

**STRAIN TYPING OF *TRYPANOSOMA CRUZI* IN THE SOUTHERN  
UNITED STATES**

An Undergraduate Research Scholars Thesis

by

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## ABSTRACT

Strain Typing of *Trypanosoma cruzi* in the Southern United States

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is endemic in Latin America and has also been documented in the United States. The disease has a wide range of symptoms, ranging from acute, cold-like symptoms, to chronic afflictions of the heart and gastrointestinal systems. While treatment options are available for humans, a low percentage of infected individuals can access and afford these treatments and no antiparasitic treatments are approved for dogs. *T. cruzi* has a large amount of genetic diversity. Seven strain types, TcI-TcVI and Tcbat, have been identified, with geographic distribution varying with the region. In South America north of the Amazon, TcI dominates, while TcV and TcVI dominate south of the Amazon. TcI and TcIV are the main strains of Central America and have also been previously identified in the Texas, yet there is little research attention on the strains in other regions of the southern United States. The goal of this project is to characterize the *T. cruzi* parasite genetic strain distribution across the southern US. The DNA from the gut tissue of the triatomine vector ('kissing bug'), collected through Texas A&M University's Citizen Science Program, was analyzed using PCR assay to identify the presence of *T. cruzi*. Then, a PCR assay with probes for the strain types was used to identify the strain type of positive samples. A geographic map of the

southern United States was created to illustrate the distribution of the strain types found in the sample. It was found that 54% of the sample (n=2129) was positive for *T. cruzi*. Of the sample size 1174 kissing bugs were tested for the strain type. Of this tested set, 41% were TcI, 36% were TcIV, 10% were co-infected with TcI and TcIV, and 13% were untypable. Co-infected bugs were only found in Texas, while TcI was localized to Texas and the southwest and TcIV was located in the southeast. There is evidence of a link between the various strains and their propensity to cause chronic symptoms, so it is critical to not just know where *T. cruzi* is cycling and infecting people and animals in the United States, but also to have a clear understanding of which strains are present where. The results of this study will help bridge the gap in knowledge on *T. cruzi* strain types and will help public health officials, doctors, and veterinarians better assess the risk of humans and animals in the southern United States.

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## NOMENCLATURE

DTUs	Discrete Typing Units
qPCR	Quantitative Polymerase Chain Reaction
Ct	Cycle Threshold
SL-IR	Spliced Leader Intergenic Region

# CHAPTER I

## INTRODUCTION

*Trypanosoma cruzi* is the causative agent for Chagas disease. *T. cruzi* has seven discrete typing units (DTUs), TcI-TcVI and TcBat, indicating a high amount of diversity within the parasite.<sup>1</sup> Prior research has indicated that TcII, TcV, and TcVI are primarily found in the domestic cycle, with TcIII and TcIV primarily found in the sylvatic cycle. TcI is found in both domestic and sylvatic cycles.<sup>2</sup> The phrase ‘strain type’ is interchangeable with DTU.

Chagas disease presents in two phases: acute and chronic. Acute symptoms include those of the common cold and flu, and so are not regularly attributed to Chagas disease. However, if untreated, Chagas disease can manifest a chronic phase with cardiac and intestinal impacts.<sup>3,4</sup> With its cardiac implications, Chagas disease is responsible for the most cases of infectious cardiomyopathy in the world.<sup>5</sup> Chagas disease can be latent in both the acute and chronic phase and approximately 30 percent of people with Chagas disease experience chronic symptoms.<sup>3,4</sup> Recent studies have concluded that specific strain types and the likelihood of developing chronic symptoms are linked, although it varies with regions. Additionally, some studies have found an association with the strain types and the category of chronic symptoms (cardiac vs. gastrointestinal).<sup>6</sup> Overall, Chagas disease has a significant impact on the Disability-Adjusted Life Years, due to the health problems and symptoms associated with Chagas disease.<sup>5</sup>

Transmission of *T. cruzi* can occur via four major methods: from the triatomine bug, from ingesting contaminated food, from a contaminated organ transplant or blood transfusion, or from an infected mother passing it to her baby. An individual, human or animal, can get infected with *T. cruzi* from the triatomine bug after exposure to infected feces or ingesting the infected bug.

Canine and other animals infected with *T. cruzi* have often eaten an infected triatomine. Treatment of the disease has been mainly limited to treatment of the acute phase and management of the chronic symptoms should they occur, yet less than one percent of infected individuals have the resources to get treatment.<sup>5</sup>

Chagas disease is endemic in Latin America and is present in the United States and is highly linked to poverty and urban migration.<sup>1,4</sup>

### **Chagas Disease in Latin America**

Seven to eight million people are infected with Chagas disease in Latin America.<sup>1</sup> Over 100 articles have been written about the distribution of the DTUs in Central and South America. While distribution in South America appears to vary by country, the general finding is that TcI is the major DTU north of the Amazon River and rain forest and TcV and TcVI predominate south of the Amazon. TcI and TcIV are the main strains in Central America.<sup>7</sup>

### **Chagas Disease in the United States**

In the United States, it is estimated that there are 300,000 to upwards of a million cases of Chagas disease.<sup>3</sup> However, relative to the amount of Chagas disease research in Central and South America, there is less research activity of *T. cruzi* transmission in the United States. So, the amount of knowledge on the distribution of the DTUs is limited. Like Central America, most research performed in the United States concludes that TcI and TcIV are the main DTUs of the Southern United States, yet more recent studies report that a wider DTU diversity and distribution may be present.<sup>8</sup> In addition to humans being diagnosed with Chagas disease in the southern United States, a significant number of canines are diagnosed with Chagas disease every year, with the number increasing.<sup>9,10</sup> Both acute and chronic canine Chagas disease has been widely reported in all the regions of Texas.<sup>10</sup>



## CHAPTER II

### METHODS

#### Specimen Collection

Specimens were collected through a citizen science program, in which submitters send one or more deceased triatomine bugs to the laboratory to be tested for *T. cruzi*.<sup>10</sup> Each individual specimen's sex, length, and species were documented. Following documentation, specimens were soaked with a 50% bleach solution and rinsed with distilled water to prevent outside DNA from contaminating the specimens. The last segment of the abdomen was removed with forceps. Then, the convexium of each bug was cut open with dissection scissors and the dorsal abdominal wall was removed. Hindgut tissue is where the *T. cruzi* parasite resides in the triatomine vector, so hindgut tissue was scraped out of the abdomen with a pair of forceps and placed into a tube with the last segment of the abdomen.<sup>9,11</sup> All instruments used were sterile. DNA from the hindgut tissue was extracted using the KingFisher Cell and Tissue Kit (Thermo Fisher, Waltham, MA).

#### Determination of the Presence of *Trypanosoma cruzi*

DNA samples were then run through quantitative polymerase chain reaction (qPCR) to identify the presence *T. cruzi* DNA. Each qPCR utilized Cruzi 1/Cruzi 2 primers with a Cruzi 3 probe to augment and identify the 166 base-pair target nuclear satellite gene. Every plate included a positive and negative *T. cruzi* control. Molecular grade water was the negative control, while *T. cruzi* Sylvio X10 CL4 (American Type Culture Collection, Manassas, VA) was the positive control. Cycle threshold (Ct) values that were less than 35 were considered positive for *T. cruzi* infection, while samples with Ct values of greater than 35 were identified as

negative.<sup>11</sup> A sample was considered borderline if the Ct value was between 33 and 35. PCR protocols utilized in this project follow the previously established protocols.<sup>9, 12, 13</sup>

### **Determination of DTUs**

Samples that were positive for *T. cruzi* were then run through a spliced leader intergenic region (SL-IR) qPCR. Borderline samples were also run through the SL-IR qPCR. Probes for each strain were utilized for identification of DTUs. Positive and negative controls were used for each plate. The negative control for all plates was molecular grade water. The positive controls were *T. cruzi* Slyvio X10 CL4 (American Type Culture Collection, Manassas, VA) for TcI, DNA from a *T. cruzi* positive *Triatoma sanguisuga* submitted through the citizen science program for TcIV, and *T. cruzi* Y (American Type Culture Collection, Manassas, VA) for TcII.<sup>9, 11</sup> If a sample was negative in the SL-IR qPCR, but had been positive in the Cruzi 123 qPCR, the sample was run through another SL-IR qPCR using a 1:10 dilution. For the dilution, one microliter of kissing bug DNA was added to nine microliters of molecular grade water. PCR protocols utilized in this project follow the ones described previously.<sup>9, 14</sup>

## CHAPTER III

### RESULTS

#### **Geographic Location of Bugs**

Kissing bugs sent in from the citizen science program were located from California to West Virginia. A total of 2129 kissing bugs were used for creating the geographical map and for data analysis. 432 of these bugs were from non-Texas locations, while the remaining 1697 bugs came from Texas counties. These kissing bugs were all a part of the citizen science program and were submitted between January 2014 to February 2019. All out-of-state and Texas records from this time period were included in this sample set, as long as the submission included an address or location. *Triatoma sanguisuga* was the most widespread species across the sample and *Triatoma gerstaeckeri* was found only in Texas (Table 1 (Appendix A)). Geographically, more bugs were found in Texas and the southwest.

#### **Bugs Positive for *Trypanosoma cruzi***

1149 (54% of sample size) kissing bugs tested positive with qPCR for *T. cruzi* (Figure 1). Indiana, Louisiana, Kansas, and Texas had at or above 50% of the submitted bugs positive for *T. cruzi*. Higher infection prevalence was found in Texas and the southwest. Within the sample size, 57.5% of male kissing bugs were positive for *T. cruzi*, while 54.4% of female kissing bugs were positive for *T. cruzi*. The variation in infection prevalence across the different species of kissing bugs, ranged from *Triatoma gerstaeckeri* and *Triatoma lecticularia* (61.1%) to *T. rubida* (16.5%) (Table 2).

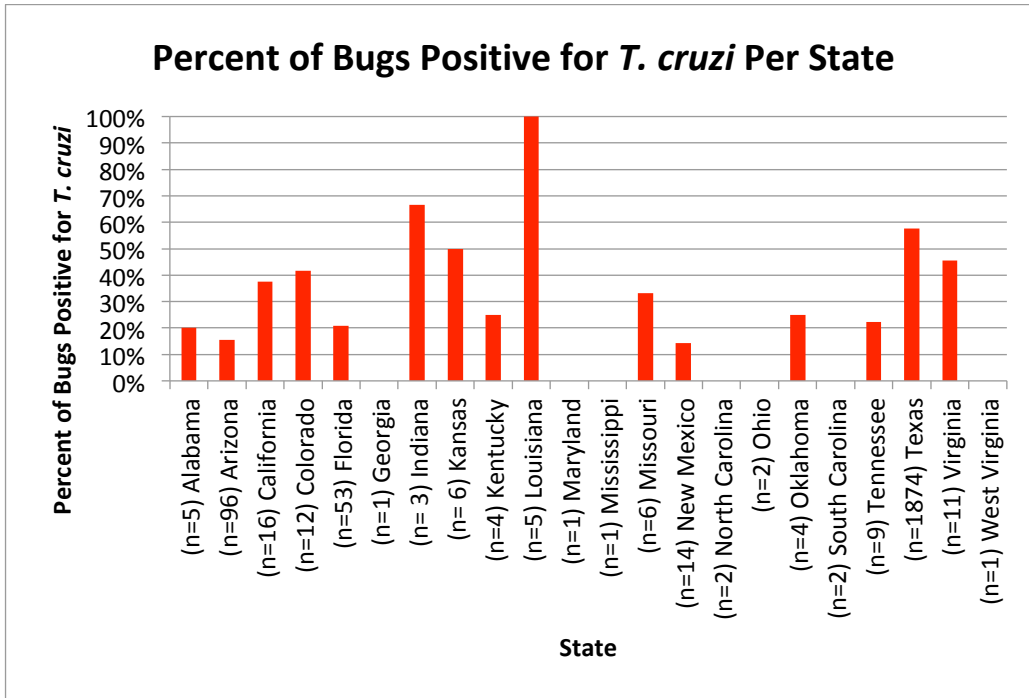


Figure 1. Graph depicting infection prevalence in each state

Table 2. Table depicting infection prevalence for sex and species of adult

Variable	Sample Size (n)	Infection Prevalence
Sex		
Female	1197	54.4%
Male	771	57.5%
Unknown	161	29.8%
Species of Adult		
<i>T. gerstaeckeri</i>	1185	61.1%
<i>T. protracta</i>	36	25.0%
<i>T. indictiva</i>	97	54.6%
<i>T. lecticularia</i>	90	61.1%
Species of Adult		
<i>T. rubida</i>	97	16.5%
<i>T. neotomae</i>	1	100%
<i>T. sanguisuga</i>	490	50.6%
Unknown	133	38.5%

### Strain Types

Of the total sample size, 1174 kissing bugs were strain typed with qPCR (positive and borderline samples). 481 (41%) of the tested kissing bugs were identified as having DTU TcI. 420 (36%) of the tested kissing bugs were identified as having DTU TcIV. 118 (10%) of the tested kissing bugs were found to be co-infected with strain types TcI and TcIV. 155 (13%) of the tested kissing bugs were untypable (Figure 2). TcIV was located primarily in the southeast, while TcI was almost exclusively found in Texas and the southwest. Texas had the only samples that were coinfecting with TcI and TcIV.

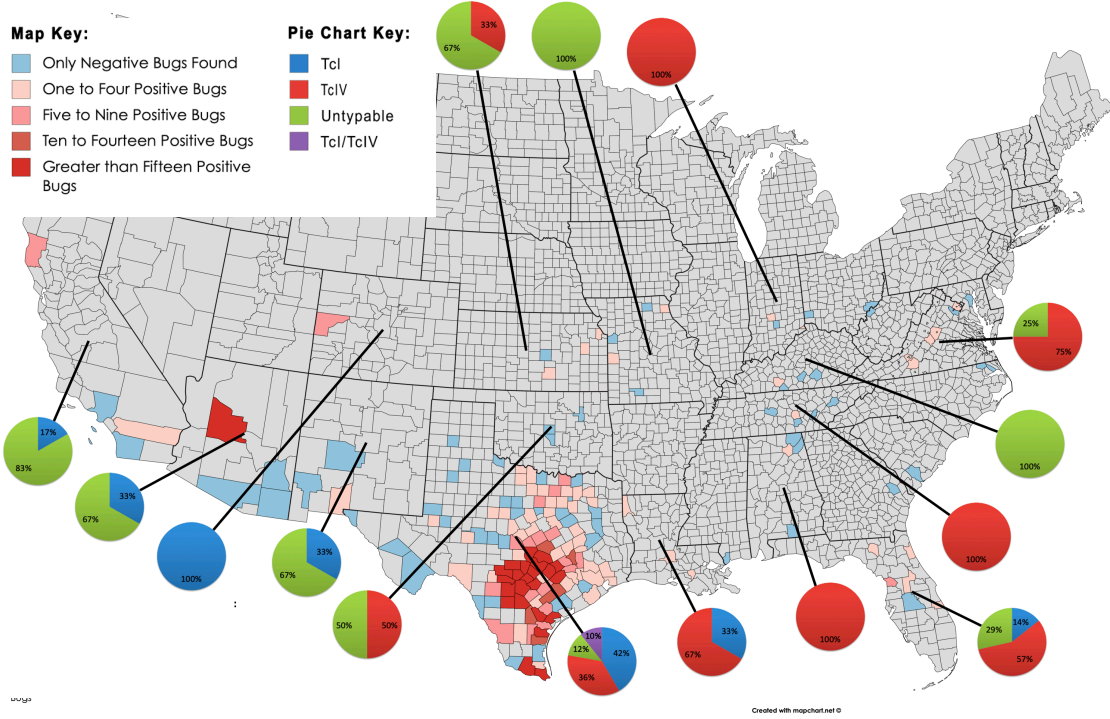


Figure 2. Geographic map of kissing bug distribution and infection prevalence. Pie charts were created to show the breakdown of *T. cruzi* strain types in states with bugs positive for *T. cruzi*. Map made using mapchart.net.

## CHAPTER IV

### DISCUSSION

This project focused on the analysis of kissing bugs sent in from southern states through the citizen science program. I tested each out-of-state kissing bug sent in during August 2018 to February 2019 for the presence of *T. cruzi* and then ran each kissing bug that was positive for *T. cruzi* through a qPCR with probes to determine the DTU. I also tested out-of-state bugs that had been stored and untested when they were received originally. I incorporated data from the existing Texas and kissing bug database for the Hamer Lab after analyzing the non-Texas kissing bugs. This database also included out-of-state bugs that had previously been dissected, tested for *T. cruzi*, and strain typed. 54% of the sample size (n=2129) was found to be positive for the parasite. Of this set of positive samples, 41% were DTU TcI, while 36% were DTU TcIV (n=1174).

Studies that have focused on Louisiana and Texas have reported similar values of infection prevalence for the sample size of the individual studies.<sup>9,16</sup> The incidence of increased infection prevalence in Texas and the southwest states could be attributed to parasite-vector interactions. It is possible that the *Triatoma* species interact with the *T. cruzi* parasite in different ways. Perhaps, the triatomine vectors that reside in the southwest and Texas have a more favorable relationship with the parasite than the other triatomine species. Other factors that could be considered are geographic distribution of hosts, feeding activities of the differing *Triatoma* species, and parasite-vector-host interactions. Previous studies have linked raccoons and opossums with *Triatoma sanguisuga* and woodrats with *Triatoma gerstaeckeri*.<sup>17</sup> The southern tip of Texas, a scrub vegetation and semi-arid climate dominated region, has been found to be

primarily associated with the woodrat. In the southeastern United States, the ecoregion is typically comprised of woodland and forest vegetation and linked with raccoons. Southwest Texas has been found to be occupied by both woodrats and raccoons.<sup>17</sup> Further studies should be conducted to better understand the distribution of hosts and their link to the different *Triatoma* species.

Comparable to previous studies, DTUs TcI and TcIV were the only DTUs found in my studies.<sup>9,16,17</sup> TcI has been found to be associated with opossums, while TcIV has been associated with raccoons.<sup>17</sup> Both DTUs have been linked to woodrats.<sup>9</sup> This distribution could be attributed to host and species distribution. Host and species interaction with canines and humans could also be driving factors in creating this geographic distribution of the DTUs. When considering host and species interactions with canines and humans, the migration patterns and location in comparison to where humans and canines live could also have a role in the DTUs found in specific regions.

This study can be used to further what is known about *T. cruzi* and to assist in the establishment of risk for people and canines contracting Chagas disease. Determination of DTU location is becoming increasingly important as studies uncover the role that DTUs play in infecting people and causing certain symptoms.<sup>6</sup> Further studies should be conducted to better understand the association between host and vector, vector-host-parasite interactions and correlation to DTUs, and localization of the different species implicated as hosts for *T. cruzi* in comparison to the habitats of canines and humans.

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## APPENDIX A

### DISTRIBUTION OF *TRIATOMA* VECTOR SPECIES

Table 1. Table depicting percentage of species found per state

State	Total Number of Bugs	<i>T. gerstaeckeri</i> (%)	<i>T. protracta</i> (%)	<i>T. indictiva</i> (%)	<i>T. leticularia</i> (%)	<i>T. rubida</i> (%)	<i>T. neotomae</i> (%)	<i>T. sanguisuga</i> (%)	Unk-nown (%)
Alabama	5	0	0	0	0	0	0	4 (80%)	1 (20%)
Arizona	96	0	13 (13.5%)	0	0	80 (83.3%)	0	0	3 (3.1%)
California	16	0	16 (100%)	0	0	0	0	0	0
Colorado	12	0	5 (41.7%)	0	0	0	0	0	7 (58.3%)
Florida	53	0	0	0	0	0	0	53 (100%)	0
Georgia	1	0	0	0	0	0	0	1 (100%)	0
Indiana	3	0	0	0	0	0	0	3 (100%)	0
Kansas	6	0	0	0	0	0	0	5 (83.3%)	1 (16.7%)
Kentucky	4	0	0	0	0	0	0	3 (75%)	1 (25%)

Table 1. Continued

State	Total Number of Bugs	<i>T.</i> <i>gerstaeckeri</i> (%)	<i>T.</i> <i>protracta</i> (%)	<i>T.</i> <i>indictiva</i> (%)	<i>T. leticularia</i> (%)	<i>T. rubida</i> (%)	<i>T.</i> <i>neotomae</i> (%)	<i>T.</i> <i>sanguisuga</i> (%)	Unk- nown (%)
Maryland	1	0	0	0	0	0	0	0	1 (100%)
Mississippi	1	0	0	0	0	0	0	1 (100%)	0
Missouri	6	0	0	0	0	0	0	6 (100%)	0
New Mexico	14	0	0	0	0	11 (78.6%)	0	1 (7.1%)	2 (14.3%)
North Carolina	2	0	0	0	0	0	0	2 (100%)	0
Ohio	2	0	0	0	0	0	0	2 (100%)	0
Oklahoma	4	0	0	0	0	0	0	3 (75%)	1 (25%)
South Carolina	2	0	0	0	0	0	0	1 (50%)	1 (50%)
Tennessee	9	0	0	0	0	0	0	5 (55.6%)	4 (44.4%)
Texas	1874	1184 (63.2%)	1 (0%)	97 (5.2%)	90 (4.8%)	6 (0.3%)	1 (0%)	385 (20.5%)	110 (5.9%)
Virginia	11	0	0	0	0	0	0	10 (90.9%)	1 (9.1%)
West Virginia	1	0	0	0	0	0	0	1 (100%)	0