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Infection rates and distribution of Trypanosoma cruzi in triatomine insects from several public parks of Starr, Hidalgo and Cameron Counties

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INFECTION RATES AND DISTRIBUTION OF *Trypanosoma cruzi* IN TRIATOMINE INSECTS FROM SEVERAL PUBLIC PARKS OF STARR, HIDALGO AND CAMERON COUNTIES

A Thesis

by

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ABSTRACT

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Chagas disease is caused by the protozoan *Trypanosoma cruzi* and is a major public health concern in many areas of the world, including the United States. The disease is transmitted by insect vectors known as kissing bugs from the subfamily *Triatominae*. While the majority of studies focus on domestic and peri-domestic collections, this study collected insect vectors from state parks in the Lower Rio Grande Valley. PCR analysis was done to obtain infection rates for collected insects, and a morphological examination was done to check insects for gender. In total 18 insects were captured with 12 of the captured insects being female, and 6 being male. A total infection rate of 67% was seen. Our results reason that the prevention of insect vectors at both the sylvatic level and domestic level may be more effective in stopping the spread of Chagas disease than prevention at the domestic level alone.

DEDICATION

The completion of my masters studies would not have been possible without the love and support of my family. My father, Carlos Guzman, my mother, Lorena Barrera, my brother, Alex Guzman, my sister Lorissa Guzman, wholeheartedly inspired, motivated and supported me by all means to accomplish this degree. Thank you for your love and patience.

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Thirdly, I would like to thank my parents and family for their support, both financially and emotionally. I would also like to thank Stephanie Gene Pedraza for her ability to keep me focused on my goal, and her unwavering support.

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CHAPTER I

INTRODUCTION

Chagas Disease, also commonly referred to as American trypanosomiasis, is a tropical parasitic disease that infects an estimated 16 – 18 million people and results in 45,000 deaths annually worldwide [1]. In the United States it is approximated that there is nearly 1 million infections with over 250,000 of them being in Texas [2]. The disease is the result of a hemoflagellate protozoan, known as *Trypanosoma cruzi* spread by insect vectors known as triatomine or kissing bugs, which feed on blood of human and animal hosts. Triatomine insects have a life-cycle that ranges from three to four months to up to two years, and live in protected environments near or inside their host's shelter, which can range from domestic and sylvatic animals to humans [3]. The most common disease cycle consists of the *T. cruzi* parasite being transmitted from vector to host through the feces of infected bugs containing metacyclic trypomastigotes that enter the body through the bite wound or mucous membranes [4] though there have been reported cases of congential, blood transfusion, organ transfusion, and oral transmission [5–7].

The geographic distribution of *T. cruzi* and its insect vectors is historically diverse throughout the tropical and subtropical regions of South and North America, and can be found approximately between the 42° and 46° latitudes [8,9]. However because of climate change there have been some shifts in the geographic distribution of many triatomine species that has allowed these vectors to push their northern latitudes [10].

In the United States, there are currently 11 recorded *Triatoma* insect vectors that have been reported to transmit *T. cruzi* to a variety of mammalian hosts [11]. The distribution of triatomine vectors is almost exclusive to the southern half of the United States [12] due to a variety of reasons including patterns of human migration into Texas from endemic regions of Latin America [1].

In Texas, there are currently 7 recorded *Triatoma* insect vectors reported: *Triatoma gerstaeckeri, T. sanguisuga, T. leticularia, T. protracta, T. indictiva, T. rubida,* and *T. neotomae* [13]. Of the 254 counties in Texas, 82 of them have recorded collections of infected vectors or hosts with the large majority of these counties being located in the southern half [1].

Spatial relative risk of Chagas disease cycle establishment was assessed in Texas using a five-stage analysis that included ecological risk using maximum entropy, incidence-based relative risk using the Bayesian Besag Tolk-Mollie model, and a variety of ecological risk and incidence based risk analysis and revealed that counties at highest risk for infection were those in South Texas and a few counties with very large populations in the north, east, and central parts of Texas. Results showed that Chagas disease risk is highly concentrated in south Texas though not necessarily constrained to it [1].

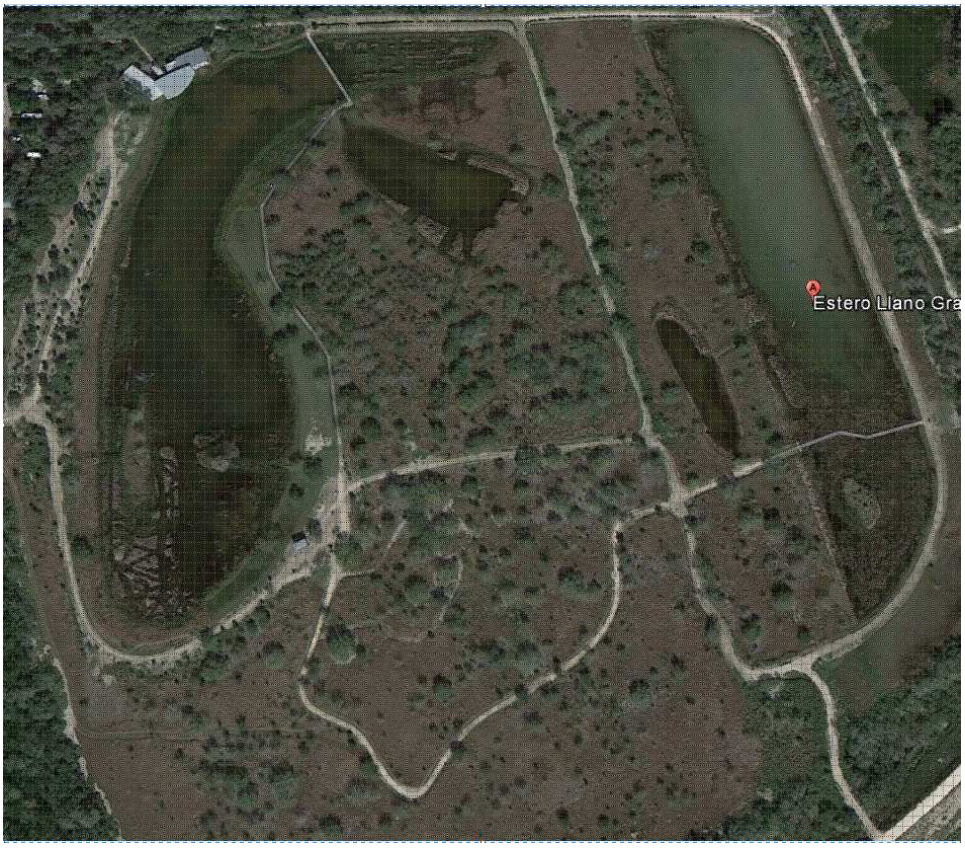
The LRGV is a group of four counties that include: Starr County, Hidalgo County, Willacy County, and Cameron County with a population of approximately 1,300,000 (2012 Census Estimates). Previous studies have shown that Chagas disease exists in the Texas Lower Rio Grande Valley [13] with several of these studies having infection rates of approximately 50% [14,15]. In 2013, a study on *Neotoma micropus* was done; 104 species were collected and tested for *T. cruzi* infection and 50 were found to be infected for a 48% infection rate. Yet today, there is a

lack of information on the distribution of triatomine vectors in parks of the Lower Rio Grande Valley, and there is no data that pertains to the infection rates of insect vectors found in sylvatic park environments in Texas. This is because the large majority of studies conducted focus on the collection of insect vectors in and around areas that are frequented by humans in domestic and peri-domestic areas [2,4,5,12,12,13,15–20]. Because of this it is possible to assume that there may be some differences in infection rates in insects that live in this habitat as opposed to insects that live in sylvatic habitats. The most commonly occurring *Triatoma* species in Texas is *T. gerstaeckeri* [9] and by large this insect vector is considered to be a sylvatic species [12] but are known to colonize and invade homes in what are commonly referred to as chagasic towns in the southern United States[21]. With no vaccine existing for Chagas disease much of the prevention for this disease has come in the form of researching the vector and reducing the number of insect vectors available to transmit the disease into humans [8]. Because most triatomine species are actually born in the sylvatic environment and over time begin to move and colonize domestic areas it is of extreme importance to determine whether insect vectors that colonize domestic areas are already infected with *T. cruzi* prior to domesticated colonization. It is also just as important to determine how much emphasis should be placed on vector control in these sylvatic environments instead of waiting for vectors to inhabit human areas before acting. As the prevalence of Chagas disease continues to rise[4,36] it is absolutely crucial that these vectors be monitored continuously. Therefore my research questions were 1) what are the distributions of triatomine insects in different parks of the Lower Rio Grande Valley and 2) what is the infection rate of the insects collected from these parks and does infection rate differ significantly between males vs females.

CHAPTER II

METHODS

Collections were taken from four parks in the Lower Rio Grande Valley and include Estero Llano State Park, Falcon State Park, Bensten State Park and Boca Chica State Park. Collection sites were chosen at random via simple random sampling that used a grid system and a random number generator. The grid system was created using photoshop and google earth, taking an overhead picture of the park and creating gridlines and then assigning a number to each individual square. An example of the map used for Estero Llano State Park is shown below:



A random function was then used in Excel in order to choose three collection sites per park. Each of the four parks were visited three times during a 6 month period from August to January. Each collection visit lasted 5 hours from approximately 7PM to 12AM.

Once a collection site was chosen, setup of light traps began at approximately 6:30. Collection traps were made using a white canvas sheet held up by metallic camping poles and weighed down by batteries to keep the sheet from moving throughout the visit. At the base of the white canvas sheet a single large block of dry ice was placed to simulate carbon dioxide release of hosts. At the top of the white canvas sheet two fluorescent lights were placed. Insects were collected using gloved hands and carefully placed inside sealed containers. These containers were then brought back to the research lab and placed in a -20 Celsius freezer the same night they were caught to avoid any possible problems related to DNA degeneration or contamination[22].

DNA extraction from triatomines was done after samples had been stored overnight in -20 Celsius. Samples were transferred to a disposable Petri dish (at this point insects were checked for morphological characteristics to determine sex) and had the posterior third of their abdomen removed using a disposable scalpel blade and transfer pipet. Once removed the abdomen was transferred into a 2.0 ml safe-lock tube and 200 ul of AL buffer from Qiagen DNeasy Blood & Tissue Kit was added. A disposable plastic pestle was used to crush the abdomen for three minutes. Once crushed all tubes containing samples were span down briefly, and 180 ul of ATL buffer along with 20 ul of proteinase K were added into the tube, samples were then vortexed for several seconds[23]. Samples were then incubated at 56°C for fifteen minutes (or more) and upon finishing were spun down for 1 minute. 300ul of supernatant were collected and transferred to a 1.5 ml tube where 150 ul of 100% ethanol was added, vortexed and

then spun down for several seconds[23]. The sample was then loaded onto a column collection tube and spun down for 2 minutes and then transferred onto a new collection tube followed by 500 ul of AW1 buffer, and again spun down for 2 minutes. After spinning, the sample was transferred to a second new collection tube and followed by 500 ul of AW2 buffer and allowed to sit at room temperature for 5 minutes. After 5 minutes, the samples were spun down for 4 minutes. Afterwards the column was transferred to a new 1.5 ml tube, and 200ul of AE buffer were added. The tube and AE buffer were allowed to sit at 1 minute at room temperature then spun down for 2 minutes, the DNA solution was then vortexed and spun down again for several seconds and then stored frozen at -20°C[23].

Once DNA has been extracted from triatomines we then began testing for *T. cruzi* detection via PCR and agarose gel analysis. TCZ1 (5' - cgagctcttgcccacacgggtgct – 3') and TCZ2 (5' – cctccaagcagcggatagttcagg – 3') primers were used directly from JC Pizarro, DE Lucero, and L Stevens 2007 paper [22,24–29].

Promega PCR components were thawed and kept inside ice, this included all primers, dNTPs, samples, molecular grade water, and buffers, but did not include our Phusion Hot Start II DNA polymerase which was kept at -20°C until needed[23]. Once PCR components were thawed, a master mix for (2xN+1) 25 ul reactions was made where N is the number of samples to be tested[23]. The master mix composition included: 17.75 ul H_2O, 5 ul 5x HF buffer, 0.5 ul 10 mM dNTP, 0.25 ul 100 uM forward primer, 0.25 ul 100 uM reverse primer, 1 ul DNA sample, and 0.25 ul Phusion Hot Start II DNA polymerase per sample to be run. After master mix was created, we aliquoted 24 ul of master mix into PCR stripes, and added 1 ul of DNA sample. For *T. cruzi* detection we took a true positive control DNA sample from colleagues at the Baylor School of Tropical Medicine, and for a negative control we used H_2O. Once DNA samples and

controls were loaded onto all PCR stripes we loaded the stripes onto the thermocycler and ran a PCR with the following conditions:

98° 30s
98° 10s |
64° 20s | 35 cycles
72° 20s |
72° 7 min

While samples were being run in thermocycler, a 1% agarose gel was prepared in TAE buffer. 5 ul of EZ-Vision One buffer was added into the agarose gel prep flask before pouring. Once sample PCRs were completed, samples were then loaded onto the gel flanking with DNA marker ladder and run at 5-7 V/cm in TAE buffer[23]. Upon gel electrophoresis completion the gel was taken and visualized under a UV gel documentation system and pictures were taken to record positives and negatives. TCZ1 – TCZ2 primers produced a 188 bp band that would indicate *T. cruzi* positive samples[23].

To determine gender the abdomen of insects were looked at [30]. Insects were taken out of collection containers and deposited on a petri dish where they were then manipulated onto their back sides so appropriate gender identification could be made. Males have a roughly ovoid abdomen while females have a pointed behind [31].

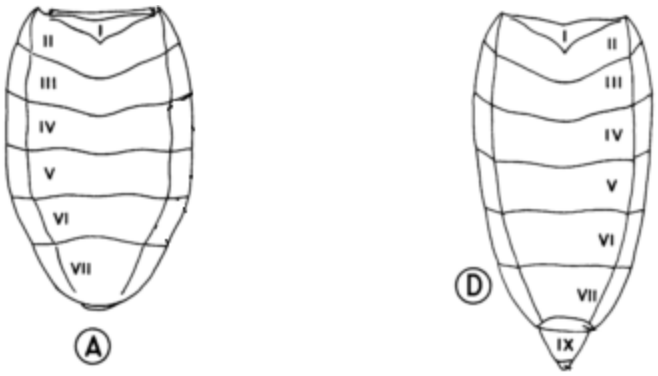


Figure 1. Images above borrowed from Lent & Wygodzinsky, 1979 paper[30]. Figure A represents abdomen of male, dorsal view. Figure D represents abdomen of female, dorsal view.

CHAPTER III

RESULTS

Parks	County	Dates Collected	Number of Insects Collected	Females	Males
Estero Llano State Park	Hidalgo	8/15/2014	1	6	2
		9/15/2014	4		
		10/15/2014	3		
Bentsen State Park	Hidalgo	8/25/2014	3	3	3
		9/25/2014	1		
		1/25/2015	2		
Falcon State Park	Starr	9/5/2014	0	3	1
		10/5/2014	3		
		11/5/2014	1		
Boca Chica State Park	Cameron	11/20/2014	0	0	0
		12/20/2014	0		
		1/3/2015	0		

Table 1: Table containing collection information regarding days trips were taken and the number of insects collected at each trip. Gender of insects collected from each site is also given.

Parks were visited a total of three times each over a six month period. A total of 18 triatomine insects were captured in four different locations (though one location had zero captures) using a dry-ice sheet collection trap explained in the methods. Figure 2 shows the distribution of collected samples and distance between parks used as collection sites.

Figure 2. All collection sites (indicated by red boxes) where species were collected. Yellow pins indicate multiple insects and numbers represent the ID of the insect that was collected there. Graph was created using google earth and ArcMap. Longtitude and Latitude data was taken from a database and incorporated into ArcMap and a KML file was then converted and applied to google earth.

A total of 12 insects tested positive for *T. cruzi*. The infection rate was 67% which is in line with the percentages that colleagues at Baylor School of Tropical Medicine were getting.

Figure 3 is a copy of the pictures taken using our gel documentation system for *T. cruzi* detection. This is an image of a gel staining after electrophoresis has been run; the bright bands characterize samples that are positive for *T. cruzi* infection. Some bands are considered to be 'faint' but are still taken as positives. Figure 4 depicts infection rate and number of positive samples vs negative samples in graph form. We collected 8 insects at Estero Llano State Park, 4 insects at Falcon State Park, and 6 insects at Bentsen State Park. Of the 8 insects collected at Estero Llano 6 were positive for *T. cruzi* infection, of the 4 insects collected at Falcon State Park 2 were positive *T. cruzi* infection, and of the 6 insects collected at Bentsen State Park 4 were positive *T. cruzi* infection.

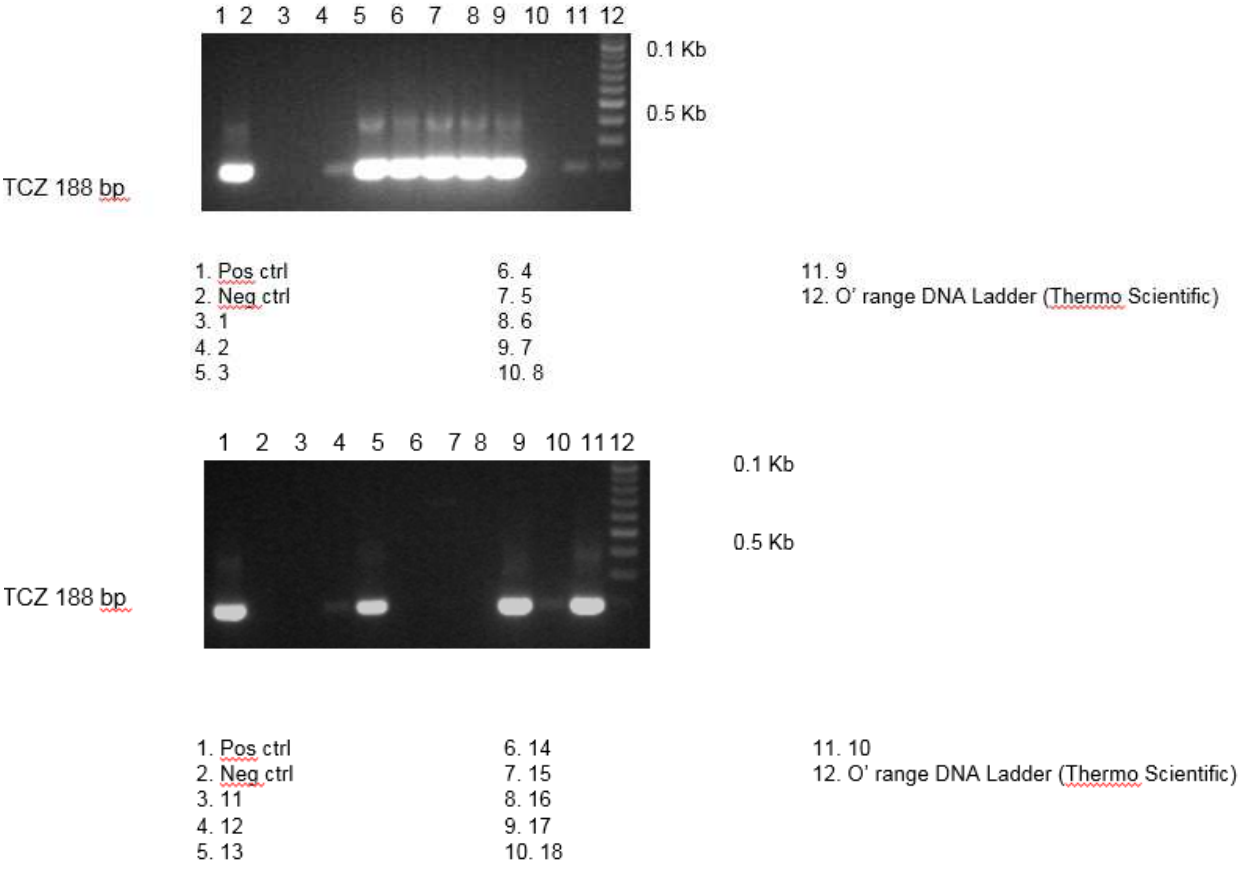


Figure 3. Gel electrophoresis pictures using a gel documentation camera. The bright bands represent samples that are positive for *T. cruzi* presence. Positive control used DNA from an insect sample previously verified as positive at Baylor Tropical School of Medicine, and the

negative control used water. Samples 2,9, 12, and 18 are considered 'faint' but still positive. PCR's and gel's were run multiple times to confirm results.

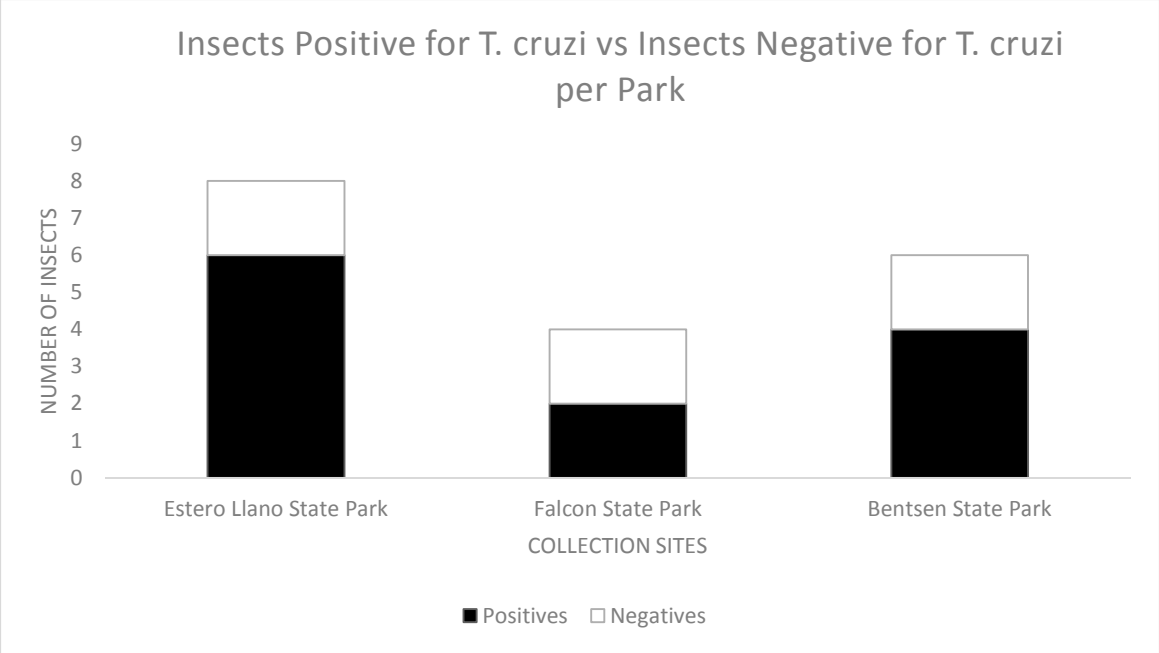


Figure 4. Bar graph that shows the number of insects positive for *T. cruzi* vs the number of insects negative for *T. cruzi*. Boca Chica State Park is not shown because no samples were collected.

Using morphological characteristics we determined the sex of each insect. Out of 18 captured insects 12 of them were female and 6 of them were male. With such a small sample size it is not possible to say with any degree of accuracy that females are the larger population of triatoma insects, or that they are more active than males.

Parks	County	Positive	Negative	Females	Males
Estero Llano State Park	Hidalgo	6	2	6	2
Bentsen State Park	Hidalgo	4	2	3	3
Falcon State Park	Starr	2	2	3	1

Boca Chica State Park	Cameron	0	0	0	0

Table 2: Table containing the number of positive infected vectors per park as well as the number of females and males collected.

We decided to take a look at *T. cruzi* infection status by gender in total. Figure 5 depicts *T. cruzi* infection status for male and females. Of the 12 female insects collected, 9 of them came back positive for an infection rate of 75%. Of the 6 male insects collected, 3 of them came back positive for an infection rate of 50%.

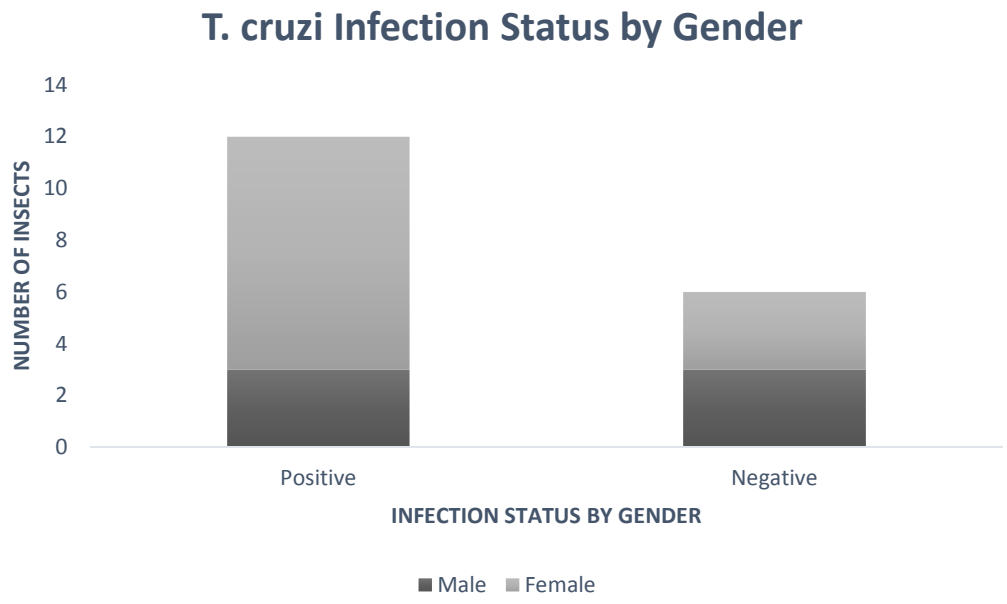


Figure 5. Bar graph depicting *T. cruzi* infection status by gender.

To continue consistency a bar graph that depicts *T. cruzi* infection status by gender per park shown on figure 5. In Estero Llano, there were two captured males and 6 captured females. One male tested positive, and 5 females tested positive for a 50% and 83% infection rate respectively. In Falcon State Park, there was 1 captured male and 3 captured female insects; 1

male was infected, and 1 female was infected for an infection rate of 100% in males and 33% in females. In Bentsen State Park, 3 males were captured, and 3 females were captured. 1 male tested positive for *T. cruzi* infection and 3 females tested positive for infection rates of 33% and 100% respectively.

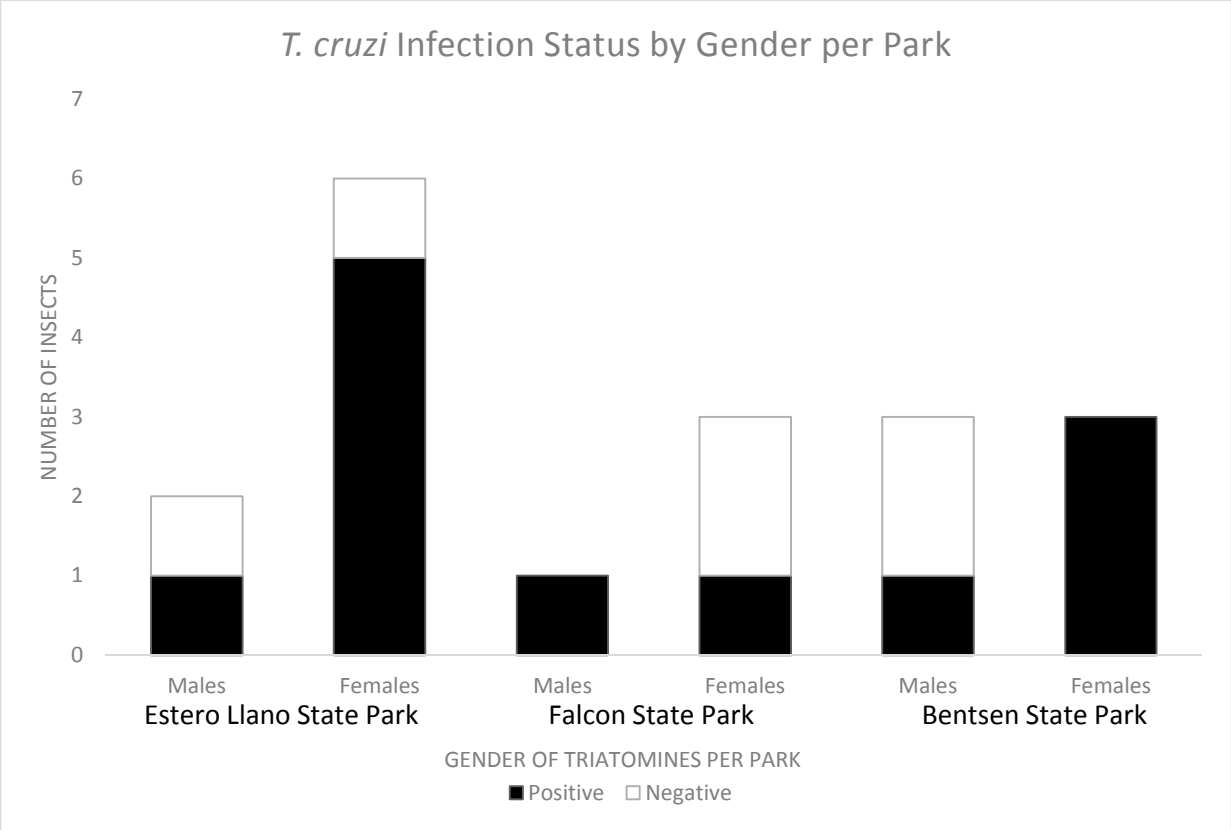


Figure 6. Bar graphc depicting *T. cruzi* infection in males vs females in different parks.

CHAPTER IV

DISCUSSION

A decrease in the frequency of Chagas disease entails the constant update and monitoring of the insect vector to expand information available for future risk assessments and introduction of appropriate prevention strategies. Our results show that infected insect vectors can be found distributed in parks of the Lower Rio Grande Valley. Three of the four parks visited had insect captures of similar numbers excluding Boca Chica State Park where zero insects were captured. There is no reason to believe that the insect vector is not found at this park as previous literature has collected *T. cruzi* positive blood donations from the nearby area of Brownsville, TX[32].

The insect's absence may be explained by many reasons but one can be seen by looking at the park's vegetation. Boca Chica State Park is a sandy peninsula with little vegetation other than small patches of black mangroves and no facilities available to visitors. Black mangroves are known for their use as shelter by some species of birds and many species of crustaceans and fish, but not for triatomine insects. The difference in vegetation here from other collection sites is quickly noticeable, small patches of mangroves are found cluttered among the sand dunes and in some areas are very separated from one another as opposed to the vegetation of the other three collection sites that all contained very high grasses, many trees, and large amounts of fallen tree carcasses.

In 2009, a study of 33 insects captured in rural parts of Texas found an infection rate of only 8%[9] which would indicate that the majority of vectors obtained the parasite only after inhabiting domestic areas. We have found a high infection rate of *T. cruzi* collected in state parks (67%) which is slightly higher than the reported infection rates in domestic and peri-domestic studies[9,14,15,24,27] and interesting for reported infection rates of sylvatic insects. Previously it made sense that most prevention control strategies for Chagas disease are focused at the domestic level, because data regarding infection rates of sylvatic insects led to the assumption that *T. cruzi* infection occurred once a vector established itself in a domestic habitat. However, as previously mentioned many *Triatoma* species are sylvatic by nature[33] and only later on in life begin to inhabit homes, and other domestic areas. Our results argue that while spraying homes with insecticides may help reduce the immediate risk of Chagas disease related infection in humans, it does not control or limit the growth of *T. cruzi* infection in other mammalian hosts or offer any long term solution to the rise in Chagas disease related incidents. This continued rise can be clearly seen in domesticated animals such as dogs, as the number of infected animal cases continues to rise from approximately 100 cases in 2000 to over 500 in 2006 as shown in one study[14]. The same rising trend in humans can be seen, and as human population number increase so may the number of cases of Chagas disease infections[2].

As the number of mammalian hosts that become infected with *T. cruzi* in the wild grows, the number of triatomine vectors that feed off these infected animals will also grow which will increase the risk of an infected vector coming into contact with a human. This study urges that strategies be put in place that allow for the control of insect vectors at a sylvatic level.

Of the twelve female insects collected, nine came back positive for an infection rate of 75% while of the six males collected, three came back positive for an infection rate of 50%.

These results may be explained by literature that states in which females tend to more actively disperse than males in search for food and due to wing-length differences that allow them to fly farther[34]. Females must more actively search out food sources than males in order to make up the energy lost during pregnancy. Our results show that female insects were caught more often in two of the three parks in which insects were collected. Bentsen State Park had a total of 6 insect captures, 3 of which were female. The equal collection of insect genders is likely due to small sampling size, but may have also been caused by feeding status. Female insects are less likely to actively disperse if they have a blood host nearby and are full, in which case they send out pheromones to male suitors[34]. While the majority of this literature is based on different triatomine species found in Latin America, and species are known to act different from one another, it is plausible that much of the literature applies to some varying degree to those triatomine species found in the United States.

Infection rates per gender show an unusual increase in the infection rate of females in comparison to males. Most literature, at least in adult triatomine insects, has a ~1:1 ratio of vector infection with *T. cruzi*[35], but it is very likely that our low sample count distorted an accurate prevalence. However the majority of this literature deals with infection rates of domestic insects, and since essentially no literature exists on this topic no real guesses can be taken on whether females are more active or in larger abundance in our collection sites.

CHAPTER V

CONCLUDING REMARKS

Although there are some apparent shortcomings that will be discussed briefly in this section, our study is among the first to study distribution and infection rates of Chagas disease vectors in State Parks of the Lower Rio Grande Valley.

Ideally, the number of insect samples collected would be much higher in order to be able to run significant statistics and to keep out distortion of inflated infection rates. Also a systematic approach to collection would have been best so that results for collection of insect vectors over a series of time could have been evaluated.

Further collection of insects and blood meal analysis are also needed to help refine distributions of species and determine the potential hosts for park insects. This information can be used to further help create a prevention control strategy to help stop rising Chagas disease infections in Texas.

REFERENCES

1. Sarkar, S. et al. Chagas disease risk in Texas. PLoS Negl. Trop. Dis. 4, (2010).

2. Hanford, E. J., Zhan, F. B., Lu, Y. & Giordano, A. Chagas disease in Texas: Recognizing the significance and implications of evidence in the literature. Soc. Sci. Med. 65, 60–79 (2007).

3. Jurberg J, G. C. Biology , ecology , and systematics of Triatominae (Heteroptera , Reduviidae), vectors of Chagas disease , and implications for human health 1. Denisia 50, 1096–1116 (2006).

4. Bern, C., Kjos, S., Yabsley, M. J. & Montgomery, S. P. Trypanosoma cruzi and chagas' disease in the united states. Clin. Microbiol. Rev. 24, 655–681 (2011).

5. Cevallos, A. M. & Hernández, R. Chagas' disease: Pregnancy and congenital transmission. Biomed Res. Int. 2014, (2014).

6. Kun, H. et al. Transmission of Trypanosoma cruzi by heart transplantation. Clin. Infect. Dis. 48, 1534–1540 (2009).

7. Roque, a. L. R. et al. Trypanosoma cruzi transmission cycle among wild and domestic mammals in three areas of orally transmitted chagas disease outbreaks. Am. J. Trop. Med. Hyg. 79, 742–749 (2008).

8. Schofield, C. J. Control of Chagas' disease vectors. Br. Med. Bull. 41, 187–194 (1985).

9. Kjos, S. a, Snowden, K. F. & Olson, J. K. Biogeography and Trypanosoma cruzi Infection Prevalence of Chagas Disease Vectors in Texas, USA. Vector Borne Zoonotic Dis. 9, 41–50 (2009).

10. Garza, M. et al. Projected Future Distributions of Vectors of Trypanosoma cruzi in North America under Climate Change Scenarios. PLoS Negl. Trop. Dis. 8, (2014).

11. Garcia, M. N., Hotez, P. J. & Murray, K. O. Potential novel risk factors for autochthonous and sylvatic transmission of human Chagas disease in the United States. Parasit. Vectors 7, 311 (2014).

12. Beard, C. et al. Chagas disease in a domestic transmisson cycle in Southern Texas, USA. Emerg. Infect. Dis. 9, 103–105 (2003).

13. Burkholder, J. E., Allison, T. C. & Kelly, V. P. Trypanosoma cruzi (Chagas) (Protozoa: Kinetoplastida) in invertebrate, reservoir, and human hosts of the lower Rio Grande valley of Texas. J. Parasitol. 66, 305–311 (1980).

14. Kjos, S. a. et al. Distribution and characterization of canine Chagas disease in Texas. Vet. Parasitol. 152, 249–256 (2008).

15. Charles, R. a, Kjos, S., Ellis, A. E., Barnes, J. C. & Yabsley, M. J. Southern plains woodrats (Neotoma micropus) from southern Texas are important reservoirs of two genotypes of Trypanosoma cruzi and host of a putative novel Trypanosoma species. Vector Borne Zoonotic Dis. 13, 22–30 (2013).

16. Alvarado-Otegui, J. a. et al. The sylvatic transmission cycle of Trypanosoma cruzi in a rural area in the humid Chaco of Argentina. Acta Trop. 124, 79–86 (2012).

17. Barnabé, C., Neubauer, K., Solari, A. & Tibayrenc, M. Trypanosoma cruzi: Presence of the two major phylogenetic lineages and of several lesser discrete typing units (DTUs) in Chile and Paraguay. Acta Trop. 78, 127–137 (2001).

18. Burgos, J. M. et al. Molecular identification of Trypanosoma cruzi discrete typing units in end-stage chronic Chagas heart disease and reactivation after heart transplantation. Clin. Infect. Dis. 51, 485–495 (2010).

19. Dorn, P. L. et al. Autochthonous transmission of Trypanosoma cruzi, Louisiana. Emerg. Infect. Dis. 13, 605–607 (2007).

20. Ez, Ä., Alvear, a L., Ramsey, J. M. & Ordon, R. Distribution of domestic Triatominae and strati ® cation of Chagas Disease transmission in Oaxaca , Mexico. Med. Vet. Entomol. (2000).

21. Ramsey, J. M. et al. Ecological Connectivity of Trypanosoma cruzi Reservoirs and Triatoma pallidipennis Hosts in an Anthropogenic Landscape with Endemic Chagas Disease. PLoS One 7, (2012).

22. Pizarro, J. C. & Stevens, L. A new method for forensic DNA analysis of the blood meal in Chagas disease vectors demonstrated using Triatoma infestans from Chuquisaca, Bolivia. PLoS One 3, 1–8 (2008).

23. Medicine, B. S. of T. Triatomine Protocols. (2015).

24. Pizarro, J. C., Lucero, D. E. & Stevens, L. PCR reveals significantly higher rates of Trypanosoma cruzi infection than microscopy in the Chagas vector, Triatoma infestans: high rates found in Chuquisaca, Bolivia. BMC Infect. Dis. 7, 66 (2007).

25. Roellig, D. M. et al. Molecular typing of Trypanosoma cruzi isolates, United States. Emerg. Infect. Dis. 14, 1123–1125 (2008).

26. Kitano, T., Umetsu, K., Tian, W. & Osawa, M. Two universal primer sets for species identification among vertebrates. Int. J. Legal Med. 121, 423–427 (2007).

27. Kjos, S. a 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. 50, 1126–1139 (2013).

28. Cura, C. I. 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. 9, e0003765 (2015).

29. Cosentino, R. O. & Agüero, F. A simple strain typing assay for Trypanosoma cruzi: Discrimination of major evolutionary lineages from a single amplification product. PLoS Negl. Trop. Dis. 6, (2012).

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

31. Bierle, S. et al. A Literature-based Key to REDUVIIDAE (Heteroptera) of Florida. 1–18 (2002).

32. Garcia, M. N. et al. Trypanosoma cruzi screening in Texas blood donors, 2008–2012. Epidemiol. Infect. 1–4 (2014). doi:10.1017/S0950268814002234

33. Crawford, B. a. & Kribs-Zaleta, C. M. Vector migration and dispersal rates for sylvatic Trypanosoma cruzi transmission. Ecol. Complex. 14, 145–156 (2013).

34. Abrahan, L. B., Gorla, D. E. & Catalá, S. S. Dispersal of Triatoma infestans and other triatominae speciesin the arid Chaco of Argentina - Flying, walking or passive carriage? Mem. Inst. Oswaldo Cruz 106, 232–239 (2011).

35. Cesa, A. K., Caillouët, K. a, Dorn, P. L. & Wesson, D. M. High Trypanosoma cruzi (Kinetoplastida: Trypanosomatidae) Prevalence in Triatoma sanguisuga (Hemiptera: Redviidae) in Southeastern Louisiana. J. Med. Entomol. 48, 1091–1094 (2011).

36. Leiby, D. A., Herron, R. M., Read, E. J., Lenes, B. A. and Stumpf, R. J. Trypanosoma cruzi in Los Angeles and Miami blood donors: impact of evolving donor demographics on seroprevalence and implications for transfusion transmission. Transfusion, 42: 549–555 (2002).

BIOGRAPHICAL SKETCH

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