TRYPANOSOMA CRUZI PREVALENCE IN MESOMAMMALS IN SOUTH TEXAS:

IS THERE A HOST SEX-BIAS?

A Thesis

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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August 2018

Major Subject: Wildlife and Fisheries Sciences

ABSTRACT

American trypanosomiasis, also known as Chagas disease, is caused by the protozoan *Trypanosoma cruzi (T. cruzi)*. American trypanosomiasis is considered a neglected tropical disease which can cause serious cardiac and digestive problems in humans and other mammalian species. The only *T. cruzi* trypanosome vectors are invertebrate insects of the subfamily Triatominae. Most species of triatomines are associated with free-ranging vertebrates which act as reservoir hosts. Virginia opossums (*Didelphis virginiana*) and raccoons (*Procyon lotor*) are frequently identified as the principal reservoir hosts across the United States. This study sought to further dissect *T. cruzi* disease dynamics in south Texas.

My objectives consisted of 1) estimating *T. cruzi* prevalence in three host species: Virginia opossum, raccoon, and striped skunk (*Mephitis mephitis*); and 2) determining if there is a sex-bias in *T. cruzi* parasitism among the three aforementioned host species. The study analyzed 94 blood and tissue samples from 30 meso-mammals captured and processed between 2012-13 using polymerase chain reaction (PCR) methodologies to screen for *T. cruzi*. The overall *T. cruzi* prevalence from 3 species of meso-mammals was 40% (12/30). Virginia opossums had the highest prevalence 71.4% (10/14) followed by raccoons 33.3% (1/3) and striped skunks 23.1% (3/13). No statistically significant difference in prevalence rates between males and females of the species tested was found. The overall prevalence in total number of females was 45.5% (5/11), and the overall prevalence in total number of males was 47.4% (9/19).

My results suggest that in south Texas, striped skunks should be considered a reservoir species of special concern in addition to raccoons and Virginia opossums. Additionally, my results indicate *T. cruzi* parasitism does not appear to differ between females and males of the mammalian reservoir species with the highest prevalence in south Texas; although, further research with a larger sample size could lead to more definitive results.

DEDICATION

A la memoria de mi padre y a mi madre.

ACKNOWLEDGEMENTS

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis committee consisting of Professor Roel R. Lopez and Professor Nova J. Silvy of the Department of Wildlife and Fisheries Sciences and Associate Professor Donald S. Davis of the Department of Veterinary Pathobiology.

A portion of the PCR analysis was conducted by Dr. Candelaria Daniels of Joint Base San Antonio. The remainder PCR analysis was conducted by Dr. Sarah Hamar at Texas A&M University and Melissa Nolan-Garcia and Rodion Gorchakov at Baylor University.

All other work conducted for the thesis was completed independently by the student.

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1. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

American trypanosomiasis, also known as Chagas disease, is caused by the protozoan *Trypanosoma cruzi* (*T. cruzi*). The disease was first described in 1909 in Brazil by Dr. Carlos Chagas (Barr 2009); although, studies of ancient DNA from human remains recovered from archeological sites in Peru and Brazil have confirmed chagasic infections in Pre-Colombian civilizations dating back to 4,000-7,000 years ago (Guhl and Aufderheide 2010).

American trypanosomiasis can cause serious cardiac and digestive problems in humans and other mammalian species. The disease is a neglected tropical disease considered a significant human health problem in Central and South America where up to 11 million people are infected (Center for Disease Control [CDC] 2011). In the United States (U.S.) there have been few reported cases; although, the CDC estimated that 300,000 people or more were infected in 2009. In the U.S., the first human reported case occurred in 1955 in Corpus Christi, Texas (Olsen et al. 1964, Dorn et al. 2007).

T. cruzi is the causative agent of Chagas disease (Figure 1). Currently, there are approximately 10 well established clades within the genus *Trypanosoma*. The *T. cruzi* clade contains several trypanosomes placed within the subgenus *Schizotrypanum*, and it includes trypanosomes that are parasitic in a diverse range of mammals including two human-infective parasites, *T. cruzi and T. rangeli*, which are restricted to the Western Hemisphere. The *T. cruzi* trypanosomes have voluminous kinetoplasts, and they typically have curved bloodstream forms in the shape of an S or C with a short, pointed

posterior end. Another distinguishing life cycle characteristic is that within vertebrate

hosts, multiplication occurs within various tissues and organs instead of in the blood

stream, as is the case with other trypanosome species (Hamilton and Stevens 2010).
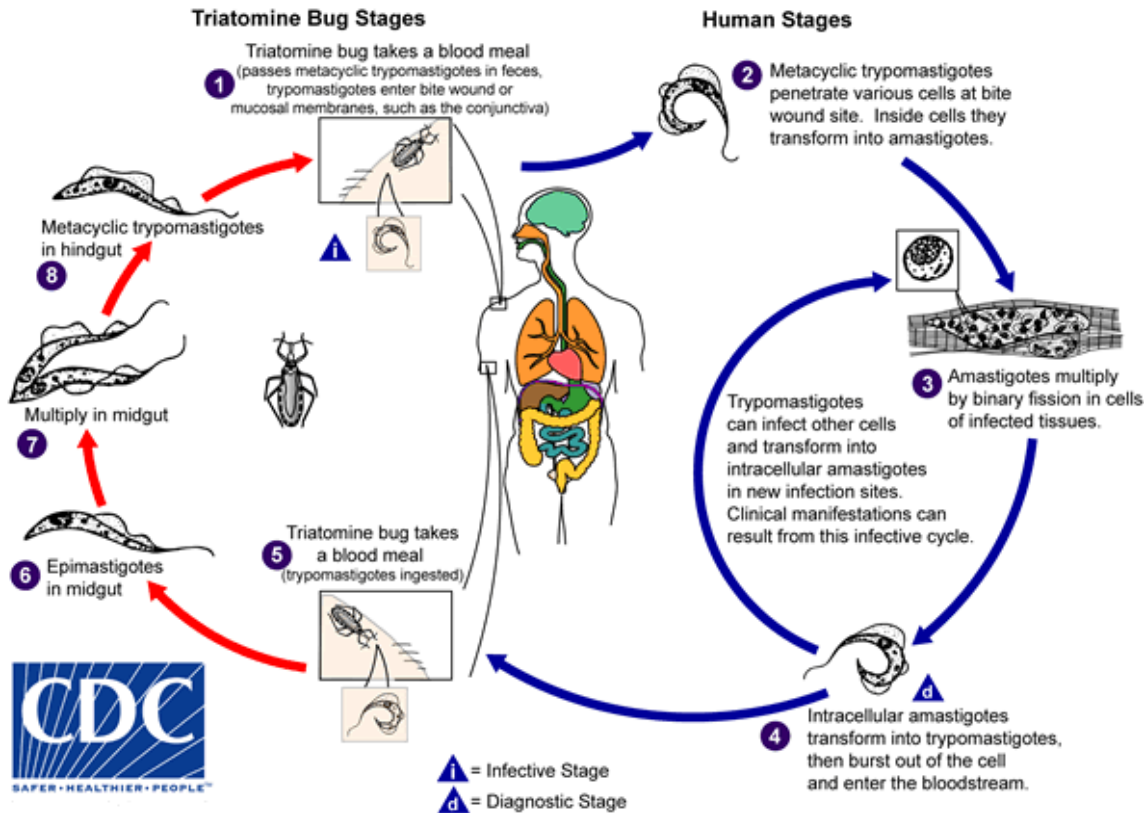
Figure 1. American trypanosomiasis life cycle. Reprinted from Centers for Disease
Control and Prevention (CDC) Official Website.

The only known *T. cruzi* trypanosome vectors are invertebrate insects of the

order Hemiptera: Reduviidae, Triatominae, Triatomine (Figure 2). The subfamily

Triatominae is classified into five tribes, 15 genera, and 141 species (Lent and

Wygodzinsky 1979; Schofield and Galvão 2009). The Triatominae are characterized by

their bloodsucking feeding habits and associated morphological adaptations such as the

straight three-segmented rostrum in which the final segment is capable of flexing

upward when the rostrum is extended for feeding purposes. Over half of the 141 species

of Triatominae have been shown capable of being naturally or experimentally infected

with *T. cruzi* (Schofield and Galvão 2009). It is important to note that, although, a

sylvatic transmission cycle has occurred for thousands of years, only a small number of

domiciliated vector species are highly important in the domestic human infection cycle

(Lent and Wygodzinky 1979).
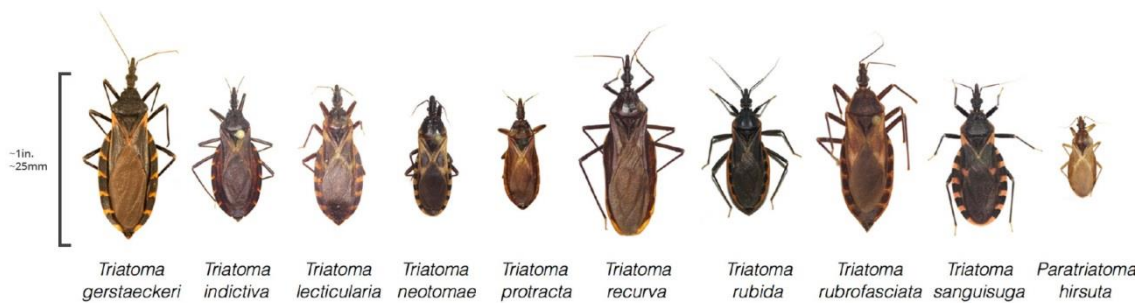
Figure 2. Examples of 10 species of kissing bugs found in the U.S. Reprinted from
Texas A&M University. Kissing Bugs and Chagas Disease in the U.S. Photo by Gabriel
Hamer.

Twelve species of triatomines occur in the U.S. across the southern two-thirds of

the continental U.S. (Figure 3). The most common vector species being *T. rubida* and *T.

protacta* in Arizona and California, and *T. gerstaeckeri* and *T. sanguisuga* in Texas and

New Mexico (The Kissing Bug Project 2011). The trypanosome carries out part of its

life cycle in the insects' digestive tract; the protozoan parasite is later transmitted to

blood meal hosts when the insect defecates during or after feeding (Figure 1).

Alternatively, *T. cruzi* is transmitted orally when animals eat infected vectors, or in the case of humans it can occur by inadvertently ingesting secretions from infected animals in contaminated food products (Hontebeyrie et al 2010).
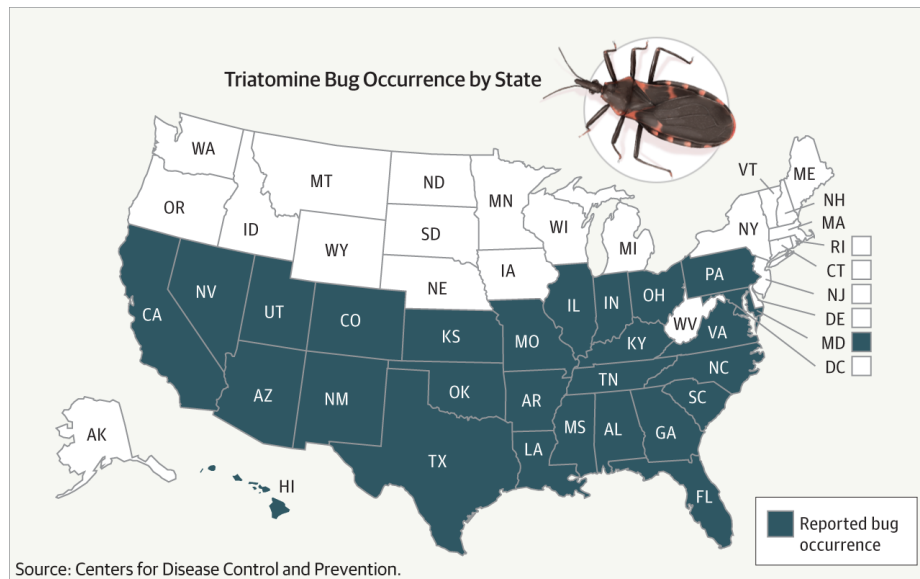
Figure 3. Kissing bug occurrence by state in the U.S. Reprinted from Centers for Disease Control and Prevention (CDC) Official Website.

Most species of triatomines are associated with free-ranging vertebrates which act as reservoir hosts (Miles et al. 1981; Rozas et al. 2005). A study in Mexico identified the blood meal origins of 47 triatomine species; the researchers identified raccoons (*Procyon lotor*) and armadillos (*Dasypus* spp.) as the main blood meal hosts (Bosseno et al. 2009). In the U.S., *T. cruzi* flagellates were first reported in triatomines found in wood rat (*Neotoma* spp.) nests in 1916 in California; although, researchers at the time did not find the parasite in the rodents' blood samples, so it was not until 2 decades later that they were able to verify the presence of *T. cruzi* in vertebrate reservoirs and triatomines in the southwestern U.S. (Olsen et al. 1964).

A U.S. study reported a higher *T. cruzi* incidence in Virginia opossums (*Didelphis virginiana*) and raccoons (*Procyon lotor*) among 11 reservoir species from six southern states (Brown et al. 2010). Karsten et al. (1992) also identified raccoons and opossums as the most frequently infected mammals out of 18 species of mammals that had been reported to be infected in the southern U.S. at the time. Yabsley and Noblet (2002) also reported that out of over 20 species of wildlife reservoirs in the U.S., raccoons and Virginia opossums are consistently identified as the most common hosts.

In south Texas, Kramm et al. (2017) tested for *T. cruzi* in 12 species of free-ranging small mammals, meso-mammals, and large mammals and found a higher prevalence rate in Virginia opossums, raccoons, and striped skunks (*Mephitis mephitis*). Additionally, studies have reported chronic *T. cruzi* infection in working dogs in south Texas (Beard et al. 2003). In 2007, it was reported that military working dogs in south Texas had a *T. cruzi* seroprevalence of 8% (McPhatter et al. 2012).

Given the results of the Kramm et al. (2017) study, and in order to better understand a specific aspect of *T. cruzi* disease dynamics in free-ranging mammals in south Texas, specifically at Joint Base San Antonio (JBSA) – Lackland Air Force Base (LAFB), my research seeks to analyze if sex-bias parasitism in free-ranging mammals is present in south Texas and if so, does this play a significant factor in the perpetuation of the *T. cruzi* disease cycle in south Texas.

Physiological, morphological, and behavioral differences between females and males could lead to sexual bias in infection levels (Poulin 1996; Wilson et al. 2002). Mammalian sex hormones affect the immune system in different ways. Estrogens

stimulate immunity, whereas androgens depress immunity; therefore, the expectation is that males will tend to exhibit higher parasitism levels compared to females (Zuk and McKean 1996; Gemmill et al. 1997; Schalk and Forbes 1997; Krasnov et al. 2005; Bordes et al. 2012). Invertebrates on the other hand lack steroids such as testosterone, and despite an absence of any endocrine-immunological interaction, a study found that male arthropods are not more susceptible to higher levels of parasitism compared to female arthropods (Sheridan et al. 2000).

My research focuses on three mammalian species identified as having the highest *T. cruzi* prevalence rate in south Texas: Virginia opossums, raccoons, and striped skunks. The null hypothesis is there is no significant difference in *T. cruzi* parasitism between males and females of the aforementioned species. The alternative hypothesis is there is a significant difference in *T. cruzi* parasitism between males and females of the aforementioned species.

1.2 Research Objectives

The objective of the overall project since its inception was to understand *T. cruzi* disease dynamics in free-ranging mammals in south Texas, specifically at JBSA Lackland Annex; therefore, the research objectives seek to further dissect disease dynamics that may or may not play a significant role in the perpetuation of the *T. cruzi* disease cycle in south Texas. Additionally, the thesis project seeks to contribute additional knowledge related to the prevalence of *T. cruzi* in key medium-sized free-ranging wildlife mammalian host species in south Texas.

The research focused on determining if parasitism in three of the medium-sized mammal species with the highest *T. cruzi* prevalence rates differs between females and males of each species. Physiological, morphological, and behavioral differences between females and males could lead to sexual bias in infection levels; for example, high testosterone levels can cause immunosuppression in males which can result in males having a higher parasite load (Poulin 1996; Wilson et al. 2002).

The research objectives consist of 1) estimate the prevalence of *T. cruzi* in three major mammalian host species: raccoon, Virginia opossum, and striped skunk; and, 2) determine if there is a sex-bias in *T. cruzi* parasitism among three major mammalian host species: raccoon, Virginia opossum, and striped skunk.

1.3 Study Area

JBSA Lackland is one of the three locations that comprise JBSA (Figure 4), and it is the home of the Air Force Basic Military Training School, AETC Non-Commissioned Officers Academy, Defense Language Institute, and Wilford Hall Medical Center. Additionally, JBSA Lackland Annex is home to the Military Working Dog (MWD) Center which consists of two primary kennel facilities and training areas utilized by the 341st Training Squadron. The Military Working Dog Center is responsible for training approximately 900 dogs annually.

JBSA Lackland is divided into three areas: the Main Base, the Lackland Training Annex, and the Kelly Field Annex. The study took place within the undeveloped area or semi-developed areas within Lackland Training Annex which is located in southwest Bexar County 14.5 km from downtown San Antonio, Texas on approximately 3,844 ha

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of land. The Lackland Training Annex is located west of the Main Base across a one-mile corridor of civilian land that includes a portion of Interstate Highway 410; it consists of a small cantonment area, storage bunkers and designated firing ranges; although, for the most part it consists of undeveloped land (Lackland AFB INRMP 2007).

Bexar County is located at the junction of three distinct ecological regions: Edwards Plateau, Blackland Prairie, and South Texas Plains. JBSA Lackland Annex is located within the Blackland Prairie ecological region; although, there are some areas where the regions overlap. The topography of the Blackland Prairie consists of gently rolling plains with elevations ranging from 91 to 244 meters above sea level. Historically, the region was a true prairie with climax species such as little bluestem. Other important species included big bluestem, Indian grass, switch grass, sideoats grama, hairy grama, tall dropseed, silver bluestem and Texas winter-grass (Lackland AFB INRMP 2007).

Currently, the vegetative community at Lackland can be divided into three main plant communities: deciduous shrublands and woodlands, riparian woodlands, and grasslands. The study transects were located within deciduous shrublands and woodlands and grasslands. The deciduous shrublands and woodlands plant community is comprised of various species including honey mesquite (*Prosopis glandulosa*), hackberry (*Celtis laevingata*), granjeno (*Celtis pallida*), and Eve's necklance (*Sophora affinis*) in the overstory. Small shrubs occupy open spaces such as the Texas colubrine (*Colubrina texensis*), whitebrush (*Aloysia gratissima*), littleleaf sumac (*Rhus microphylla*), and

Texas persimmon (*Diospyros texana*). Herbaceous cover includes King Ranch bluestem (*Bothriochloa ischaemum* var. *songarica*), buffalograss (*Buchloe dactyloides*), and Texas wintergrass (*Stipa leucotricha*).

The grasslands vegetative community consists of some native remnants as well as non-native species. The species include plains lovegrass (*Eragrostis intermedia*), panicums (*Panicum* spp.), big cenchrus (*Cenchrus myosuroides*), little bluestem (*Schizachyrium scoparium*), swithchgrass (*Panicum virgatum*), Johnsongrass (*Sorghum halepenense*), southwestern bristlegrass (*Setaria* spp.), knotroot bristlegrass (*Setaria leuchtricha*), sideoats grama (*Bouteloua curtipendula*), and buffalograss (*Buchloe dactyloides*) (Lackland AFB INRMP 2007).
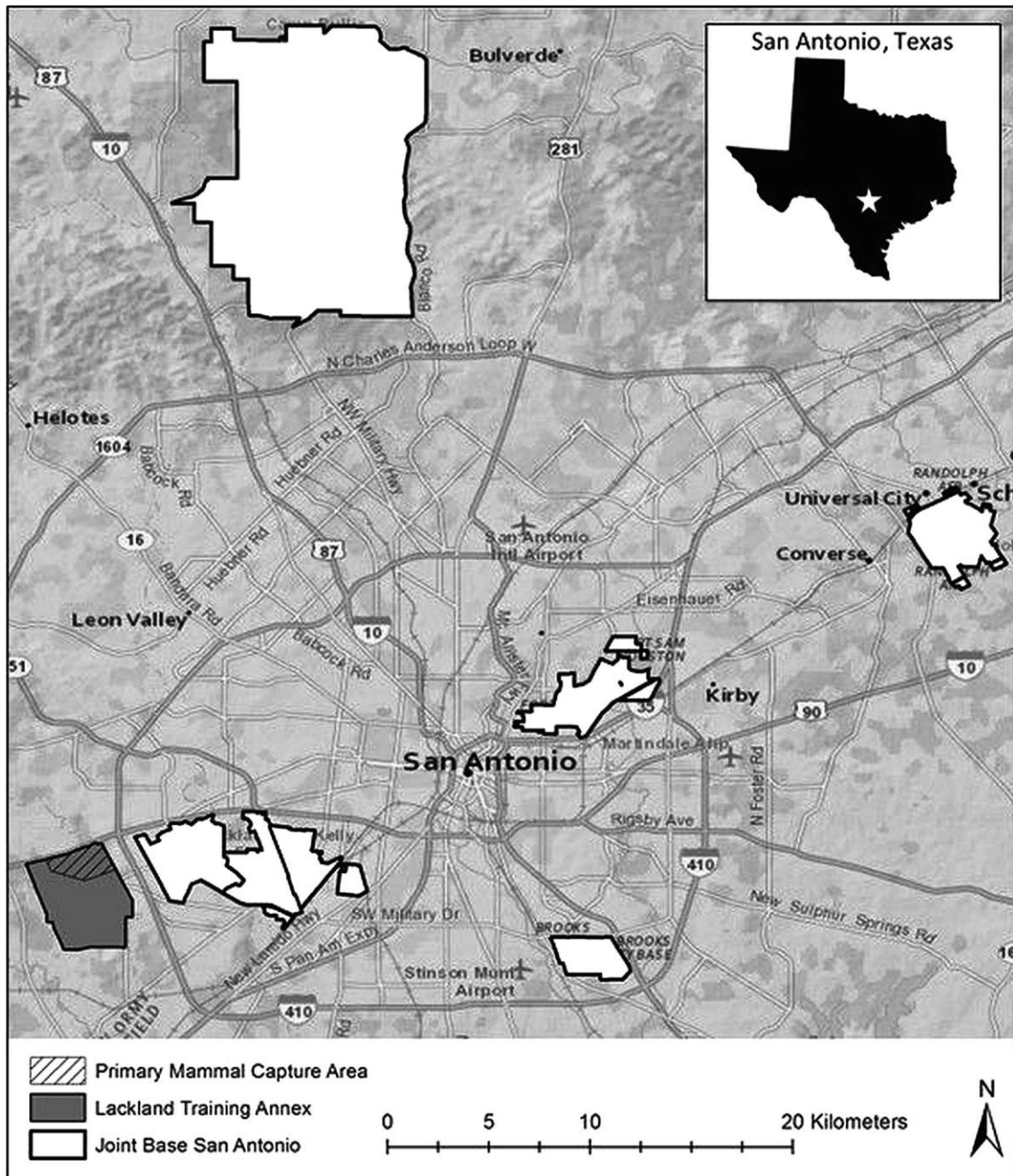
Figure 4. Study area at Joint Base San Antonio (JBSA) Lackland Training Annex in San Antonio, Texas. Reprinted from Kramm et al. 2017. *Trypanasoma cruzi* in free-ranging mammalian populations in South Texas, USA. *Journal of Wildlife Diseases* 53:788-794.

2. *TRYPANOSOMA CRUZI* PREVALENCE IN MESOMAMMALS IN SOUTH TEXAS: IS THERE A HOST SEX-BIAS?

2.1 Introduction

T. cruzi is the causative agent of American trypanosomiasis. The causative agent relies on insects of the order Hemiptera as vectors. The insect vectors rely on securing blood meals from various mammal species which in turn become reservoir hosts for the disease. Generalist meso-mammals such Virginia opossums and raccoons tend to exhibit the highest *T. cruzi* prevalence rates in the U.S. Physiological, morphological, and behavioral differences between females and males could lead to sexual biases in infection levels (Poulin 1996; Wilson et al 2002). Since mammalian sex hormones affect the immune system differently, estrogens stimulate immunity whereas androgens depress immunity; the expectation is that males will tend to exhibit higher levels of parasitism compared to females (Zuk and McKean 1996; Gemmill et al 1997; Schalk and Forbes 1997; Krasnov et al. 2005; Bordes et al. 2012).

2.2 Materials and Methods

2.2.1 Mammal Trapping

As part of the original study design and project to study mammal densities and species abundance in the different vegetative communities surrounding the Military Working Dog Training Complex in 2011-2013, which also sought to obtain baseline data regarding *T. cruzi* prevalence in potential mammal reservoirs, I conducted seasonal small mammal and medium-sized mammal trapping (Institutional Animal Care and Use

Committee IACUC 2011-294). A total of 9 transects was distributed in the study area, each one 100m long.

With regards to medium-sized animal trapping, I placed 3 Tomahawk live traps (48 x 15 x 15 cm; Tomahawk, WI) along the beginning, middle, and end of each transect line (Schemnitz et al. 2012). The Tomahawk live traps were baited with cat food or sardines. Trapping was conducted once or twice seasonally (fall, winter, spring, and summer) between the spring of 2012 and summer of 2013, and each trapping session consisted of 9 consecutive trap nights. Medium-sized mammals were marked with temporary external marks (Indian ink) and when handled, by clipping fur from a small section on the rear so as to aid in recapture identification (Silvy et al. 2012). All of the procedures utilized conform to the Guidelines of the American Society of Mammalogists for Use of Wild Mammals in Research (Sikes RS et al. 2011).

2.2.2 Sample Collection and Disease Prevalence Analysis

Captured medium-sized mammals were anesthetized with Telazol (tiletamine hydrochloride) at a species appropriate dosage (Institutional Animal Care and Use Committee IACUC 2011-294). I collected 0.5 to 1 mL of blood via femoral or jugular vein puncture. Blood samples were placed in BD Vacutainer tubes (Becton, Dickison and Company, Franklin Lakes, NJ, USA). Additionally, blood and tissue samples from medium-sized mammals were collected opportunistically from on-going management actions conducted by Air Force Pest Control Personnel.

Polymerase chain reaction (PCR) analyses were conducted by the Department of the Army Public Health Command Region – South – Ft. Sam Houston, San Antonio,

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Texas. The samples were processed according to Quiagen DNeasy manufacturer instructions (Quiagen, Valencia, California, USA). The samples were screened for 2 *T. cruzi* genomic targets using conventional PCR methods, and they were designated as positive if 2 separate PCRs coincided with 330 bp kDNA and 188 bp nDNA (Reisenman et al. 2010).

2.2.3 Data Analysis

 T. cruzi prevalence results of males and females of raccoons, Virginia opossums, and skunks were compared using a Chi-square test ($\alpha = 0.05$). The results of the PCR analysis relevant to objective one are presented in Table 1 which includes the *T. cruzi* prevalence for each species expressed as percentages.

2.3 Results

2.3.1 T. cruzi prevalence in Virginia opossums, striped skunks, and raccoons.

 T. cruzi was found in the three species of meso-mammals that were trapped. A total of 30 animals was tested and the overall prevalence was 40%. *T. cruzi* prevalence in Virginia opossums was 10/14 (71.4%) of the animals sampled; in striped skunks 3/13 (23.1%) of the animals sampled; and, in raccoons 1/3 (33.3%) of the animals sampled (Table 1).

2.3.2 Trypanosoma cruzi in males and females of three meso-mammal species.

 A total of ($n = 19$) males of three species of meso-mammals was tested for *T. cruzi* and the overall prevalence rate was 47.4% (Table 2). A total of ($n = 11$) females of three species of meso-mammals were tested for *T. cruzi* and the overall prevalence rate was 45.5% (Table 3). The prevalence results differentiated by the sex of each species

were as follows: Virginia opossum females 5/7 (71.4%) and Virginia opossum males 5/7

(71.4%); raccoon females 0/2 (0%) and raccoon males 1/1 (100%); striped skunk

females 0/2 (0%) and striped skunk males 3/11 (27.3%) (Table 2 and Table 3).

A Chi-square test (α =0.05) for *T. cruzi* parasitism prevalence between male and

female Virginia opossums was found to be not significant since p > 0.05 (X^2 = 1.3694, P

= 0.24). The Chi-square test could not be performed to compare the parasitism

prevalence rates between sexes in raccoons due to the small sample size ($n = 2$ females;

$n = 1$ male), nor in striped skunks because of the zero number of positive samples in

female skunks. A Chi-square test (α=0.05) for *T. cruzi* parasitism between all females

and all males of the three species (total females $n = 11$; total males $n = 19$) was found to

be not significant since p > 0.05 (X^2 = 0.4654, P = 0.495); therefore, I failed to reject the

null hypothesis.

Table 1 *Trypanosoma cruzi* prevalence in meso-mammal species, Joint Base San
Antonio − Lackland Annex, San Antonio, Texas, 2012 − 2013

Meso-mammal Species	Prevalence +/*n* (%)	# of PCR Samples	# of Positive PCR Samples	% of PCR Positive Samples
Virginia oppposum	10/14 (71.4)	44	28	63.6
Raccoon	1/3 (33.3)	11	1	9.1
Striped skunk	3/13 (23.1)	39	9	23.1
Total	14/30 (40.0)	94	38	40.4

Prevalence: Positives/n total by species.

Table 2 *Trypanosoma cruzi* prevalence in males of three species of meso-mammals, Joint Base San Antonio – Lackland Annex, San Antonio, Texas, 2012 – 2013

Mesomammal Species	Males +/*n* (%)	# of PCR Samples	# of Positive Samples	% of PCR Positive Samples
Virginia opossum	5/7 (71.4)	17	9	52.9
Raccoon	1/1 (100)	1	1	100
Striped skunk	3/11 (27.3)	33	9	27.8
TOTAL	9/19 (47.4)	51	19	37.3

Prevalence: Positives/n total males per species.

Table 3 *Trypanosoma cruzi* prevalence in females of three species of meso-mammals, Joint Base San Antonio – Lackland Annex, San Antonio, Texas, 2012 – 2013

Mesomammal Species	Females +/*n* (%)	# of PCR Samples	# of Positive Samples	% of PCR Positive Samples
Virginia opossum	5/7 (71.4)	27	19	70.4
Raccoon	0/2 (0)	10	0	0
Striped skunk	0/2 (0)	6	0	0
TOTAL	5/11 (45.5)	43	19	44.2

Prevalence: Positives/n total females per species.

2.4 Discussion

The high *T. cruzi* prevalence in Virginia opossums (71.4%) found in my study is consistent with results found in other studies conducted throughout the southern U.S. which consistently identify this species as one of the principal mammalian reservoirs (Olsen et al. 1964; Karsten et al. 1992; Brown et al. 2010). Raccoons also are consistently identified as one of the main mammalian reservoirs across the southern U.S. (Yabsley and Noblet 2002; Brown et al. 2010; Maloney et al. 2010).

In my study in south Texas only 3 raccoons were tested and only one tested positive resulting in a 33.3% *T. cruzi* prevalence (Table 1). Maloney et al. (2010) in Tennessee, collected serum samples from 706 raccoons of which 206 or 29.2% were seropositive for *T. cruzi*, and Yabsley and Noblet (2002) in South Carolina and Georgia collected serum samples from 221 raccoons of which 104 or 47% were seropositive. Despite my low raccoon sample size, raccoon samples yielded a high prevalence rate compared to other species that have been tested in south Texas; therefore, it can be inferred that an increased raccoon sample size would have resulted in higher *T. cruzi* prevalence rates consistent with other U.S. studies.

In my study, striped skunks had the third highest *T. cruzi* prevalence (23.1%) among the three species (Table 1). Striped skunks have been shown to be experimentally susceptible to *T. cruzi* infection (Davis et al. 1980). However, in the wild Brown et al. (2010) identified 11 potential reservoir species in six southern states: Arizona, California, Florida, Georgia, Missouri, and Virginia, and only found a 9% seroprevalence rate in striped skunks from Arizona out of 34 tested and a 33%

seroprevalence rate out of 3 striped skunks tested for antibodies in Georgia. Overall, the researchers tested 43 striped skunks in Arizona, California, and Georgia; of the 10 striped skunks tested for *T. cruzi* antibodies in California none were positive (Brown et al. 2010). My study in south Texas tested 13 striped skunks, and the *T. cruzi* prevalence was 23.1% (Table 1). Given the relatively high prevalence rate for the sample size, it can be inferred that striped skunks should be considered a key mammalian reservoir host in south Texas.

The second part of my research consisted on determining if there was a statistically significant sex bias in *T. cruzi* parasitism in the three meso mammal species discussed previously. The null hypothesis states there is no significant difference in *T. cruzi* parasitism between Virginia opossum, raccoon, and striped skunk males and females. The alternative hypothesis states there is a significant difference in *T. cruzi* parasitism between males and females of the aforementioned species. In total, 11 females (Table 3) and 19 males (Table 2) was tested, and even though males showed a slightly higher *T. cruzi* prevalence (47.4%) compared to females (45.5%), the results were not statistically significant; therefore, I failed to reject the null hypothesis.

Since mammalian sex hormones affect the immune system differently, estrogens stimulate the female immune system and testosterone suppresses the male immune system (Zuk and McKean 1996; Gemmill et al. 1997; Schalk and Forbes 1997; Krasnov et al. 2005; Bordes et al. 2012) I expected males to show a significantly higher level of parasitism compared to females. The overall sample size in my study was small ($n = 30$), and it was not evenly divided between sexes nor species; therefore, an overall larger

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sample size with even representation between females and males of each species might have yielded different results.

Sex biased parasitism in mammals is a complex topic and even though males are more prone to parasitism, in most published studies, the differences are not statistically significant (Bordes et al. 2012); although, exceptions do exist, for example a study of bladder nematodes in masked shrews (*Sorex cinereus*) found an extreme male-biased parasitism (Cowan et al. 2007). In other instances where a male biased statistical significance was detected, the differences tended to be small and not considered an applicable rule across different species. A meta-analysis of sex biased focused published studies reported that out of 136 field based studies, only 8.8% of male-biased parasitism found was statistically significant. Male biases were more clearly defined if infections were experimentally performed compared to results from field studies. Additionally, protozoan and arthropod parasitism was identified as being more likely to be male-biased versus helminthes parasitism (Schalk and Forbes 1997).

Studies solely focused on *T. cruzi* sex-biased parasitism are for the most part none existent; instead some studies report sex differences in parasitism as an incidental note. Yabsley and Noblet (2002) found female raccoons exhibited a higher *T. cruzi* seroprevalence rate than male raccoons, but the differences were not statistically significant. Maloney et al. (2010) tested 587 raccoons and found a statistical significance between males and females, 36.1% of females were seroprevalence compared to 22.4% of male raccoons. In my study, the raccoon sample size was extremely small ($n = 3$), and

the 1 male tested positive for *T. cruzi* infection whereas the 2 females tested negative (Table 2 and 3).

Undoubtedly, a larger sample size would have provided a more definitive means of comparison between female and male raccoons in south Texas. Brown et al. (2010) also found that female raccoons and Virginia opossums had a higher *T. cruzi* parasitism level compared to males, but the differences were not statistically significant. My study found no statistically significant difference between female and male Virginia opossums; although, the percentage of positive PCR samples did differ between female Virginia opossums (70.4%) (Table 3) and male Virginia opossums (52.9%) (Table 2). The higher percentage of positive PCR samples in females could be attributed to different stages of the disease in individual animals i.e. acute versus chronic stage and to the denning habits of female Virginia opossums that may lead to a prolonged exposure to insect vectors compared to males of the same species (Brown et al. 2010).

The two female striped skunks in my study tested negative for *T. cruzi* (Table 3); therefore, and in addition to the small size, a comparison between male and female striped skunk parasitism was not possible. Finally, no striped skunk sex-biased *T. cruzi* parasitism studies conducted in the wild could be found to elaborate on this point. An experimental infection of 3 male and 1 female captive raised striped skunks was conducted in Texas, and after 24 days of being inoculated with trypomastigotes, all striped skunks were seropositive. Additionally, the authors suggested that striped skunks should be considered a reservoir host in Texas and the American Southwest based on

their susceptibility, asymptomatic nature, and prolonged *T. cruzi* parasitemia (Davis et al. 1980).

3. SUMMARY AND CONCLUSIONS

3.1. Summary

American trypanosomiasis, or Chagas disease, is found in south Texas in a sylvatic disease cycle in which the main reservoir hosts are Virginia opossums, raccoons, and striped skunks. The causative agent is *Trypanosoma cruzi* which is transmitted to hosts via invertebrate vectors. In south Texas, *Triatoma gerstaeckeri* and *Triatoma sanguisuga* are the most common vector species. *T. cruzi* parasitism does not appear to distinguish between the sexes of the three mammalian reservoir species with the highest prevalence rate in south Texas.

3.2 Conclusions

No insight into how disease dynamics could potentially be affected by sex-biased *T. cruzi* parasitism levels could be drawn from the results of my study; therefore, it is not possible to make specific management recommendations. However, I do recommend investment in further vector host disease dynamics research in south Texas and beyond. Further research efforts could contribute to obtaining a better understanding of the possible implications and repercussions to public health as a result of anthropogenically derived disruptions of the sylvatic disease cycle in south Texas. The three species identified as having the highest prevalence rates in my study are generalist species that are known to adapt to urban or peri-urban settings, so I recommend continued monitoring of *T. cruzi* parasitism in Virginia opossum, raccoon, and striped skunk populations in south Texas.

Specifically, I recommend studying Chagas disease in striped skunks in south Texas and in northern Mexico. Galaviz-Silva et al. (2017) working in Nuevo León, a Mexican state which borders Texas' Rio Grande Valley, identified striped skunks as the species with the highest *T. cruzi* prevalence rate in Nuevo León, Mexico, so a bi-national Chagas study focused on a common reservoir species, such as striped skunks could contribute new knowledge and valuable insights regarding transboundary *T. cruzi* disease dynamics.

Finally, I recommend analyzing disease dynamic models that factor in how climate change may affect the distribution of the invertebrate vectors in the U.S., since an expanded northerly distribution could pose public health risks in regions of the country where the vectors are not currently distributed (Figure 3).

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