/ajtmh.17-0526

24. Afonso AM, Ebell MH, Tarleton RL. A systematic review of high quality diagnostic tests for Chagas disease. *PLoS Negl Trop Dis*. 2012;6(11):e1881. doi:10.1371/journal.pntd.0001881

25. Guzmán-Gómez D, López-Monteon A, Lagunes-Castro M de la S, et al. Highly discordant serology against Trypanosoma cruzi in central Veracruz, Mexico: role of the antigen used for diagnostic. *Parasites and Vectors*. 2015;8(1):1-8. doi:10.1186/s13071-015-1072-2

26. Cardinal M V, Reithinger R, Gürtler RE. Use of an immunochromatographic dipstick test for rapid detection of Trypanosoma cruzi in sera from animal reservoir hosts. *J Clin Microbiol*. 2006;44(8):3005-3007. doi:10.1128/JCM.00552-06

27. WHO Expert Committee, Coura JR, Dias JCP, et al. *Control of Chagas Disease.* Vol 905.; 2002. doi:10.1016/S0065-308X(05)61004-4

CHAPTER IV

THE EPIDEMIOLOGY OF *TRYPANOSOMA CRUZI* AND ASSOCIATED PATHOLOGY OF URBAN-DWELLING VIRGINIA OPOSSUMS (*DIDELPHIS VIRGINIANA*) OF THE RIO GRANDE VALLEY OF TEXAS

INTRODUCTION

Trypanosoma cruzi is a hemoflagelate parasite that is transmitted by triatomine insects commonly known as a kissing bugs. The hematophagous vector is distributed across the Americas, including the southern United States, and infection of humans or animals with *T. cruzi* may cause a potentially deadly disease known as Chagas disease. The disease affects an estimated 8 million people worldwide (Montgomery et al. 2014). In Argentina and Mexico, where Chagas disease is prevalent, domestic cats and dog, have been identified as key hosts that maintain the domestic transmission (Cardinal et al. 2007; Gurtler et al. 2007; Jimenez-Coello et al. 2010). In contrast, true domiciliation of vectors is relatively rare in the southern US, and *T. cruzi* is maintained in sylvatic cycles by several wildlife species (Hodo and Hamer 2017) with dogs as key hosts in peridomestic cycles (Curtis-Robles et al. 2017b).

In Latin America, peridomestic species and urban wildlife like *Didelphis sp.* opossums have long been recognized as *T. cruzi* reservoirs where they bridge transmission cycles to domestic animals and humans (Herrera and Urdaneta-Morales 1992; Roque et al. 2008). Furthermore, the lineages of *T. cruzi* discrete typing unit TcI are thought to have originated in opossums, and are consistently isolated from opossums (Yeo et al. 2005). Opossums have been identified as important reservoir hosts in the Americas including some parts of the southern US (Herrera and Urdaneta-Morales 1992; Houk et al. 2010) but the pathological consequence of

infection on animal health has not been thoroughly investigated. The Virginia opossum (*Didelphis virginiana)* has a wide geographic range spanning from central to north America and is the only marsupial found in the United States (Siciliano 2013). They are primarily nocturnal animals that thrive in urban environments in and around human dwellings. Opossums are opportunistic omnivores and insectivores, which could increase their risk of infection if they consume infected kissing bugs. Opossums and other peridomestic animals can increase the number of kissing bugs in the environment thus increasing the risk of transmission to humans and other domestic animals (Ruiz-Pina and Cruz-Reyes 2002). Being natural hosts of *T. cruzi,* opossums living in urban areas pose a public health threat in areas where risk factors are already present (Yeo et al. 2005). In the southern United States, particularly in Texas, autochthonous human, domestic animal, and wildlife cases have been reported (Curtis-Robles et al. 2016; Nolan et al. 2018; Hodo et al. 2019). More so, areas in Texas along the US-Mexico border, like the Lower Rio Grande Valley (RGV), experience a higher than expected density of *Triatoma gerstaeckeri*, a species of kissing bug with more than 50% infection rate, and at least four triatomine species occur within the region (Curtis-Robles et al. 2017a, 2018a). The substantial infection rate in dogs of this region (6.7%-19.6%) indicate that other animals, like opossums, in the area with greater exposure to vectors may serve a potential reservoir hosts (Tenney et al. 2014; Curtis-Robles et al. 2017b). However, not much is known about the involvement of opossums in Chagas disease ecology of the RGV.

The objectives of this study were to assess the epidemiology of Chagas disease in Virginia opossums (*Didelphis virginiana*) in a transmission hotspot of the southern United States and determine if infection is associated with pathology that may impact animal health. Further we wished to determine if the anal glad secretions in *T. cruzi*-infected Virginia opossums

harbored parasites, thereby creating the potential for transmission via exposure to these secretions as has been shown for other opossum species. Identifying the prevalence of *T. cruzi* and understanding the pathology of the parasite will help us identify the role Virginia opossums play in the disease ecology and transmission cycle within the region.

MATERIALS & METHODS

Study Site:

A large animal shelter located in RGV (Hidalgo Co.) intakes over 30,000 animals a year including an average (2016-2018) of 6,750 opossums annually. Animals come to the shelter from multiple animal control agencies but the majority of opossums come from an urban metropolitan area (McAllen, TX) where animal control is highly active. Animal control agencies respond to resident complaints about nuisance opossums and trap or pick up trapped opossums and drop them off at the animal shelter.

Opossum necropsy and tissue sampling:

We conducted a repeated cross-sectional study of cats from an RGV shelter in the winter, spring, and summer of 2017. The opossums (n=100) used in this study were euthanized for reasons unrelated to our study. Place of animal origin (city) and season of collection was noted. Age was determined by shelter staff through weight approximation (<5lbs=juvenile, >5lbs=adult); only adults were used in this study. Necropsies were performed 20-60 minutes post euthanasia. Heart tissue, blood, and anal gland secretions were collected from all 100 animals; in addition, anal gland tissue and intercostal muscle were collected from 43 animals for which time allowed the extra tissue collection. Anal gland secretions were collected by manual compression of the anal

glands into a sterile vial. Using standard necropsy procedures, the heart, anal glands, and approximately 2-3 anterior ribs with attached muscle were completely excised from the body and placed into separate specimen bags. All cutting tools and forceps used were sterilized between subjects. Approximately 8-12 milliliters of blood was collected from the thoracic cavity with a syringe and placed into sterile vials that were maintained on ice. Blood was centrifuged at 7000 rpm for 8 minutes to recover the blood clot which was saved in -80 °C until processing.

Histopathology:

After thawing, an incision was made along the coronary sulcus of the heart to expose the interior of the right ventricle followed by an incision towards the right atrium. An incision was then made from the apex to the left auricle to expose the interior of the left ventricle and atrium. A 1.5-2mm section of tissue from each chamber was collected with half preserved in formalin (10%) for histopathology, and half placed into a nuclease free tube for molecular analysis. Anal glands and intercostal muscle excised from a rib section were evaluated with separate sections preserved in formalin and prepared for molecular evaluation. The formalin fixed tissues were routinely processed and stained with hematoxylin and eosin. Inflammation was evaluated on twelve opossums that were PCR-positive on at least one tissue type and on twelve randomly selected opossums who were negative on all PCR tests.

Inflammation was semiquantitatively scored (Inflammation Score= *IS*) for each heart chamber, intercostal muscle, and anal glands 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.

Detection of parasite DNA in blood clot, anal gland secretions, and tissues:

From each tissue, we sampled different areas of the heart (four chambers), intercostal muscle, and anal glands to yield a final volume of approximately 30mg; samples were macerated in a nuclease free tube. For heart samples, all four chambers were prepared and macerated together to represent all parts of the heart. DNA was extracted from the blood clot and tissues using the E.Z.N.A. kit (Omega Bio- Tek, Norcross, GA). All extraction steps were conducted as per manufacturer instructions with the addition of a longer lysis duration (18-24 hours). To detect *T. cruzi* DNA in samples, we used a two-step process starting with a screening qPCR to amplify a 166-bp segment of the *T. cruzi* 195-bp repetitive satellite DNA (Duffy et al. 2013). PCR reactions (20 μL) contained 5 μL of DNA, with Cruzi 1, 2, and 3 primers and probe (0.75 μM each) and iTaq Universal Probes Supermix (BioRad Laboratories, Hercules, CA). Next, any sample that screened positive (Ct value less than 40 with a sigmoidal amplification curve) was then subjected discrete typing unit (DTU) determination using multiplex assay amplifying the spliced leader intergenic region (SL-IR) (Cura et al. 2015). Finally, any sample that screened positive yet was negative on the SL-IR assay was then subjected to a final PCR using the *T. cruzi* 121/122 primers to amplify a 330bp region of kinetoplast DNA (Wincker et al. 1994; Virreira et al. 2003; Curtis-Robles et al. 2016). As previously described in (Curtis-Robles et al. 2016) reactions consisted of 1 μL template DNA, primers at a final concentration of 0.75 μM each, and FailSafe PCR Enzyme Mix with PreMix E (Epicentre, Madison, WI) in a final volume of 15 μL. Visualization of PCR products were conducted by using a 1.5% agarose gel stained with

ethidium bromide. Only samples that had positive results on two independent PCRs for different gene regions were considered to be infected.

Epidemiological analysis:

Molecular prevalence in blood clot was calculated as the total number of opossums that had PCR-positive blood clot, over the total number of opossums enrolled in the study. Parasite DNA located in blood clot could signal circulating parasite which could be used as a metric useful in estimating the prevalence of animals that are infectious to vectors.

Molecular prevalence in tissues and anal gland secretions was calculated as the total number of opossums with a PCR-positive tissue type, over the total number of opossums with that specific tissue type collected. This metric may be useful in estimating the prevalence of animals that have parasite localized in specific tissue; tissue tropism of the parasite could vary by parasite genetic strain (Vera-Cruz et al. 2003).

Overall infection prevalence was calculated as the total number of opossums that were either molecularly positive on any blood clot, anal gland secretion, or tissue type, over the total number of opossums enrolled in the study.

Statistical Analysis:

Statistical analysis was performed in RStudio version 1.1.423. (R Development Core Team 2008) Bivaritate analysis using χ^2 or Fisher's exact test was used to evaluate the relationship between risk factors (sex & PCR results) and bilateral cardiac inflammation.

Opportunistic triatomine vector collection and processing:

Ongoing education and distribution of educational materials were provided to shelter staff to raise awareness of triatomine vectors and Chagas disease. Passive vector surveillance was conducted by engaging shelter staff in the safe collection of kissing bugs on the premises using

methods detailed through our kissing bug citizen science program.(Curtis-Robles et al. 2018a) Kissing bugs were identified morphologically to species, sexed, surface-sterilized, and dissected.(Lent and Wygodzinsky 1979; Curtis-Robles et al. 2015) At the time of insect dissection, the presence of host blood in the hindgut was noted and scored from 1 (no blood observed) to 5 (fully engorged bug). DNA was extracted (KingFisher Cell and Tissue DNA kit, Thermo Fisher Scientific, Waltham, MA) from the vector hindguts and tested for infection and DTU of *T. cruzi* through using the molecular methods described above. In order to determine the vertebrate hosts upon which kissing bugs have previously fed, a molecular bloodmeal analysis (BMA) was conducted using PCR amplification of host cytochrome B sequences using 'herp' and 'BM' primers and Sanger sequencing (Eton Bioscience Inc., San Diego, CA, USA) as previously detailed.(Curtis-Robles et al. 2018b) Samples were considered positive for a human blood meal only when yielding two independently acquired human DNA sequences. (Curtis-Robles et al. 2018b)

RESULTS

Demographic Results

Of the 100 opossums sampled, 48 were female and 52 were male. Number of opossums collected by season are as follows: winter (42%), spring (27%), summer (31%). The majority of the opossums originated from McAllen, TX (92%) while the rest originated from Alamo, TX (6%) and Palmview, TX (2%).

Molecular prevalence in blood clot

Of 100 opossums, 9% of the blood clots were PCR positive. The DTU for all PCR positive blood clot was TcI.

Molecular prevalence in tissues and anal gland secretions

Heart tissue and anal gland secretions were collected from 100 opossums. The molecular prevalence in heart tissue was 10% and of those, all ten samples typed as TcI. Of the anal gland secretions, 12% were PCR-positive of which the DTU was determined for five- all TcI. The rest of the anal gland secretions (n=7) did not amplify on the SLIR PCR that is used for DTU determination, yet were confirmed positive on the third PCR assay to amplify kinetoplast DNA. Intercostal muscle and anal glands were sampled from 43 opossums. The molecular prevalence was 16.3% (n=7) in intercostal muscle and 9.3% (n=4) in anal glands; all positive samples were infected with *T. cruzi* DTU TcI.

Overall infection prevalence

Of 100 opossums, 15 (15%) had at least one PCR positive sample, including 9 (9%) animals that were PCR positive on at least 2 tissues and five animals (5%) that were positive in all tissues tested (Table 5). All of the tissues in which DTU was determined (n=35) were exclusively TcI.

Table 5. Opossums of the Rio Grande Valley, TX (2017) PCR positive on at least one tissue and associated inflammation scores (*IS*)

ID	Sex	Blood Clot	Anal Gland Secretion	Heart Tissue	*IS*		Intercostal Muscle	*IS*	Anal Gland Tissue	*IS*	Number of PCR Positive Tissues
					L	R					
OP03	F	Negative	**Positive***	Negative	**	**	N/A	N/A	N/A	N/A	1/3
OP05	F	Negative	**Positive***	Negative	**	**	N/A	N/A	N/A	N/A	1/3
OP06	F	Negative	**Positive***	**Negative**	**	**	N/A	N/A	N/A	N/A	2/3
OP07	M	**Positive**	**Positive**	**Positive**	3	2	N/A	N/A	N/A	N/A	3/3
OP09	F	Negative	**Positive**	Negative	0	1	N/A	N/A	N/A	N/A	1/3
OP17	F	**Positive**	**Positive***	**Positive**	2	2	N/A	N/A	N/A	N/A	3/3
OP34	M	Negative	**Positive**	Negative	1	0	N/A	N/A	N/A	N/A	1/3
OP63	F	**Positive**	Negative	**Positive**	0	2	**Positive**	0	Negative	2	3/5
OP69	F	**Positive**	**Positive**	**Positive**	2	2	**Positive**	0	**Positive**	3	5/5
OP72	M	**Positive**	**Positive***	**Positive**	2	3♦	**Positive**	0•	**Positive**	2	5/5
OP81	M	**Positive**	**Positive**	**Positive**	2	2	**Positive**	0•	**Positive**	1	5/5
OP82	M	Negative	Negative	**Positive**	1	1	Negative	0•	Negative	2	1/5
OP84	M	**Positive**	**Positive***	**Positive**	2	3	**Positive**	0	Negative	0	4/5
OP91	M	**Positive**	Negative	**Positive**	2	2	**Positive**	2	**Positive**	1	4/5
OP94	M	**Positive**	**Positive***	**Positive**	1	0	**Positive**	1•	Negative	0	4/5

*** No DTU detected, confirmed positive using 121/122 PCR** **N/A= Not available**
**** No histology performed** **• Sarcocysts present**
♦Eosinophils present

Table 6. Opossums of the Rio Grande Valley, TX (2017) PCR negative tissue and associated inflammation scores (*IS*)

ID	Sex	Heart *IS*		Intercostal Muscle *IS*	Anal Gland Tissue *IS*
		L	R		
OP01	M	0	1	N/A	N/A
OP11	F	0	0	N/A	N/A
OP23	M	0	1	N/A	N/A
OP38	M	0	0	N/A	N/A
OP58	M	0	1	1	1
OP60	F	3♦	1	1	3
OP66	F	0	1	0	2
OP74	M	2	0	0	2
OP88	M	1	0	0	2
OP95	M	0	2♦	1	2
OP99	M	0	0	0	2
OP100	M	0	0	1	1

♦Eosinophils present
N/A= Not available

Histology

Inflammation was evaluated on twelve infected opossums (had at least one tissue type; positive group) and twelve randomly selected opossums that were negative on all PCR tests (negative group). All 12 (100%) of the hearts from the positive group showed inflammation in either left chambers, right chambers, or both chambers of the heart, whereas 8 (66.7%) of the hearts from the negative group showed inflammation. Opossums from the positive groups were significantly more likely to exhibit bilateral multifocal inflammation (66.7%) when compared to opossums in the negative group (8.3%; P-value= 0.009, OR=18.8, CI= 1.70-1053.64).-Ten of the hearts from the positive group showed left sided inflammation ranging from minimal (n=3), mild (n=6), and moderate (n=1) inflammation and ten showed right sided inflammation ranging from minimal (n=2), mild (n=6), and moderate (n=2) inflammation. The heart from the positive opossum that had moderate inflammation in the left chamber showed myocardial fiber loss with replacement fibrosis (Figure 7). Few eosinophils were observed in one the right chambers of a heart from a positive opossum (OP72) with dual sided inflammation. Three of the hearts from the negative group showed left sided inflammation ranging from minimal (n=1), mild (n=1), and moderate (n=1) inflammation and six showed right sided inflammation ranging from minimal (n=5) to mild (n=1) inflammation. The heart from the negative opossum that had left sided moderate inflammation and the heart from the negative opossum that had right sided mild inflammation showed the presence of substantial eosinophils.

Minimal to mild inflammation was observed in intercostal muscles from two animals in the positive group and four animals in the negative group. *Sarcocystis* sp. cysts (Sarcocysts) were observed in the intercostal muscle of four positive animals, in one case associated with mild

inflammation. Minimal to moderate inflammation was observed in 6 of 8 anal gland tissues from the positive group (Table 5) and all anal gland tissues of the negative group. (Table 6)

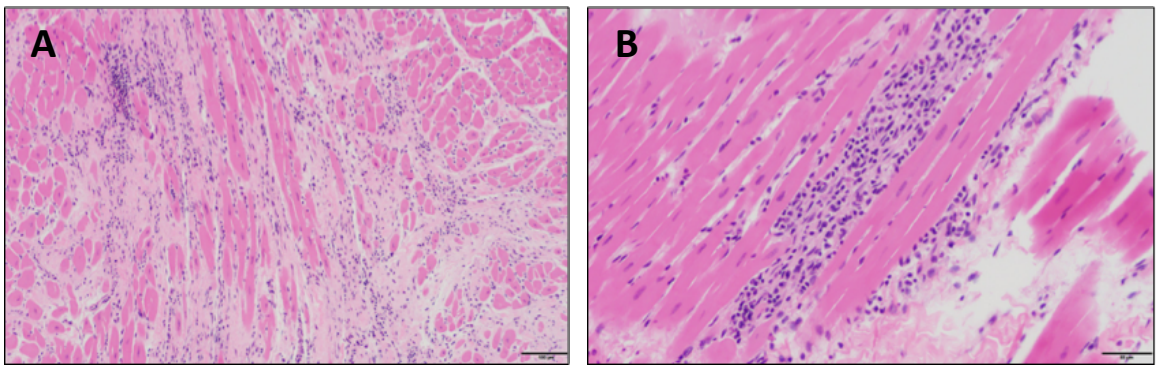

Figure 7. Opossum 07 Pathology, Hematoxylin and eosin stain. **A** (Opossum- Left Ventricle (LV)) 10x: There is marked myocardial fiber loss with replacement fibrosis. **B** (Same Opossum-LV) 20X: Myocardial inflammation is characterized by lymphocytes and plasma cells.

Triatomine vector results

A total of 3 kissing bugs were collected by shelter staff, including one prior to the start of the study and two across the study period, and all identified as *Triatoma gerstaeckeri* female adults (Table 7). All three kissing bugs showed engorgement: two were assigned a blood meal score (BMS) of 4 and one was assigned a BMS of 5, indicative of recent meals. Two (66.7%) kissing bugs were PCR positive for *T. cruzi;* both were infected with DTU TcI. Bloodmeal analysis of one PCR positive kissing bug showed the presence of domestic canine (*Canis lupus familiaris*) DNA, and the other PCR-positive bug showed opossum (*Didelphis virginiana*) DNA. Blood meal analysis in the PCR-negative bug revealed human DNA.

Table 7. Triatomines found alive in an outdoor kennel of the Rio Grande Valley, Texas animal shelter

Triatomine ID	Date Captured	Life stage	Species	Sex	BMS	PCR	DTU	BMA
PS0126	6/2013	Adult	*T. gerstaeckeri*	F	4	+	TcI	opossum
PS3031	4/2017	Adult	*T. gerstaeckeri*	F	5	+	Tc1	canine
PS3035	5/2017	Adult	*T. gerstaeckeri*	F	4	-	n/a	human

DISCUSSION

Opossums are recognized to play diverse roles in the ecology of zoonotic disease. For example, studies of opossums in the Lyme disease system concluded that opossums serve as 'ecological traps' because they groom off and consume attached ticks, thereby reducing the risk of Lyme disease (Keesing et al. 2009). In contrast, opossums in Galveston, TX carry fleas infected with *Rickettsia typhi* that are thought to contribute to recent cases of murine typhus in humans. (Blanton et al. 2016) Here, we show *T. cruzi* infected 15% of opossums across multiple biologic samples, including blood clot, heart, anal gland secretions intercostal muscle, and anal glands in opossums from a Chagas-endemic region along the Texas-Mexico border. Approximately 6,750 opossums come through the RGV shelter yearly; based on extrapolation from our study, over 1000 of these may be infected with *T. cruzi*. Given that opossum populations thrive in urban environments, there is potential for a large number of *T. cruzi* infected opossums to occur in the southern US in close proximity to human populations.

The first documented autochthonous case of Chagas disease in the United States was in 1955 in a child in Corpus Christi, Texas, which is approximately 275 kilometers north of the US-Mexico border and the RGV (Woody and Woody 1955). The case was speculated to be attributed to a kissing bug infected by opossums around the home of the victim, as the authors of the original report stated "*Many opossums have been seen in the area at night. Some time before the child's*

illness the carcass of a dead opossum was seen being fed upon by triatomid bugs. These insects, locally called blood-suckers, have been a source of trouble in the family home for several months, often biting the occupants during sleep". (Woody and Woody 1955) Despite historical suggestion of opossums contributing to the transmission cycle in the region, the role they play in the ecology of *T. cruzi* within the RGV has been largely unknown.

Although PCR positivity in blood does not demonstrate the presence of viable parasites, the 10% of opossums with PCR-positive blood clots indicates a population that may be parasitemic and could potentially be infective to vectors, thus increasing the risk to humans and other animals. Further, the presence of parasite in the anal gland secretions may pose a risk to humans and other animals since opossums frequently use the secretions as a territory marker and release secretions at defecation (Steindel et al. 1988; Urdaneta-Morales and Nironi 1996). For example, dogs are known to roll in and eat feces of other animals, which is a behavior that may increases the risk of zoonosis for parasites like *Toxoplasma* (Frenkel and Parker 1996) and could serve as a risk factor for *T, cruzi* transmission. A study also observed that inoculation with infected anal gland secretions or anal gland secretion cultures from opossums caused the mortality of laboratory mice (Urdaneta-Morales and Nironi 1996).

All infections for which the parasite DTU was ascertained were comprised of 'TcI', the DTU associated with human disease in the United States, despite DTU TcI and TcIV being near equal abundance in kissing bugs (Curtis-Robles et al. 2018a) and dogs of the region (Hodo et al. 2019). Our data, and that of others (Roellig et al. 2009; Hodo et al. 2018) support a strong host association of TcI in Virginia opossums.

Through histopathologic examination, we show evidence of bilateral inflammation in 66.7% of the hearts from the positive group and only 8.33% in the hearts from the negative group. suggesting that a multifocal distribution of inflammation is more commonly found in *T. cruzi*-infected opossums. We found that pathology of skeletal muscle (intercostal muscle) from PCR-positive opossums was minimal when compared to the pathology described from skeletal muscle in *Didelphis marsupialis* (Carreira et al. 1996), but variation in *T. cruzi* strains and being infected with multiple strains can impact pathology outcomes (Roellig et al. 2009). Almost all of the anal glands in the positive and negative group showed lymphoplasmacytic inflammation with few eosinophils. Inflammation in tissues that were not PCR positive could be as a result of other pathogens like *Besnoitia darlingi* and *Sarcocystis* sp. or due to *T. cruzi* infection that was not detectable using PCR methods (Barr et al. 1991).

Three kissing bugs were collected in outdoor kennels at the shelter, including two *T. cruzi*-infected bugs both of which harbored TcI- the same DTU that infected the opossums at the shelter. The three bugs had bloodmeals from an opossum, dog, and human. At the shelter, dogs and opossums are housed in outdoor environments. Although the bloodmeal analysis procedure cannot inform where these insects fed on these hosts, their high levels of engorgement suggest the bloodmeals were recently acquired and may have been obtained at the shelter. Alternately, insects may have fed in the surrounding environment\ before dispersing to the shelter.

We show opossums are infected with *T. cruzi*, sometimes associated with pathology in multiple organs or secretions, in the RGV where ongoing autochthonous human and canine transmission

occurs. The high levels of *T. cruzi* infection in anal gland secretions of infected animals may allow signal a potential source of infection for humans and animals without the need of a vector. Future research will determine the relative importance of opossums as reservoirs of *T. cruzi* in the United States and the threat they pose to domestic animals and public health.

REFERENCES

1. Barr S, Brown CC, Dennis VA, Klei TR. 1991. The lesions and prevalence of Trypanosoma cruzi in opossums and armadillos from southern Louisiana. *J Parasitol* 77:624–627.
2. Blanton LS, Idowu BM, Tatsch TN, Henderson JM, Bouyer DH, Walker DH. 2016. Opossums and cat fleas: New insights in the ecology of murine typhus in Galveston, Texas. *Am J Trop Med Hyg*.
3. Cardinal M, Lauricella M, Marcet P, Orozco M, Kitron U, Gürtler R. 2007. Impact of community-based vector control on house infestation and Trypanosoma cruzi infection in Triatoma infestans, dogs and cats in the Argentine Chaco. *Acta Trop* 103:201–211. http://linkinghub.elsevier.com/retrieve/pii/S0001706X0700157X. Accessed.
4. Carreira JCA, Jansen AM, Deane MP, Lenzi HL. 1996. Histopathological study of experimental and natural infections by Trypanosoma cruzi in Didelphis marsupialis. *Mem Inst Oswaldo Cruz*.
5. Cura CI, Duffy T, Lucero RH, Bisio M, Péneau J, Jimenez-Coello M, Calabuig E, Gimenez MJ, Valencia Ayala E, Kjos SA, et al. 2015. Multiplex real-time PCR assay using TaqMan probes for the identification of Trypanosoma cruzi DTUs in biological and clinical samples. *PLoS Negl Trop Dis* 9:e0003765. http://dx.plos.org/10.1371/journal.pntd.0003765. Accessed.
6. Curtis-Robles R, Aukland LD, Snowden KF, Hamer GL, Hamer SA. 2018a. Analysis of over 1500 triatomine vectors from across the US, predominantly Texas, for Trypanosoma cruzi infection and discrete typing units. Elsevier. *Infect Genet Evol* 58:171–180. https://doi.org/10.1016/j.meegid.2017.12.016. Accessed.

7. Curtis-Robles R, Lane S, Levy M, Hamer S, Hamer G. 2017a. Bionomics and spatial distribution of Triatomine vectors of Trypanosoma cruzi in Texas and other southern states, USA. *Am J Trop Med Hyg* In press.
8. Curtis-Robles R, Lewis BC, Hamer SA. 2016. High Trypanosoma cruzi infection prevalence associated with minimal cardiac pathology among wild carnivores in central Texas. Elsevier Ltd. *Int J Parasitol Parasites Wildl* 5:117–123. http://linkinghub.elsevier.com/retrieve/pii/S2213224416300098. Accessed.
9. Curtis-Robles R, Meyers AC, Auckland LD, Zecca IB, Skiles R, Hamer SA. 2018b. Parasitic interactions among Trypanosoma cruzi, triatomine vectors, domestic animals, and wildlife in Big Bend National Park along the Texas-Mexico border. Elsevier. *Acta Trop* 188:225–233. https://doi.org/10.1016/j.actatropica.2018.09.002. Accessed.
10. Curtis-Robles R, Wozniak EJ, Auckland LD, Hamer GL, Hamer SA. 2015. Combining public health education and disease ecology research: using citizen science to assess Chagas disease entomological risk in Texas. *PLoS Negl Trop Dis* 9:e0004235.
11. Curtis-Robles R, Zecca IB, Roman-Cruz V, Carbajal ES, Auckland LD, Flores I, Millard A V, Hamer SA. 2017b. Trypanosoma cruzi (Agent of Chagas Disease) in sympatric human and dog populations in "colonias" of the Lower Rio Grande Valley of Texas. *Am J Trop Med Hyg* 96:805–814. http://www.ajtmh.org/lookup/doi/10.4269/ajtmh.16-0789. Accessed.
12. Duffy T, Cura CI, Ramirez JC, Abate T, Cayo NM, Parrado R, Bello ZD, Velazquez E, Muñoz-Calderon A, Juiz NA, et al. 2013. Analytical performance of a multiplex Real-Time PCR assay using TaqMan probes for quantification of Trypanosoma cruzi satellite DNA in blood samples. *PLoS Negl Trop Dis* 7:e2000.

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3547845&tool=pmcentrez&rendertype=abstract. Accessed May 2014.

13. Frenkel JK, Parker BB. 1996. An apparent role of dogs in the transmission of Toxoplasma gondii. The probable importance of xenosmophilia. In: *Annals of the New York Academy of Sciences*.
14. Gurtler R, Cecere MC, Lauricella M a, Cardinal M V, Kitron U, Cohen JE, Gürtler RE, Cecere MC, Lauricella M a, Cardinal M V, et al. 2007. Domestic dogs and cats as sources of Trypanosoma cruzi infection in rural northwestern Argentina. *Parasitology* 134:69–82. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2669415&tool=pmcentrez&rendertype=abstract. Accessed August 2012.
15. Herrera L, Urdaneta-Morales S. 1992. Didelphis marsupialis: A primary reservoir of Trypanosoma cruzi in urban areas of Caracas, Venezuela. *Ann Trop Med Parasitol* 86:607–612.
16. Hodo CL, Hamer SA. 2017. Toward an ecological framework for assessing reservoirs of vector-borne pathogens: wildlife reservoirs of Trypanosoma cruzi across the southern United States. *ILAR J* 58:379–392.
17. Hodo CL, Rodriguez JY, Curtis-Robles R, Zecca IB, Snowden KF, Cummings KJ, Hamer SA. 2019. Repeated cross-sectional study of Trypanosoma cruzi in shelter dogs in Texas, in the context of Dirofilaria immitis and tick-borne pathogen prevalence. *J Vet Intern Med* 33:158–166.
18. Hodo CL, Wilkerson GK, Birkner EC, Gray SB, Hamer SA. 2018. Trypanosoma cruzi Transmission Among Captive Nonhuman Primates, Wildlife, and Vectors. Springer US.

Ecohealth 15:426–436. https://doi.org/10.1007/s10393-018-1318-5. Accessed.

19. Houk AE, Goodwin DG, Zajac AM, Barr SC, Dubey JP, Lindsay DS. 2010. Prevalence of Antibodies to Trypanosoma cruzi, Toxoplasma gondii, Encephalitozoon cuniculi, Sarcocystis neurona, Besnoitia darlingi, and Neospora caninum in North American Opossums, Didelphis virginiana, from Southern Louisiana. *J Parasitol* 96:1119–1122. http://www.bioone.org/doi/abs/10.1645/GE-2515.1. Accessed.
20. Jimenez-Coello M, Ortega-Pacheco A, Guzman-Marin E, Guiris-Andrade DM, Martinez-Figueroa L, Acosta-Viana KY. 2010. Stray Dogs as Reservoirs of the Zoonotic Agents Leptospira interrogans, Trypanosoma cruzi, and Aspergillus spp. in an Urban Area of Chiapas in Southern Mexico. *Vector-borne Zoonotic Dis* 10:135–141.
21. Keesing F, Brunner J, Duerr S, Killilea M, LoGiudice K, Schmidt K, Vuong H, Ostfeld RS. 2009. Hosts as ecological traps for the vector of Lyme disease. *Proc R Soc B Biol Sci* 276:3911–3919.
22. Lent H, Wygodzinsky PW. 1979. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' Disease. *Bull Am Museum Nat Hist* 163:123–520.
23. Montgomery SP, Starr MC, Cantey PT, Edwards MS, Meymandi SK, Cantey PT, Meymandi SK, Montgomery SP. 2014. Neglected Parasitic Infections in the United States: Chagas Disease. The American Society of Tropical Medicine and Hygiene. *Am J Trop Med Hyg* 90:814–818. http://www.ajtmh.org/content/journals/10.4269/ajtmh.13-0726. Accessed February 2018.
24. Nolan MS, Aguilar D, Brown EL, Gunter SM, Ronca E, Hanis CL, Murray KO. 2018. Continuing evidence of Chagas disease along the Texas-Mexico border. :1–8.

25. R Development Core Team. 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computin, Vienna, Austria. https://www.r-project.org. Accessed.
26. Roellig DM, Ellis AE, Yabsley MJ. 2009. Genetically different isolates of Trypanosoma cruzi elicit different infection dynamics in raccoons (Procyon lotor) and Virginia opossums (Didelphis virginiana). Australian Society for Parasitology Inc. *Int J Parasitol* 39:1603–1610. http://dx.doi.org/10.1016/j.ijpara.2009.06.007. Accessed.
27. Roque ALR, Xavier SCC, Da Rocha MG, Duarte ACM, D'Andrea PS, Jansen AM. 2008. Trypanosoma cruzi transmission cycle among wild and domestic mammals in three areas of orally transmitted chagas disease outbreaks. *Am J Trop Med Hyg*.
28. Ruiz-Pina HA, Cruz-Reyes A. 2002. The opossum Didelphis virginiana as a synanthropic reservoir of Trypanosoma cruzi in Dzidzilche, Yucatan, Mexico. *Mem Inst Oswaldo Cruz* 97:613–620.
29. Siciliano M. 2013. Didelphis virginiana. https://animaldiversity.org/accounts/Didelphis_virginiana/. Accessed May 2019.
30. Steindel M, Scholz AF, Toma HK, Schlemper BR. 1988. Presence of Trypanosoma cruzi in the anal glands of naturally infected opossum (Didelphis marsupialis) in the state of Santa Catarina, Brazil. *Mem Inst Oswaldo Cruz*.
31. Tenney TD, Curtis-Robles R, Snowden KF, Hamer SA. 2014. Shelter dogs as sentinels for Trypanosoma cruzi transmission across Texas. *Emerg Infect Dis* 20:1323–6. http://www.ncbi.nlm.nih.gov/pubmed/25062281. Accessed.
32. Urdaneta-Morales S, Nironi I. 1996. Trypanosoma cruzi in the anal flands of urban opossums. I-isolation and experimental infections. *Mem Inst Oswaldo Cruz*.

33. Vera-Cruz JM, Magallon-Gastelum E, Grijalva G, Rincon AR, Ramos-Garcia C, Armendariz-Borunda J. 2003. Molecular diagnosis of Chagas' disease and use of an animal model to study parasite tropism. *Parasitol Res* 89:480–486. http://www.ncbi.nlm.nih.gov/pubmed/12658460%5Cnhttp://www.springerlink.com/content/hv06j3qlpmprbagw/fulltext.pdf. Accessed.

34. Virreira M, Torrico F, Truyens C, Alonso-Vega C, Solano M, Carlier Y, Svoboda M. 2003. Comparison of polymerase chain reaction methods for reliable and easy detection of congenital Trypanosoma cruzi infection. *Am J Trop Med Hyg* 68:574–82. http://www.ncbi.nlm.nih.gov/pubmed/12812349. Accessed.

35. Wincker P, Britto C, Pereira JB, Cardoso MA, Oelemann W, Morel CM. 1994. Use of a simplified polymerase chain reaction procedure to detect Trypanosoma cruzi in blood samples from chronic chagasic patients in a rural endemic area. *Am J Trop Med Hyg* 51:771–777.

36. Woody NC, Woody HB. 1955. American Trypanosomiasis (Chagas' Disease): First indigenous case in the United States. *J Am Med Assoc* 159:676–677.

37. Yeo M, Acosta N, Llewellyn M, Sánchez H, Adamson S, Miles G a J, López E, González N, Patterson JS, Gaunt MW, et al. 2005. Origins of Chagas disease: Didelphis species are natural hosts of Trypanosoma cruzi I and armadillos hosts of Trypanosoma cruzi II, including hybrids. *Int J Parasitol* 35:225–33. http://www.ncbi.nlm.nih.gov/pubmed/15710443. Accessed July 2012.

CHAPTER V

DOMESTIC CAT *TRYPANOSOMA CRUZI* INFECTION FREQUENTLY ASSOCIATED WITH INFLAMMATION AND CARDIOMYOPATHY, LOWER RIO GRANDE VALLEY OF TEXAS

INTRODUCTION

Trypanosoma cruzi is a zoonotic protozoal parasite and etiologic agent of Chagas disease, a potentially debilitating and deadly cardiac disease of humans, dogs, and other mammals. The vector borne parasite affects approximately 8 million people in the Americas including an estimated 300,000 in the United States.[1,2] Transmission occurs through contact with infectious feces of the bloodsucking triatomine insect or consumption of these insects, commonly known as kissing bugs. Other forms of transmission include vertical transmission, via blood transfusion, organ transplant, and through ingestion of contaminated foods.[2] In humans and dogs, initial infection may cause acute nonspecific disease that is typically followed by an asymptomatic, indeterminate phase. Approximately 30% of infected people develop chronic Chagas disease, characterized by various cardiac or, less commonly, gastrointestinal or neurological complications.[2]

The veterinary health burden of Chagas disease in the US is largely unknown, but infected wild and domestic mammals have been described across the endemic range of triatomines. The wild hosts of *T. cruzi* in nature across the southern US are diverse, with raccoons (*Procyon lotor*), opossums (*Didelphis virginiana*), and woodrats (*Neotoma* sp.) among the most commonly

studied and infected wild species.[3] Recent studies in the southern US report a range of infection in wildlife, with *T. cruzi*-infected heart tissue identified in over 70% of raccoons (*Procyon lotor*) and 12-14% of bobcats (*Lynx rufus*), gray fox (*Urocyon cinereoargenteus*) and coyotes (*Canis latrans*) showing *T. cruzi*-infected heart tissue.[4] Among domestic animals, canine Chagas disease has been described from experimental animal models and epidemiological studies, with dogs of many breeds showing exposure to the parasite. Acute and chronic heart disease has been attributed to *T. cruzi* infection in young and older dogs.[5] In the southern US, prevalence of canine infection appears highly variable across populations and diagnostic methods used. In Texas, some shelter dogs show a seroprevalence of 8.8%-18.1% while some multi-dog kennels with vector infestations show a seroprevalence in excess of 55% of dogs.[6–8] Dogs of the southern US undoubtedly have high levels of contact with triatomine vectors, and may serve as sentinels of human disease risk in the same regions, yet far less is known about the involvement of other domestic animals in Chagas disease ecology in the southern US.

Natural infection with *T. cruzi* is reported in a small number of wild felids, including ocelot (*Leopardus pardalis)* in Brazil and bobcat (*Lynx rufus)* in the United States.[4,9] Feral and free roaming domestic cats pose threats to wildlife and human health thorough predation of animals and spreading zoootic pathogens to humans,[10,11] yet the epidemiology of *T. cruzi* in domestic cats and the degree to which they serve as sentinels of human disease risk is largely unknown in the United States.More importantly, domesticated felids are described as potential household reservoirs of *T. cruzi* and key sources of infection for triatomines in peridomestic environments

aiding in domestic transmission.[12,13] Studies in Argentina and Brazil have documented *T. cruzi* in domestic cats of rural villages.[13,14] Further, studies conducted in Southeast Mexico and Brazil have documented *T. cruzi* in urban dwelling domestic cats.[15,16] These few studies of cats in Latin America show that cats play an important role in the epidemiology of *T. cruzi* across both rural or urban environments.

The objectives of this study were to quantify domestic cat infection with *T. cruzi* using serologic and molecular approaches in a Chagas-endemic region of the southern United States and evaluate cardiac pathology in naturally infected versus uninfected cats. Cats were sampled at a large urban shelter. Knowledge of the role that cats may play in the ecology and epidemiology of Chagas disease, and the extent to which they are clinically impacted by *T. cruzi* infections, could provide new information for veterinary and public health interventions.

MATERIALS & METHODS

Study site:

The Lower Rio Grande Valley (RGV) of Texas is an area of the US-Mexico border with documented *T. cruzi* cases among humans and dogs, and is located within an endemic region of at least four triatomine species.[17–19] The largest animal shelter in the RGV is in Hidalgo County and intakes approximately 33,000 animals per year, including an annual average of 9,000 domestic cats. Cats are admitted to the shelter predominately by drop off by animal control agencies across the RGV, and are largely comprised of stray cats trapped off the streets in the

outdoor environment. Additionally, some cats at the shelter are relinquished by owners or members of the public.

Cat necropsy and tissue sampling:

We conducted a repeated cross-sectional study of cats from the RGV shelter in the winter, spring, and summer of 2017. Cats that were euthanized for reasons unrelated to our study were sampled. Demographic data was collected including cat breed (domestic short hair (DSH), domestic medium hair, domestic longhair, american shorthair)), sex, size assigned by shelter staff based on body weight (small (<6lbs), medium (6-9lbs), and large (>9lbs)), intake type (stray or owner/guardian surrender), season of sample collection (winter, spring, summer), place of origin (city/town), and presence or absence of fleas. Flea presence was documented since flea infestations are a risk factor for vector-borne diseases, such as *Bartonella henselae*, that could affect the immune system and overall health of a cat.[20] Only adult cats, determined by absence of deciduous canines, were selected for the study. Necropsies were performed 20-60 minutes after euthanasia. At the time of necropsy, ectoparasites were collected into ethanol and gross abnormalities were noted. For the winter sampling points, blood and heart were collected from all cats. Using standard necropsy procedures, the heart was completely excised from the body and placed into a specimen bag. All cutting tools and forceps used were sterilized between subjects using a 50% bleach solution, then rinsed with 70% ethanol, followed by flame sterilization using a handheld torch. Approximately 8-12 milliliters of blood were collected from the thoracic cavity with a syringe and placed into sterile vials. The vials were the placed in a cooler with ice for transportation and centrifuged within 24-36 hours.

More comprehensive tissue sampling was conducted for cats sampled during the spring and summer months. In addition to the collection of blood and cardiac tissue as described above, a section of intercostal muscle, biceps femoris, sciatic nerve, esophagus, mesentery, and distal colon was collected with sterilization of tools between sample collection. In these spring and summer sampling rounds, we performed a rapid blood test for *T. cruzi* at the time of necropsy, the results of which determined whether additional tissues beyone the list above were also collected. We used a rapid immunochromatographic assay (Chagas Stat-Pak; Chembio Diagnostic Systems, Inc., Medford, NY) to detect anti-*T. cruzi* antibodies. This assay was designed for humans and has widely been used on dogs and wildlife.[17,21–23] It has not been validated in cats.

The test was run using 5μL of serum. Any cat that produced a band after the 15 minute test development time, including faint bands, was flagged as suspect-positive and additional tissues were collected including sections of diaphragm, trachea, lung, liver, stomach, small intestine (proximal, middle, and distal), spleen, kidneys, urinary bladder, reproductive organs (uterus, ovaries, testicles), mammary tissue, and milk if from a lactating cat. All organs were placed on dry ice for transportation and frozen at -80°C upon arrival to the laboratory.

Detection of anti-T. cruzi antibodies:

Blood samples were centrifuged at 7000 rpm for 8 minutes with serum and clot prepared into aliquots. All serum was tested using three independent serologic tests. First, samples from the fall were tested using the Chagas Stat-Pak (described above), whereas samples from spring and summer had already been tested with this tool at the time of euthanasia at the shelter and were not re-tested in the laboratory. Second, each serum sample was run on to the Chagas Detect Plus

Rapid Test (InBios International, Inc., Seattle, WA). This assay is a rapid immunochromatiographic test designed for humans use and used in animal research.[21] Samples were scored as positive if there was development of a band of any color intensity, including faint bands. Finally, an aliquot of serum was submitted to the Texas Veterinary Medical Diagnostic Laboratory (TVMDL, College Station, TX) for testing of IgG antibodies using indirect fluorescent antibody (IFA) test in which anti-cat conjugated antibody was used on a testing platform that was optimized for the detection of anti-*T. cruzi* antibodies in dogs. For this assay, samples were screened at dilutions of 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, and 1:1280. We considered a cat as serologically positive when two or more independent serological tests produced positive results and we report the discordance among assays.

Histopathology:

After thawing, hearts were evaluated grossly for abnormalities. An incision was made along the coronary sulcus to expose the interior of the right ventricle followed by an incision towards the right atrium. An incision was then made from the apex to the left auricle to expose the interior of the left ventricle and atrium. A 1.5-2mm section of tissue from each chamber was collected with half preserved in formalin (10%) for histopathology, and half placed into a nuclease free tube for molecular analysis. Adult heartworms (*Dirofilaria immitis*) were collected if observed in heart chambers or pulmonary arteries. All other sampled tissues (intercostal muscle, biceps femoris, sciatic nerve, esophagus, mesentery, distal colon; in the case of suspect-seropositive cats, also diaphragm, trachea, lung, liver, stomach, small intestine (proximal, middle, and distal), spleen, kidneys, urinary bladder, reproductive organs (uterus, ovaries, testicles), and mammary tissue) were evaluated with separate sections preserved in formalin and prepared for molecular

evaluation. The formalin fixed tissues were routinely processed and stained with hematoxylin and eosin.

The hearts of cats showing a seropositive result on at least two serological assays plus approximately 20% of randomly selected hearts from seronegative cats were examined histologically. Two slides from each cat, representing right and left sides of the heart, were examined using light microscopy. Inflammation was semi quantitatively scored (Inflammation Score= *IS*) 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. We performed histological examination and assigned *IS* on an expanded set of tissue types from a subset of 15 cats comprised of 5 randomly selected from each of the following groups: cats seropositive on at least 2 tests; seropositive on 1 test; and seronegative across all tests. The expanded set of tissues examined histologically included intercostal muscle, biceps femoris, sciatic nerve, mesenteric fat, esophagus, and colon. All histopathology interpretation was performed by a board certified veterinary pathologist (CLH) who was blinded to the serostatus and molecular status of the samples at the time of initial scoring.

Detection of parasite DNA in blood clot and tissues:

From each tissue, we sampled multiple areas to yield a final volume of approximately 30mg; samples were macerated in a nuclease free tube. For heart samples, all four chambers were macerated together to represent all parts of the heart. DNA was extracted from the blood clot and

tissues using the E.Z.N.A. kit (Omega Bio- Tek, Norcross, GA). All extraction steps were conducted as per manufacturer instructions with the addition of a longer lysis duration (18-24 hours). To detect *T. cruzi* DNA in samples, we used a two-step process starting with a screening qPCR to amplify a 166-bp segment of the *T. cruzi* 195-bp repetitive satellite DNA.[24] PCR reactions (20 µL) contained 5 µL of DNA, with Cruzi 1, 2, and 3 primers and probe (0.75 µM each) and iTaq Universal Probes Supermix (BioRad Laboratories, Hercules, CA). Based on internal laboratory validations, a Ct value less than 40 with a sigmoid-shaped amplification curve was flagged as suspect positive for parasite DNA. For all suspect positive samples, a multiplex qPCR amplifying the spliced leader intergenic region (SL-IR) was used to confirm positivity and determine *T. cruzi* discrete typing unit (DTU). A clot or tissue sample was considered molecularly positive if the Ct value was less than 40 in the initial PCR and a DTU was determined in the secondary PCR. If the SLIR assay failed, those samples that screened positive were subjected to a third and final PCR by using the *T. cruzi* 121/122 primers to amplify a 330bp region of kinetoplast DNA. [4,25,26] As previously described in [4] reactions consisted of 1 µL template DNA, primers at a final concentration of 0.75 µM each, and FailSafe PCR Enzyme Mix with PreMix E (Epicentre, Madison, WI) in a final volume of 15 µL. Visualization of PCR products were conducted by using a 1.5% agarose gel stained with ethidium bromide. Only samples that had positive results on two independent PCRs for different gene regions were considered to be infected.

Epidemiological analysis:

Seroprevalence was calculated as the total number of cats that were serologically positive over the total number of cats enrolled in the study. In the absence of gold standard serologic methods,

serologic positivity was defined as having two or more positive test results on independent testing platforms.

Molecular prevalence in blood was calculated as the total number of cats that had PCR-positive blood clot, over the total number of cats enrolled in the study. Given that parasite DNA is likely to circulate in the blood when hosts are parasitemic or in the acute stage of infection, this metric may be useful in estimating the prevalence of animals that are infectious to vectors.

Molecular prevalence in tissues was calculated as the total number of cats with a PCR-positive tissue type, over the total number of cats with that specific tissue type collected. This metric may be useful in estimating the prevalence of animals that have parasite localized in specific tissue; tissue tropism of the parasite could vary by parasite genetic strain.[27]

Overall infection prevalence was calculated as the total number of cats that were seropositive and/or molecularly positive on any blood or tissue type, over the total number of cats enrolled in the study. Because infection is assumed to be life-long, antibody positive animals are assumed to be currently infected, and therefore the seropositive animals are included in the overall infection prevalence. Statistical analysis was performed in RStudio version 1.1.423.[28] Bivaritate analysis using chi-squared or Fisher's exact test was performed to evaluate the relationship between assumed risk factors and cat serostatus. Putative risk factors were: cat type (dichotomized into DSH vs. other), sex, size, intake type, season, flea presence, and human population size (United States Census Bureau)[29] where the cat originated from (<20,000 & >20,000) as a proxy for level

of urbanization. Bivariate analysis was used to evaluate the relationship between serostatus and cardiac inflammation, with separate analyses for overall inflammation, left sided inflammation, right sided inflammation, and dual sided inflammation. Generalized linear models were used to further investigate risk factors with P value ≤ 0.25 in the bivariate analysis or those with sufficient justification for inclusion, followed by calculating odds ratios and 95% confidence intervals.

RESULTS

Demographic Results

A total of 167 adult cats were sampled of which 87 were female (52.1%) and 80 were male (47.9%). The majority of the cats were classified as DSH (93.4%), while the remaining were classified as "other type" (6.6%) which included domestic medium hair (n=6), domestic long hair (n=4), and American short hair (n=1). Most of the cats were sampled in the winter (71.9%) with the rest of the samples collected in the spring (18.6%) and the summer (9.6%). Cats sampled ranged in size: small (n=6; 3.6%), medium (n=70; 41.9%), and large (n=91; 54.5%). Stray cats (n=146; 87.4%) represented most of the sample population and the rest were surrendered by their owner/guardian (n=21; 12.6%). Cats were obtained from 14 different Texas cities/census-designated places in Hidalgo and Cameron counties. The majority of cats (n=140; 83.8%) came from locations with a human population greater than 20,000 while 27 (16.2%) came from locations with a human population less than 20,000.

Seroprevalence

A total of 19 (11.4%) cats met the definition for serological positivity with positive results on two or more serologic tests. Of these, 16 (9.6%) were reactive on two tests and three (1.8%) cats were reactive on all three serologic assays. Additionally, 38 cats (22.8%) were reactive on only a single serologic assay and were thus considered negative in the seroprevalence estimate. In a bivariate analysis, we found potential associations (P-value <0.25) between serostatus and intake type, sex, size, and human population size where the cat originated; these factors were then included in a logistic regression model (Table 8). In contrast, season, presence of fleas, and cat breed had P values >0.25 and were not considered further. In the intake type category, the odds of seropositivity in strays was approximately six times less (OR=0.18, CI= (0.05-0.64), pvalue=0.006) than cats who were surrendered by their owners/guardians. The odds of seropositivity in medium and large cats were 0.08 (12) and 0.05 (20) times less when compared to small cats, respectively (OR=0.08, CI=(0.01-0.62), pvalue=0.015; OR=0.05, CI=(0.01-0.41), pvalue=0.005). Cats that originated from areas with human population >20,000 were five times less likely to be seropositive than cats that originated from areas with human population <20,000 (OR=0.22, CI=0.07-0.73, pvalue= 0.012). The odds of seropositivity in male cats is marginally greater than for female cats (OR=3.13, CI=1.04-10.91, pvalue=.052). Seroprevalence was not associated with cardiac inflammation.

Table 8. Bivariate analysis of serology results and demographic factors of feral cats (n=167) from the Rio Grande Valley, Texas 2017

Demographic factor		No. tested	No. seropositive (%)	Odds ratio	95% CI	p value
Intake type						
	Owner/Guardian Surrender	21	6 (28.6%)			referent
	Stray	146	13 (8.90%)	0.18	(0.05-0.64)	0.006***
Sex						
	Female	87	13 (14.9%)			referent
	Male	80	6 (6.9%)	3.13	(1.04-10.91)	0.052*
Size						
	Small	6	3 (50%)			referent
	Medium	70	8 (11.4%)	0.08	(0.01-0.62)	0.015**
	Large	91	8 (8.8%)	0.05	(0.01-0.41)	0.005***
Human Population Size (cat origin)						
	<20,000	27	7 (25.93%)			referent
	>20,000	140	12 (8.57%)	0.22	(0.07-0.73)	0.012**

Molecular prevalence in blood

The blood clot samples from all 167 cats were negative on the initial qPCR assay, resulting in a molecular prevalence in blood of 0%.

Molecular prevalence in tissues

Tissues in three cats (1.8%) were PCR positive (Table 9). Of these, two had positive PCR results in two or more tissues for a total of six positive PCR reactions across all tissue samples tested. Although all three of these PCR positive cats were reactive on at least one serological assay, only

one (F133) was positive on two or more tests and therefore considered seropositive under study criteria. Tissues that were PCR positive include esophagus (2.1%; 1/47), bicep femoris (4.3%; 2/47), sciatic nerve (4.3%; 2/47), mesentery (2.1%; 1/47), and cardiac tissue (0.6%; 1/167). All PCR positive tissues were DTU TcI. All adipose (epicardial, splenic, kidney), intercostal muscle, mesentery, trachea, lung, diaphragm, stomach, spleen, liver, small intestine (proximal, medial, and distal), colon, and reproductive organs (testes, ovary, uterus) tested were PCR negative.

Overall Infection Prevalence.

A total of 21 of 167 cats (12.6%) were either seropositive and/or PCR positive in one or more tissues.

Table 9. Details of serology and histology for PCR positive feral cats from the Rio Grande Valley, Texas with PCR positive tissues 2017

	Serological Tests							
Cat ID	**StatPAK**	**InBios**	**IFA**	**PCR Positive Tissue**	**Inflammation Score**	**Ct Value**	**DTU**	**PCR Negative Tissue**
F124	Negative	Negative	**Positive**	Esophagus	2	26.39	TcI	Clot, Cardiac m., Epicardial Adipose, Intercostal m., Mesentery, Colon
				Biceps femoris m.	0	31.60	TcI	
				Sciatic n.	0	29.79	TcI	
F129	**Positive**	Negative	Negative	Cardiac m.	2	26.99	TcI	Clot, Epicardial Adipose, Intercostal m., Mesentery, Colon, Trachea, Lung, Esophagus, Biceps femoris m., Testes, Spleen, Splenic Adipose, Small Intestine, Liver
F133	Negative	**Positive**	**Positive**	Biceps femoris m.	0	25.49	TcI	Clot, Cardiac m., Epicardial Adipose, Intercostal m., Colon, Esophagus
				Sciatic n.	0	27.41	TcI	
				Mesentery	0		-	

Histology Results

Histology was performed on PCR-positive tissues from three cats, and hearts of all 19 seropositive cats, and randomly selected 28 seronegative cats. Additionally, histologic examination across an expanded set of 35 tissues was performed in 15 cats, including 5 in each of the three serogroups. Of the seven PCR-positive tissue samples from three different cats, two tissues from two different cats exhibited inflammation when examined histologically. In one cat (F124), mild (*IS*:2) lymphoplasmacytic inflammation was observed in the muscle near the serosal surface of esophagus (Figure 8). The other cat (F129) exhibited mild (*IS*:2) inflammation in the right heart sections and multifocal mild (*IS*:2) inflammation in left heart sections (Figure 8; Table 9).

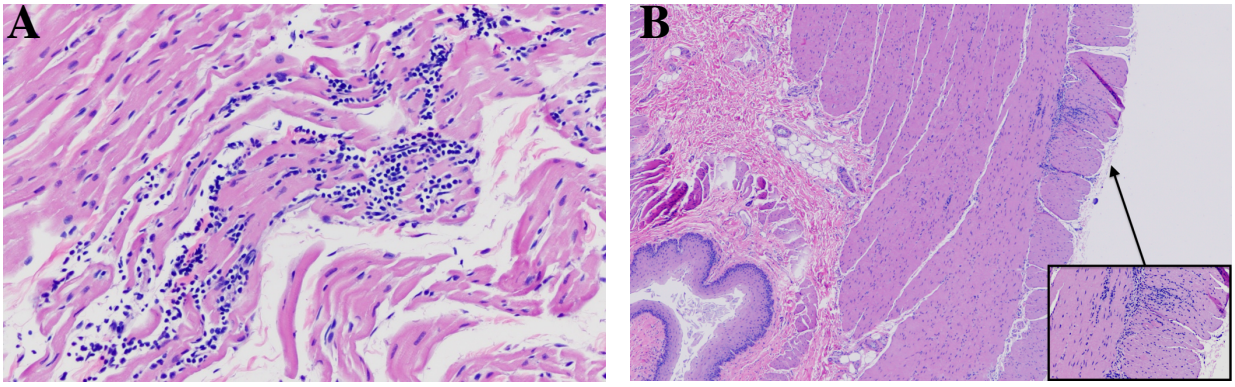

Figure 8. Cat tissues- hematoxylin and eosin stain: ***A*** *(Feline-Left Atrium) 20x: Cardiac myofibers are separated by moderate numbers of lymphocytes and plasma cells, with myocyte degeneration and loss (Inflammation Score= 2).*
B *(Esophagus) 20x: Mild lymphoplasmacytic inflammation infiltrates the muscularis of the esophagus (Inflammation Score= 2)*

The hearts of 28 seronegative cats (defined as having none or only a single reactive serological test) and all 19 seropositive cats (defined as having two or more reactive serological tests) were examined histologically. Of the 28 seronegative cats, eight had cardiac pathology documented. Among the eleven cats that were negative on all serological assays, two (18.2%) had minimal lymphoplasmacytic inflammation in the cardiac tissue sampled from the right side of the heart. Among the 17 cats that were interpreted as seronegative because they were only seroreactive on a single assay, six (35.3%) had focal to multifocal, minimal to severe lymphoplasmacytic inflammation. Of the 19 seropositive cats (≥2 reactive serological tests), eight (42.1%) exhibited minimal to moderate, multifocal, subacute lymphoplasmacytic inflammation with some fibrosis (Table 10). The three cats that were reactive on all three serological tests did not have significant lesions. Individual inflammation scores are detailed in Table 10.

Table 10. Individual histopathological assessment of feral cats from the Rio Grande Valley, Texas based on serological tests

Number of Reactive Serologic Tests	Cat ID	Left Heart Inflammation (Score)	Right Heart Inflammation (Score)	Bilateral Inflammation
0	F08	0	1	No
	F158	0	1	No
1	F31	2	0	No
	F67	3	4	Yes
	F100	1	0	No
	F124	1	0	No
	F129	2	2	Yes
	F146	2	0	No
2	F06	0	1	No
	F19	0	2	No
	F91	2	2	Yes
	F101	3	0	No
	F111	3	2	Yes
	F133	2	2	Yes
	F137	1	1	Yes
	F142	2	2	Yes
3	F26	0	0	No
	F35	0	0	No
	F113	0	0	No

Concerning histopathologic examination of the extended set of tissues, of the 5 seronegative cats, two showed minimal inflammation, one in intercostal muscle and the other in heart muscle. Of the 5 cats reactive on only one serological test, three showed minimal to mild inflammation. Interestingly, while two of the five cats in this category had other tissue types that were PCR positive (described above) the histopathology survey showed inflammation only in PCR negative tissues (Table 11).

Table 11. Histopathological examination (inflammation score) of an expanded set of tissues across five randomly selected felines from each of three serogroups: those reactive on no tests; those reactive on only a single test; And those reactive on 2 or more tests (and therefore considered seropositive for this study

ID	Left Heart	Right Heart	Intercostal muscle	Bicep femoris	Sciatic nerve	Mesenteric Fat	Esophagus	Colon
Serogroup 1: Reactive on no tests (seronegative)								
F149	0	0	0	0	0	0	0	0
F152	0	0	**1**	0	0	0	0	0
F158	0	**1**	0	0	0	0	0	0
F163	0	0	0	0	0	0	0	0
F166	0	0	0	0	0	0	0	0
Serogroup 2: Reactive on a single test (seronegative)								
F124	1	0	0	0♦	0♦	0	2♦	0
F129	2	2♦	0	2	0	0	1	2
F146	2	0	0	0	0	0	0	0
F154	0	0	0	0	0	0	0	0
F159	0	0	0	0	0	0	0	0
Reactive on 2 or more tests (seropositive)								
F133	2	2	1	0♦	0♦	0	0	0
F137	1	1	0	0	1	1	0	0
F141	0	0	0	0	0	0	0	0
F142	2	2	0	0	0	0	0	0
F160	0	0	0	0	0	0	0	0

♦PCR positive tissue

Of the 5 seropositive cats, one cat (F133) showed inflammation in intercostal muscle and cardiac tissues; these tissues were not PCR-positive, and no pathology was observed in biceps femoris muscle and sciatic nerve which were PCR positive. Two other seropositive cats showed minimal to mild inflammation in tissues including heart, sciatic nerve, and mesenteric tissue while the other two showed no pathology.

Other findings

Adult heartworms (*Dirofilaria immitis*) were found in heart chambers or pulmonary arteries of 3.6% (n=6) of the cats. Half of the cats with heartworms were seropositive for *T. cruzi.*

DISCUSSION

We report for the first time Chagas seropositive domestic cats (11.4%) from a Chagas-endemic region along the Texas-Mexico border. Through detailed molecular investigations of various tissues, we documented parasite DNA in four tissue types (heart, esophagus, sciatic nerve, biceps femoris) in which all infections were comprised of parasite DTU TcI and one tissue (mesentery) where DTU was not determined. Through histopathologic examination, we saw mild to moderate inflammation in hearts of 42.1% (8/19) of the examined seropositive cats and 28.6% (8/28) of seronegative cats.

There are no gold standard serologic testing methods for human or animal Chagas disease, and no validated serologic tests for cats. Accordingly, we used multiple independent antibody-detection testing platforms for cats and looked for two independent lines of evidence to consider a cat as seropositive for research purposes. These tests included two that were validated in

humans and one that was validated in dogs but used with cat-specific reagents. The sensitivity and specificity of this testing approach for cats is unknown. True seroprevalence may be higher (22.8%) if reaction on any single test signals positivity. Considering that 2 of the 3 PCR-positive cats were seropositive on only 1 test, the potential for false negatives within the conservative interpretation criteria we applied is possible. Approximately 9,000 cats are admitted to the RGV shelter yearly. Extrapolating from this serological study, over 1,000 of these may be infected with *T. cruzi*. With feral cat populations in the US estimated to be approximately 80 million[30], there is potential for a large number of *T. cruzi*-infected cats to occur in the southern US.

While both parasite DTUs TcI and TcIV have been found in triatomine vectors in the region[31], the infected cats in this study were exclusively infected with TcI - the DTU previously associated with human disease in the United States.[17] TcI was found in bicep femoris muscle, sciatic nerve, esophagus, and cardiac tissue from three cats. Parasite was not detected in any blood clots indicating no (or very low level of) circulating parasite was present in the cats, despite the abundance of seropositive cats and the presence of three cats with PCR-positive tissues. PCR sensitivity may be affected by the volume of blood taken for analysis especially if the sample is obtained from chronically infected individuals with low circulating parasite loads. The contrast seen between the relatively high seroprevalence and absence of a molecular prevalence in blood could be attributed to unique immune responses observed in cats. For example, immunocompromised cats, such as those suffering from common feral cat diseases like feline leukemia, produce complement dependent antibodies that may be useful to combat other pathogens like *T. cruzi.*[15] Complement dependent antibodies in addition to any *T. cruzi* antibodies formed could reduce circulating parasite load. While not systematically measured, the

cats that comprised the study population were often in poor health owing to a lack of veterinary care, lack of regular access to food, and other health threats that accompany a stray lifestyle.

Cats that originated from areas with human population less than 20,000, potentially more rural areas, were more likely to be seropositive (OR=0.22, CI=0.07-0.73, pvalue= 0.012). In contrast, a previous canine study conducted in the same area found that dogs from more urban areas were more likely to be seropositive.[17] However, our findings are consistent with studies in South America that found that cats have higher parasitemia in rural areas (39.3%) when compared to cats in urban areas (20%).[12,16] Larger cats were more likely to be seronegative when compared to smaller cats (OR=0.05, CI=0.01-0.41, pvalue=0.005); this may be because smaller cats may be more susceptible to vectors. Male cats were marginally more likely to be seropositive than female cats (OR=3.13, CI=1.04-10.91, pvalue=.052). Similarly, in a previous study, intact male cats with outdoor access were more likely to be exposed to infectious diseases like FIV and FeLV.[32] Although we unexpectedly found stray cats were less likely to be seropositive (OR=0.18, CI= (0.05-0.64), pvalue=0.006) than owner surrendered cats, classification of intake type may be biased due to clerical classification error at intake.

Several of the cats exhibited histopathologic changes consistent with those reported for *T. cruzi* infection in humans, dogs, and other species, including myocyte degeneration and lymphoplasmocytic inflammation in the cardiac tissue.[4,33] *T. cruzi* amastigotes were not observed in tissue, but failure to observe the parasite in tissue of infected animals is not uncommon, especially in chronic infections.[4] Although *T. cruzi* is most commonly associated

with cardiac tropism in humans and animals, only heart tissue from one cat was PCR positive. However, the distribution of the parasite is multifocal even in confirmed infected animals, and this may explain the lack of correlation between PCR status and inflammatory lesions. Another possible explanation is that the inflammation was caused by another etiology. While lymphoplasmacytic inflammation is characteristic of *T. cruzi* infections, it is not entirely specific to this parasite and other feline pathogens such as *Bartonella henselae* and feline panleukopenia virus (*protoparvovirus*) in cats may cause cardiac inflammation that appears similar.[34,35] One cat (F124) was PCR positive on 3 tissues but inflammation was only observed in the esophagus. Inflammation in the esophagus was reported in experimentally-infected dogs,[36] and observed in chronic Chagas disease in humans with chagasic megaesophagus.[37] Similarly, F124 and F133 sciatic nerve and skeletal muscle were PCR-positive but had no apparent inflammation. A limitation in our investigation into the potential pathological impacts of infection is that the most severely impacted cats may not have been healthy enough to be active and captured by animal control officers to arrive at the animal shelter; accordingly, any severely impacted individuals may not reflect in the current study.

We show feral cats are infected with *T. cruzi*, sometimes associated with pathology in multiple organs, in south Texas where ongoing autochthonous human and canine transmission occurs. Vectors collected from the shelter that housed the cats showed *T. cruzi* infection and evidence of feeding on wildlife (opossum), domestic animals (dog), and humans, such that cats may be at the intersection of sylvatic and domestic transmission cycles. Future research will determine the impact of *T. cruzi* on the health of domestic cats, the relative importance of cats as reservoirs of *T. cruzi*, and the threat they pose to public health.

REFERENCES

1. Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United States. Clin Infect Dis 2009;49:e52-4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19640226. Accessed August 23, 2012.

2. Montgomery SP, Starr MC, Cantey PT, et al. Neglected Parasitic Infections in the United States: Chagas Disease. Am J Trop Med Hyg 2014;90:814–818. Available at: http://www.ajtmh.org/content/journals/10.4269/ajtmh.13-0726. Accessed February 2, 2018.

3. Hodo CL, Hamer SA. Toward an ecological framework for assessing reservoirs of vector-borne pathogens: wildlife reservoirs of Trypanosoma cruzi across the southern United States. ILAR J 2017;58:379–392.

4. Curtis-Robles R, Lewis BC, Hamer SA. High Trypanosoma cruzi infection prevalence associated with minimal cardiac pathology among wild carnivores in central Texas. Int J Parasitol Parasites Wildl 2016;5:117–123. Available at: http://linkinghub.elsevier.com/retrieve/pii/S2213224416300098.

5. Vitt JP, Saunders AB, O'Brien MT, et al. Diagnostic features of acute Chagas myocarditis with sudden death in a family of Boxer dogs. J Vet Intern Med 2016;30:1210–1215.

6. Tenney TD, Curtis-Robles R, Snowden KF, et al. Shelter dogs as sentinels for Trypanosoma cruzi transmission across Texas. Emerg Infect Dis 2014;20:1323–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25062281.

7. Hodo CL, Rodriguez JY, Curtis-Robles R, et al. Repeated cross-sectional study of Trypanosoma cruzi in shelter dogs in Texas, in the context of Dirofilaria immitis and tick-borne pathogen prevalence. J Vet Intern Med 2019;33:158–166.

8. Curtis-Robles R, Snowden KF, Dominguez B, et al. Epidemiology and molecular typing of

Trypanosoma cruzi in naturally-infected hound dogs and associated triatomine vectors in Texas, USA. PLoS Negl Trop Dis 2017;11:e0005298.

9. Rocha FL, Roque ALR, Arrais RC, et al. Trypanosoma cruzi TcI and TcII transmission among wild carnivores, small mammals and dogs in a conservation unit and surrounding areas, Brazil. Parasitology 2013;140:160–70. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23062278. Accessed February 22, 2014.

10. Case JB, Chomel B, Nicholson W, et al. Serological survey of vector-borne zoonotic pathogens in pet cats and cats from animal shelters and feral colonies. J Feline Med Surg 2006;8:111–117.

11. Loyd KAT, Hernandez SM, Carroll JP, et al. Quantifying free-roaming domestic cat predation using animal-borne video cameras. Biol Conserv 2013;160:183–189.

12. Gürtler RE, Cécere MC, Petersen RM, et al. Chagas disease in north-west Argentina: association between Trypanosoma cruzi parasitaemia in dogs and cats and infection rates in domestic Triatoma infestans. Trans R Soc Trop Med Hyg 1993;87:12–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8465382.

13. Gurtler R, Cecere MC, Lauricella M a, et al. Domestic dogs and cats as sources of Trypanosoma cruzi infection in rural northwestern Argentina. Parasitology 2007;134:69–82. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2669415&tool=pmcentrez&rendertype=abstract. Accessed August 8, 2012.

14. Mott KE, Mota EA, Sherlock I, et al. Trypanosoma cruzi infection in dogs and cats and household seroreactivity to T. cruzi in a rural community in northeast Brazil. Am J Trop Med Hyg 1978;27:1123–1127. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/103445%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/10344
6.

15. Jiménez-Coello M, Acosta-Viana KY, Guzman-Marin E, et al. Epidemiological survey of Trypanosoma cruzi infection in domestic owned cats from the tropical southeast of Mexico. Zoonoses Public Health 2012;59 Suppl 2:102–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22958254. Accessed August 13, 2014.

16. Eloy LJ, Lucheis SB. Hemoculture and Polymerase Chain Reaction Using Primers TCZ1/TCZ2 for the Diagnosis of Canine and Feline Trypanosomiasis. ISRN Vet Sci 2012;2012:419378.

17. Curtis-Robles R, Zecca IB, Roman-Cruz V, et al. Trypanosoma cruzi (Agent of Chagas Disease) in sympatric human and dog populations in "colonias" of the Lower Rio Grande Valley of Texas. Am J Trop Med Hyg 2017;96:805–814. Available at: http://www.ajtmh.org/lookup/doi/10.4269/ajtmh.16-0789.

18. Beard CB, Pye G, Steurer FJ, et al. Chagas disease in a domestic transmission cycle, southern Texas, USA. Emerg Infect Dis 2003;9:103–5. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2873735&tool=pmcentrez&rendertype=abstract.

19. Curtis-Robles R, Hamer SA, Lane S, et al. Bionomics and spatial distribution of triatomine vectors of Trypanosoma cruzi in Texas and other southern states, USA. Am J Trop Med Hyg 2018;98:113–121. Available at: http://www.ncbi.nlm.nih.gov/pubmed/29141765.

20. Foley JE, Pedersen NC, Pedersen NC, et al. Seroprevalence of bartonella henselae in cattery cats: Association with cattery hygiene and flea infestation. Vet Q 1998.

21. Nieto PDPD, Boughton R, Dorn PL, et al. Comparison of two immunochromatographic

assays and the indirect immunoflourscence antibody test for diagnosis of Trypanosoma cruzi infection in dogs in south central Louisiana. Vet Parasitol 2009;165:241–247.

22. Barbabosa-Pliego A, Díaz-Albiter HM, Ochoa-García L, et al. Trypanosoma cruzi circulating in the southern region of the State of Mexico (Zumpahuacan) are pathogenic: a dog model. Am J Trop Med Hyg 2009;81:390–5. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2784919&tool=pmcentrez&rendertype=abstract.

23. Lopez RR, Davis DS, Cooper SM. PREVALENCE OF TRYPANOSOMA CRUZI IN FREE-RANGING MAMMALIAN POPULATIONS IN SOUTH TEXAS A Dissertation by MATHEW M . KRAMM III Submitted to the Office of Graduate and Professional Studies of Texas A & M University in partial fulfillment of the requirements f. 2015.

24. Duffy T, Cura CI, Ramirez JC, et al. Analytical performance of a multiplex Real-Time PCR assay using TaqMan probes for quantification of Trypanosoma cruzi satellite DNA in blood samples. PLoS Negl Trop Dis 2013;7:e2000. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3547845&tool=pmcentrez&rendertype=abstract. Accessed May 3, 2014.

25. Wincker P, Britto C, Pereira JB, et al. Use of a simplified polymerase chain reaction procedure to detect Trypanosoma cruzi in blood samples from chronic chagasic patients in a rural endemic area. Am J Trop Med Hyg 1994;51:771–777.

26. Virreira M, Torrico F, Truyens C, et al. Comparison of polymerase chain reaction methods for reliable and easy detection of congenital Trypanosoma cruzi infection. Am J Trop Med Hyg 2003;68:574–82. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12812349.

27. Vera-Cruz JM, Magallon-Gastelum E, Grijalva G, et al. Molecular diagnosis of Chagas'

disease and use of an animal model to study parasite tropism. Parasitol Res 2003;89:480–486. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12658460%5Cnhttp://www.springerlink.com/content/hv06j3qlpmprbagw/fulltext.pdf.

28. R Development Core Team. R: A language and environment for statistical computing. 2008. Available at: https://www.r-project.org.

29. US Census Bureau. State and County Quickfacts: Hidalgo County, Cameron County, Starr County, Willacy County, TX. 2014. Available at: http://quickfacts.census.gov. Accessed September 14, 2016.

30. Chu K, Anderson WM, Rieser MY. Population characteristics and neuter status of cats living in households in the United States. J Am Vet Med Assoc 2009;234:1023–1030.

31. Curtis-Robles R, Aukland LD, Snowden KF, et al. Analysis of over 1500 triatomine vectors from across the US, predominantly Texas, for Trypanosoma cruzi infection and discrete typing units. Infect Genet Evol 2018;58:171–180. Available at: https://doi.org/10.1016/j.meegid.2017.12.016.

32. Levy JK, Scott HM, Lachtara JL, et al. Seroprevalence of feline leukemia virus and feline immunodeficiency virus infection among cats in North America and risk factors for seropositivity. J Am Vet Med Assoc 2006.

33. Higuchi M de L, De Brito T, Martins Reis M, et al. Correlation between Trypanosoma cruzi parasitism and myocardial inflammatory infiltrate in human chronic chagasic myocarditis: Light microscopy and immunohistochemical findings. Cardiovasc Pathol 1993;2:101–106.

34. Varanat M, Broadhurst J, Linder KE, et al. Identification of Bartonella henselae in 2 Cats With Pyogranulomatous Myocarditis and Diaphragmatic Myositis. Vet Pathol 2012.

35. Meurs KM, Fox PR, Magnon AL, et al. Molecular screening by polymerase chain reaction detects panleukopenia virus DNA in formalin-fixed hearts from cats with idiopathic cardiomyopathy and myocarditis. Cardiovasc Pathol 2000.

36. Nogueira-Paiva NC, da Fonseca KS, de Vieira PMA, et al. Myenteric plexus is differentially affected by infection with distinct Trypanosoma cruzi strains in Beagle dogs. Mem Inst Oswaldo Cruz 2014.

37. Lages-Silva E, Crema E, Amirez LE, et al. Relationship between Trypanosoma cruzi and human chagasic megaesophagus: Blood and tissue parasitism. Am J Trop Med Hyg 2001.

CHAPTER VI

TRYPANOSOMA CRUZI (AGENT OF CHAGAS DISEASE) IN ENDANGERED OCELOTS (*LEOPARDUS PARDALIS*) OF SOUTH TEXAS

INTRODUCTION

The ocelot (*Leopardus pardalis*) is an elusive small spotted feline with a geographic range from south America to sparse areas of the southern US. Ocelots are carnivores and typically eat small rodents and birds but their diets also include larger mammals, reptiles, and insects. [1]

Although ocelots are listed as "least concern" by the International Union for Conservation of Nature-Red List,[2] they are listed as endangered with the US Fish and Wildlife Service, with factors including habitat loss and vehicle collisions attributed to the diminishment of the populations.[3] Historically in the US, ocelot populations extended across eastern Texas and some southern parts of Arkansas and Louisiana.[4] In present day, ocelots mostly in small pockets of thorn shrub habitat in the Rio Grande Valley (RGV) of south Texas. (Figure 9) Ocelots have been spotted in Arizona, but breeding populations only occur in south Texas with less than 80 individuals remaining in the region.[5] Current conservation efforts focus on road modifications along transportation corridors since most ocelot deaths in Texas occur as a result of ocelot-vehicle collisions.[6]

Figure 9. Geographical range of ocelots (*Leopardus pardalis*) in the Americas.

Paviolo, A., Crawshaw, P., Caso, A., de Oliveira, T., Lopez-Gonzalez, C.A., Kelly, M., De Angelo, C. & Payan, E. 2015. *Leopardus pardalis* (errata version published in 2016). *The IUCN Red List of Threatened Species* 2015: e.T11509A97212355. http://dx.doi.org/10.2305/IUCN.UK.20154.RLTS.T11509A50653476.en. Downloaded on 09 July 2019

Infectious diseases are attributable to the decline of several endangered taxa. For example, viral diseases like canine distemper virus have been known to infect Ethiopian wolves (*Canis simensis*), Amur Tigers (*Panthera tigris altaica*), and the Iberian lynx (*Lynx pardinus*)[7–9] A study assessing animals in the IUCN Red List of Threatened and Endangered species concluded that carnivore species were more likely to be affected by parasite-mediated declines than other taxa.[10] However, particular challenges are present in the study of parasites or pathogens in populations with small animal numbers and in which stressful trapping or invasive sampling are not desired.[10]

Few studies have assessed the impact diseases have on south Texas ocelot populations. Diseases caused by bacterial infections and helminth parasitemia have been detected in the population; for example death in south Texas ocelots has been attributed to heartworms (*Dirofilaria immitis)* and infections of *Toxscaris leonine* and *Taenia rileyi* have been noted.[11,12] Additionally, Mexican ocelot populations directly south of the Texas border were found to be heavily parasitized by *Toxoplasma gondi.*[13]

Trypanosoma cruzi, the zoonotic protozoan parasite that causes Chagas disease, is a vector borne disease known to infect a wide range of mammals including humans, domestic animals, and wildlife in Texas.[14–16] South Texas is an area with robust transmission of *T. cruzi* and our previous studies highlighted high prevalence among domestic dogs (>20%) and triatomine 'kissing bug' vectors (>55%) in the region.[17,18] Further, we found that up to 11.4% of south Texas feral cats may be infected, with some having cardiac inflammation or fibrosis, yet the impact of this parasite on the health of sympatric wild cats is largely unknown. In South America, *T. cruzi* is known to infect ocelots [19,20], yet the degree to which this parasite infects and

causes pathology in ocelots of Texas is unknown. Through use of salvaged animals scientific collections, we assesssed the prevalence of *T. cruzi* and associated pathology in south Texas ocelots to further understand parasitic diseases in ocelots.

MATERIALS & METHODS

Sample source:

We requested any available ocelot tissue from salvaged animals that were installed in the Biodiversity Research & Teaching Collections (BRTC) at Texas A&M University and the Glady's Porter Zoo (GPZ); all carcasses were from wild ocelots that were collected typically by United States Fish and Wildlife Service biologists following death at various sites across the RGV. Tissues from BRTC ocelots were collected at the time of that the ocelots were installed in the BRTC and prepped into study specimens in 2018 after variable lengths of the whole carcass being frozen (Table 1). Tissues from GPZ ocelots were collected at the time of necropsy shortly after the animal's death and were fresh-frozen since that time (Table 1).

Ocelots used in this study died primarily by vehicle collision from 2010-2017 around Laguna Atascosa National Wildlife Refuge in South Texas and had been stored in the freezer since that time. From 21 total individuals, we collected skeletal muscle (n=15), heart tissue (n=5), lung (n=1), kidney (n=1), spleen (n=1), liver (n=1), blood clot (n=7), and serum (n=1). Five ocelots had more than one tissue type: three with skeletal muscle, heart, and clot samples; one with skeletal muscle and heart; and one ocelot with skeletal muscle, heart, kidney, spleen, and liver samples. Basic demographic data (sex and year of collection for all; county records for 15 animals) was obtained from the collections record.

Sample preparation

After thawing, an incision was made along the coronary sulcus of the heart to expose the interior of the right ventricle followed by an incision towards the right atrium. An incision was then made from the apex to the left auricle to expose the interior of the left ventricle and atrium. A 1.5-2mm section of tissue from each heart chamber collected with half preserved in formalin (10%) for histopathology, and the rest of the halves placed together into a nuclease free tube for molecular analysis. A 1.5-2mm section of tissue from the lung, spleen, kidney, and liver were evaluated with separate sections preserved in formalin and prepared for molecular evaluation.

Detection of parasite DNA in tissues, blood clot, and serum:

From each sample type, we sampled different areas of the organ to yield a final volume of approximately 30mg; samples were macerated in a nuclease free tube. For heart samples, all four chambers were prepared and macerated together to represent all parts of the heart. DNA was extracted from the samples using the E.Z.N.A. kit (Omega Bio- Tek, Norcross, GA). All extraction steps were conducted as per manufacturer instructions with the addition of a longer lysis duration (18-24 hours). To detect *T. cruzi* DNA in samples, we used a two-step process starting with a screening qPCR to amplify a 166-bp segment of the *T. cruzi* 195-bp repetitive satellite DNA.[21] PCR reactions (20 μL) contained 5 μL of DNA, with Cruzi 1, 2, and 3 primers and probe (0.75 μM each) and iTaq Universal Probes Supermix (BioRad Laboratories, Hercules, CA). Any sample with a Ct value less than 40 with a sigmoidal amplification curve was next subjected to discrete typing unit (DTU) determination using multiplex assay amplifying the spliced leader intergenic region (SL-IR) [22]. If the SLIR assay failed, those samples that screened

positive were subjected to a third and final PCR by using the *T. cruzi* 121/122 primers to amplify a 330bp region of kinetoplast DNA. [15,23,24] As previously described in [15] reactions consisted of 1 μL template DNA, primers at a final concentration of 0.75 μM each, and FailSafe PCR Enzyme Mix with PreMix E (Epicentre, Madison, WI) in a final volume of 15 μL. Visualization of PCR products were conducted by using a 1.5% agarose gel stained with ethidium bromide. Only samples that had positive results on two independent PCRs for different gene regions were considered to be infected.

Histopathology:

The formalin fixed tissues were routinely processed and stained with hematoxylin and eosin and examined by a board-certified pathologist.

Prevalence Metrics:

Tissue-specific infection prevalences were calculated as the total number of ocelots with a PCR sample type, over the total number of ocelots with that specific sample type collected. Serum and blood clot data were analyzed together since a positive result in either would indicate circulating parasite. This metric may be useful in estimating the prevalence of animals that have parasite localized in specific tissue; tissue tropism of the parasite could vary by parasite genetic strain [25]. Additionally, the *overall infection prevalence* was calculated as the total number of ocelots that had at least one positive sample over the total number of ocelots represented in the study.

RESULTS

A total of 21 salvaged ocelots were represented in the study, with variable sample types available for each animal (Table 1). There were 15 (71.4%) males, 4 (23.8%) female, and the sex of one (4.8%) was unknown. Our ocelot samples were collected between 2010-2017: 2010 (19%, n=4), 2012 (4.8%, n=1), 2013 (19%, n=4), 2014 (4.8%, n=1), 2015 (33.3%, n=7), 2016 (14.3%, n=3), 2017 (4.8%, n=1). *T. cruzi* DNA was detected in 9.5% (n=2) of the ocelot samples (Table 12), in which positivity was defined on the basis of having two positive PCR results for different genetic regions. Five other samples showed Ct value less than 40 with a sigmoidal amplification curves on the screening assay but were considered negative since they were negative on the two subsequent tests.

One of the infected ocelots was a male collected in 2010 in Cameron county and had positive skeletal muscle. The parasite DTU present in this sample was 'TcI'- the DTU previously implicated in human Chagas disease in the US. This ocelot had five other tissues (heart, lung, spleen, kidney, liver) test PCR negative. This was the only infected skeletal muscle sample of 15 collected, yielding a *T. cruzi* prevalence in skeletal muscle of 6.7%. The second infected ocelot was a female in from an undisclosed location collected in 2013 and had a positive blood clot. The parasite DTU was not able to be determined for this sample, but positive status was confirmed through the secondary PCR assay for kinetoplast DNA. This was the only positiblood sample among the eight that were tested, yielding a *T. cruzi* prevalence in blood of 12.5%. Advanced autolysis hindered the histologic examination the PCR-positive skeletal muscle sample. Lymphoplasmacytic inflammation was noted in skeletal muscle of two other animals who were not PCR positive.

Table 12. Ocelots that died in the Rio Grande Valley between 2010-2017 that were sampled for *Trypanosom cruzi* investigation						
Ocelot ID	**Sex**	**Year**	**County**	**Skeletal Muscle**	**Heart**	**Other**
OC01	F	2012	Cameron	Y	N	N
OC02	M	2014	Cameron	Y	N	N
OC03	M	2015	Willacy	Y	Y	lung, kidney, spleen, liver
OC04	M	2016	Cameron	Y	N	N
OC05	M	2015	Cameron	Y	N	N
OC06	M	2010	Cameron	Y	N	N
OC07	M	2016	Cameron	Y	N	N
OC08	M	2010	Cameron	Y*	N	N
OC09	U	2010	Willacy	Y	N	N
OC10	M	2015	Cameron	Y	Y	Clot
OC11	M	2015	Cameron	Y	Y	Clot
OC12	M	2010	Cameron	Y	N	N
OC13	M	2013	Cameron	Y	Y	N
OC14	M	2016	Willacy	Y	N	N
OC15	M	2017	Cameron	Y	Y	Clot
OC16	F	2013	U	N	N	Serum
OC17	M	2013	U	N	N	Clot
OC18	F	2013	U	N	N	Clot*
OC19	M	2015	U	N	N	Clot
OC20	F	2015	U	N	N	Clot
OC21	F	2015	U	N	N	Clot
* PCR positive U= Unknown						

DISCUSSION

We discovered *T. cruzi* in two (9.5%) of 21 south Texas ocelots. The prevalence of Texas ocelots is similar to the prevalence (14.3%) in bobcats (*Lynx rufus*) of east central Texas as well as the prevalence (11.4%) in stray domestic cats of the RGV; both sympatric felid species.[15] In contrast, the prevalence (30%)[20] of *T. cruzi* in ocelots of Brazil was higher than what we

observed in south Texas, perhaps reflecting variation in habitat, different transmission dynamics, and use of fresher samples.

Parasite DTU 'TcI' was identified from one of the infected ocelots. TcI is the DTU that has been most commonly associated with human disease in the United States[26] and is the dominant DTU found in triatomines of south Texas.[18,26] Similarly, only TcI was observed in blood clot of ocelot populations of Brazil in the same area where TcII was observed in dogs (*Canis lupus familiaris*).[27] In Texas, strong host associations with DTUs have been observed in raccoons (TcIV)[15] and opossums (TcI)[28]. Expanded feline sampling across regions where multiple DTUs circulate would assist in deterineing the degree to which cats are associated with TcI or other DTUs.

Two cats showed lymphoplasmacytic inflammation in skeletal muscle. Lymphoplamacytic inflammation is commonly associated with chronic Chagas disease in humans and animals, [29,30] yet these two cats tested negative for *T. cruzi* using PCR methods. The observed inflammation could be attributed to other parasites noted in the population that may cause similar pathology, [12] or from *T. cruzi* infection at an undetectable level.

A limitation to this study is the potential degradation of DNA from samples that were collected from roadkill or have been conventionally frozen for many years. Degradation of DNA may result in false negative status of samples, and the prevalence estimates presented here should therefore be interpreted as conservative. Also, degradation of tissue will hinder histological assessment as seen in our PCR positive samples. An additional limitation of our study is that the

sampling approach relied on salvaged animals encountered by biologists such that cats would most likely need.to be out and active prior to death to facilitate their encounter. Cats with debilitating infections or severe pathology may therefore not be represented in our sample.

The south Texas ocelot population continues to decline as a result of habitat destruction and ocelot-vehicle collisions. The construction of roads through habitats and destruction of habitats for agriculture has created habitat fragmentation, which has been known to decrease biodiversity and increase the prevalence of diseases in wild mammals including *T. cruzi*.[4,31] Furthermore, plans to build a liquefied natural gas (LNG) terminal near the Laguna Atascosa Wildlife Refuge where ocelots live may disrupt important habitat. Additionally, the plans to build a border wall within the range of the Texas and Mexican ocelot populations could reduce transborder connectivity, affect genetic diversity, alter ecological communities and potentially result in extirpation.[32]

Further, habitat destruction also contributes to genetic erosion of the ocelot population thus increasing susceptibility to infectious diseases.[33] Some areas of private land may have higher densities of ocelots because of less habitat fragmentation but many of these properties are inaccessible to researchers. More so, studying the sparse populations of these elusive creatures in public and federal land is difficult. For this reason, scientific collections are essential in facilitating endangered species infectious disease research. Coupled with live animal surveillance and analysis of sympatric felid species, we may advance the understanding of parasitic disease in ocelots.

REFERENCES

1. de VILLA MEZA A, MARTINEZ MEYER E, LÓPEZ GONZÁLEZ CA. Ocelot (Leopardus pardalis) Food Habits in a Tropical Deciduous Forest of Jalisco, Mexico. *Am Midl Nat*. 2006. doi:10.1674/0003-0031(2002)148[0146:olpfhi]2.0.co;2
2. Paviolo, A., Crawshaw, P., Caso, A., de Oliveira, T., Lopez-Gonzalez, C.A., Kelly, M., De Angelo, C. & Payan E. Leopardus pardalis (errata version published in 2016). The IUCN Red List of Threatened Species 2015: e.T11509A97212355. http://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T11509A50653476.en. Published 2015.
3. Haines AM, Tewes ME, Laack LL, Horne JS, Young JH. A habitat-based population viability analysis for ocelots (Leopardus pardalis) in the United States. *Biol Conserv*. 2006. doi:10.1016/j.biocon.2006.04.035
4. Haines AM, Tewes ME, Laack LL, Grant WE, Young J. Evaluating recovery strategies for an ocelot (Leopardus pardalis) population in the united states. *Biol Conserv*. 2005. doi:10.1016/j.biocon.2005.06.032
5. Haines AM, Janecka JE, Tewes ME, Grassman LI, Morton P. The importance of private lands for ocelot Leopardus pardalis conservation in the United States. *ORYX*. 2006. doi:10.1017/S0030605306000044
6. Michael E, Kleberg C, Cl EI, Garrett P, Kp M, Carolina N. Ocelot management and conservation along transportation corridors in Southern Texas. *ICOWET*. 2001. doi:10.1080/110241500750008583
7. Laurenson K, Sillero-Zubiri C, Thompson H, Shiferaw F, Thirgood S, Malcolm J. Disease as a threat to endangered species: Ethiopian wolves, domestic dogs and canine pathogens.

Anim Conserv. 1998. doi:10.1017/S1367943098000250

8. Seimon TA, Miquelle DG, Chang TY, et al. Canine Distemper Virus: an Emerging Disease in Wild Endangered Amur Tigers (Panthera tigris altaica). *MBio*. 2013. doi:10.1128/mbio.00410-13

9. Millán J, Candela MG, Palomares F, et al. Disease threats to the endangered Iberian lynx (Lynx pardinus). *Vet J*. 2009. doi:10.1016/j.tvjl.2008.04.005

10. Pedersen AB, Jones KE, Nunn CL, Altizer S. Infectious diseases and extinction risk in wild mammals. *Conserv Biol*. 2007. doi:10.1111/j.1523-1739.2007.00776.x

11. HAINES AM, TEWES ME, LAACK LL. SURVIVAL AND SOURCES OF MORTALITY IN OCELOTS. *J Wildl Manage*. 2005. doi:10.2193/0022-541x(2005)069<0255:sasomi>2.0.co;2

12. Pence DB, Tewes ME, Laack LL. Helminths of the Ocelot from Southern Texas. *J Wildl Dis*. 2013. doi:10.7589/0090-3558-39.3.683

13. Rendón-Franco E, Caso-Aguilar A, Jimenéz-Sánchez NG, Hernandez-Jauregui DMB, Sandoval-Sánchez AL, Zepeda-López HM. Prevalence of Anti-Toxoplasma gondii Antibody in Free-ranging Ocelots (Leopardus pardalis) from Tamaulipas, Mexico. *J Wildl Dis*. 2013. doi:10.7589/0090-3558-48.3.829

14. Tenney TD, Curtis-Robles R, Snowden KF, Hamer SA. Shelter dogs as sentinels for Trypanosoma cruzi transmission across Texas. *Emerg Infect Dis*. 2014;20(8):1323-1326. doi:10.3201/eid2008.131843

15. Curtis-Robles R, Lewis BC, Hamer SA. High Trypanosoma cruzi infection prevalence associated with minimal cardiac pathology among wild carnivores in central Texas. *Int J Parasitol Parasites Wildl*. 2016;5(2):117-123. doi:10.1016/j.ijppaw.2016.04.001

16. Garcia MN, Aguilar D, Gorchakov R, et al. Case report: Evidence of autochthonous Chagas disease in southeastern Texas. *Am J Trop Med Hyg*. 2015;92(2):325-330. doi:10.4269/ajtmh.14-0238

17. Curtis-Robles R, Zecca IB, Roman-Cruz V, et al. Trypanosoma cruzi (Agent of Chagas Disease) in sympatric human and dog populations in "colonias" of the Lower Rio Grande Valley of Texas. *Am J Trop Med Hyg*. 2017;96(4):805-814. doi:10.4269/ajtmh.16-0789

18. Curtis-Robles R, Aukland LD, Snowden KF, Hamer GL, Hamer SA. Analysis of over 1500 triatomine vectors from across the US, predominantly Texas, for Trypanosoma cruzi infection and discrete typing units. *Infect Genet Evol*. 2018;58:171-180. doi:10.1016/j.meegid.2017.12.016

19. Rocha FL, Roque ALR, de Lima JS, et al. Trypanosoma cruzi infection in neotropical wild carnivores (Mammalia: Carnivora): at the top of the T. cruzi transmission chain. *PLoS One*. 2013;8(7):e67463. doi:10.1371/journal.pone.0067463

20. Herrera HM, Rocha FL, Lisboa C V., Rademaker V, Mourão GM, Jansen AM. Food web connections and the transmission cycles of Trypanosoma cruzi and Trypanosoma evansi (Kinetoplastida, Trypanosomatidae) in the Pantanal Region, Brazil. *Trans R Soc Trop Med Hyg*. 2011. doi:10.1016/j.trstmh.2011.04.008

21. Duffy T, Cura CI, Ramirez JC, et al. Analytical performance of a multiplex Real-Time PCR assay using TaqMan probes for quantification of Trypanosoma cruzi satellite DNA in blood samples. *PLoS Negl Trop Dis*. 2013;7(1):e2000. doi:10.1371/journal.pntd.0002000

22. Cura CI, Duffy T, Lucero RH, et al. Multiplex real-time PCR assay using TaqMan probes for the identification of Trypanosoma cruzi DTUs in biological and clinical samples. *PLoS*

Negl Trop Dis. 2015;9(5):e0003765. doi:10.1371/journal.pntd.0003765

23. Wincker P, Britto C, Pereira JB, Cardoso MA, Oelemann W, Morel CM. Use of a simplified polymerase chain reaction procedure to detect Trypanosoma cruzi in blood samples from chronic chagasic patients in a rural endemic area. *Am J Trop Med Hyg*. 1994;51(6):771-777.

24. Virreira M, Torrico F, Truyens C, et al. Comparison of polymerase chain reaction methods for reliable and easy detection of congenital Trypanosoma cruzi infection. *Am J Trop Med Hyg*. 2003;68(5):574-582. http://www.ncbi.nlm.nih.gov/pubmed/12812349.

25. Vera-Cruz JM, Magallon-Gastelum E, Grijalva G, Rincon AR, Ramos-Garcia C, Armendariz-Borunda J. Molecular diagnosis of Chagas' disease and use of an animal model to study parasite tropism. *Parasitol Res*. 2003;89(6):480-486. doi:10.1007/s00436-002-0787-0

26. Garcia MN, Burroughs H, Gorchakov R, et al. Molecular identification and genotyping of Trypanosoma cruzi DNA in autochthonous Chagas disease patients from Texas, USA. *Infect Genet Evol*. 2017;49:151-156. doi:10.1016/j.meegid.2017.01.016

27. Rocha FL, Roque ALR, Arrais RC, et al. Trypanosoma cruzi TcI and TcII transmission among wild carnivores, small mammals and dogs in a conservation unit and surrounding areas, Brazil. *Parasitology*. 2013. doi:10.1017/S0031182012001539

28. Hodo CL, Wilkerson GK, Birkner EC, Gray SB, Hamer SA. Trypanosoma cruzi Transmission Among Captive Nonhuman Primates, Wildlife, and Vectors. *Ecohealth*. 2018;15(2):426-436. doi:10.1007/s10393-018-1318-5

29. Santana RAG, Magalhães LKC, Magalhães LKC, et al. Trypanosoma cruzi strain TcI is associated with chronic Chagas disease in the Brazilian Amazon. *Parasites and Vectors*.

2014. doi:10.1186/1756-3305-7-267

30. Cassin Duz AL, Duz ALC, Vieira PMDA, et al. The TcI and TcII Trypanosoma cruzi experimental infections induce distinct immune responses and cardiac fibrosis in dogs. *Mem Inst Oswaldo Cruz*. 2014;109(8):1005-1013. doi:10.1590/0074-02760140208

31. Vaz VC, D'Andrea PS, Jansen AM. Effects of habitat fragmentation on wild mammal infection by Trypanosoma cruzi. *Parasitology*. 2007;134(12):1785-1793. doi:10.1017/S003118200700323X

32. Lasky JR, Jetz W, Keitt TH. Conservation biogeography of the US-Mexico border: A transcontinental risk assessment of barriers to animal dispersal. *Divers Distrib*. 2011. doi:10.1111/j.1472-4642.2011.00765.x

33. Janecka JE, Tewes ME, Laack L, Caso A, Grassman LI, Honeycutt RL. Loss of genetic diversity among ocelots in the United States during the 20th century linked to human induced population reductions. *PLoS One*. 2014;9(2):e89384-e89384. doi:10.1371/journal.pone.0089384

CHAPTER VII

SUMMARY

This dissertation work has resulted in identifying the prevalence of *Trypanosoma cruzi* among human, domestic animal, and wildlife populations of the Rio Grande Valley of Texas. The One Health approach allowed use transdisciplinary methods to assess the interconnection of populations affected by *T. cruzi.*

Working in RGV colonias through the aid of a promotora, a culturally competent community health worker, helped our research team establish critical relationships within the communities that allowed us to continuously work in the area. Using culturally competent education was crucial to work in the colonias and helped us recruit participants for our research. Our 2015 research in seven colonias discovered a low prevalence of *T. cruzi* infections in humans 0.4-1.3% (n=233) and 19.6-31.6% (n=209) in sympatric dog populations. One of the human cases identified in our study was a potential autochthonous case since the individual had no record of extensive travel history. We discovered that more urban colonias may be more at risk. Parasite DNA was detected in five dogs, indicating potential parasitemia. The seroprevalence and parasitemia observed in these dogs indicated that they may play a role in transmission cycle of *T. cruzi* in the colonia communities. Furthermore, through our education efforts, some colonia members learned to identify kissing bugs and submit them to our citizen science program. Finding infected humans, animals, and vectors within colonias shows that these at-risk border communities need additional aid and intervention measures to reduce vector burden and disease risk.

Concentrating on dog populations, we continued surveillance in 2016-2019 across different seasons. Using the same culturally competent approaches, we sampled 340 dogs from eight new RGV colonias. The overall prevalence was 32.4% (n=110) and prevalence among varying colonias ranged from 20-55.9%, indicating varying transmission dynamics among colonia communities. Parasite DNA was detected in ten dogs. Dogs sampled in fall seasons, dogs from three colonias (ER, CM2, SC), and older dogs were more likely to be seropositive. The high seroprevalence within dogs in these communities led us to believe that other urban dwelling animals, like opossums and cats, could contribute to the disease ecosystem.

In 2017, we worked with a high intake animal shelter in the RGV to opportunistically sample cats (n=167) and opossums (n=100) that were euthanized for reasons unrelated to our study. The animals that come into the shelter include animals that live in or around colonia communities. In opossums, we detected parasite DNA in at least one tissue in 15%. Of 100 opossums, parasite DNA was found in blood clot (9%), heart tissue (10%), and anal gland secretions (12%). A subset of opossums (n=43), showed infection in 16.3% of intercostal muscle and 11.6% of anal gland tissue. Our histological findings concluded that bilateral multifocal cardiac lymphoplasmacytic inflammation was more likely for opossums with a PCR positive sample. Opossums are abundant in the area and thrive in communities like colonias. The presence of potentially circulating DNA in 9% of the opossums indicate a higher risk of infection to vectors than observed in dogs of the same area.

Cats were less likely to be infective to vectors since no parasite DNA was detected in their blood. However, 19 cats (11.4%) were seropositive on at least two independent serological assays. Cats from rural environments and small cats were more likely to be seropositive. Further, parasite DNA was found in tissues (heart, bicep femoris muscle, sciatic nerve, mesentery, and esophagus) of three cats (1.8%) with lymphoplasmacytic inflammation, a characteristic consistent with *T. cruzi* infection, Identifying *T. cruzi* in opossum and cat populations indicates that both species may play a role in the ecoepidemiology of the parasite. However, the elevated prevalence of parasite DNA in opossums when compared to dogs and cats indicates that they may serve as more efficient reservoir hosts in the RGV.

The presence of *T. cruzi* in opossums indicates the potential infection of other wildlife. Although we do not fully understand how the parasite impacts the health of wildlife, endangered wildlife may be at risk. The fragile populations of ocelots in the RGV are affected by many human factors but the effects of disease burdens are unclear. Using ocelot tissues obtained through scientific collections (n=21), we were able to detect the presence of *T. cruzi* in tissues of two (9.5%) ocelots. We detected parasite in the skeletal muscle of one ocelot, but tissue autolysis hindered our histopathological assessment. Another ocelot had parasite DNA detected in blood clot, which was not observed in sympatric domestic feline populations. *T. cruzi* may impact the health outcomes of ocelots but further research is needed.

The research of this dissertation indicates the presence of *T. cruzi* across multiple populations of the RGV. Most importantly, we observed that the discrete taxonomic unit detected across all

study populations was unit ‘TcI’- the DTU previously implicated in human Chagas disease in the US. Through this research we hope to raise awareness and continue to educate communities at-risk. The One Health approach provides crucial theoretical and practical tools for addressing Chagas disease in people and animals in the US. Understanding the disease ecology of *T. cruzi* among human and animal populations will help us develop future culturally competent interventions.