Identification Of Leishmania Spp. And T. Cruzi Parasites In Bats Captured In El Paso, Texas Region: Bats As A New Reservoir

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IDENTIFICATION OF *Leishmania* spp. and *T. cruzi* parasites in bats captured in El Paso, Texas region:

**BATS AS A NEW RESERVOIR**

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Master’s Program in Public Health

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DEDICATION

To my mother, Rosa I. Chavira: forever grateful to you mom.
IDENTIFICATION OF \textit{Leishmania Spp.} AND \textit{T. Cruzi} PARASITES IN BATS CAPTURED IN EL PASO, TEXAS REGION:

BATS AS A NEW RESERVOIR

By

EDITH SANDOVAL, MD

THESIS

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ABSTRACT

Background: T. cruzi and Leishmania spp. parasites are the causative agents for Chagas disease and leishmaniasis respectively. Recognized by the World Health Organization (WHO) as two of the world’s thirteen most neglected tropical diseases (NTD) and they continue to rank among the most important public health problems in South America.

Problem Statement: Studies have provided evidence that these parasites are found in this border region and that dogs, cats and sylvatic animals are reservoirs for them. However, bats have not been studied in this region even though bats are mammals that migrate every year from South America where they have been documented to be novel reservoirs for Leishmania spp. and known reservoirs for T. cruzi.

Objectives: To determine the presence of Leishmania spp. and T. cruzi parasites in bats captured in the El Paso, Texas region by testing the heart, spleen and skin tissue from bats using molecular technology.

Method: This cross sectional, observational study collected tissue samples of wild bats that tested negative for rabies, were donated by the Texas Department of State Health Services (TDSHS). Tissue samples were obtained from the heart, spleen, and skin. DNA was extracted, Polymerase Chain Reaction (PCR) was used to identify the presence of Leishmania spp. and T. cruzi. Electrophoresis was performed to visualize the amplified DNA.

Results: Out of 32 bats, 19 (59%) were positive for T. cruzi and 5 (16%) tested positive for Leishmania spp.

Conclusion: We present evidence that bats are potential reservoirs for Leishmania spp. and T. cruzi parasites. This study may serve as a platform from which to continue research and surveillance for both parasites.
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS............................................................................................................ v
ABSTRACT .................................................................................................................................. vi
LIST OF TABLES ......................................................................................................................... ix
LIST OF FIGURES ........................................................................................................................ x
INTRODUCTION .......................................................................................................................... 1

## BACKGROUND AND SIGNIFICANCE

1. Chagas Disease ..................................................................................................................... 2
   1.1 Chagas Disease and T. cruzi Worldwide ................................................................. 2
   1.2 Chagas Disease and T. cruzi in Texas ................................................................. 3
   1.3 Chagas Disease and T. cruzi in El Paso, Texas. .................................................. 4
   1.4 T. cruzi in Bats......................................................................................................... 4
   1.5 Vector for Transmission of T. cruzi ................................................................. 5
   1.6 T. cruzi Life Cycle ............................................................................................ 6
   1.7 Clinical Manifestations of Chagas Disease .................................................. 8

2. Leishmaniasis ......................................................................................................................... 10
   2.1 Leishmaniasis Worldwide ............................................................................ 10
   2.2 Leishmaniasis in Texas .............................................................................. 10
   2.3 Leishmaniasis in El Paso, Texas ............................................................. 11
   2.4 Leishmania spp. in Bats .............................................................................. 11
   2.5 Leishmania spp. Life Cycle ...................................................................... 14
   2.6 Leishmaniasis Clinical Manifestations .................................................. 14

3. Environmental Factors ......................................................................................................... 15
   3.1 Climate Change ............................................................................................ 15
   3.2 Social and Economic Burden of Leishmaniasis and Chagas Disease ......... 15

## STUDY RATIONALE

Problem Statement .................................................................................................................... 17
Research Question ...................................................................................................................... 18
Overall Aim of the Research .................................................................................................... 18
Objectives .................................................................................................................................. 18
Expected results ......................................................................................................................... 18

## METHODOLOGY

............................................................................................................................................. 19
LIST OF TABLES

TABLE 1. SAMPLE DATA SHEET ............................................................................. 30
TABLE 2. GENE AND PRIMERS UTILIZED FOR PCR ........................................ 33
TABLE 3. RESULTS TABLE .................................................................................... 34
LIST OF FIGURES

FIG 1. *T. cruzi* LIFE CYCLE ................................................................. 17
FIG 2. *T. cruzi* VECTOR, KISSING BUG .................................................. 17
FIG 3. CDC: CHILD WITH ROMANA’S SIGN .............................................. 19
FIG 4. *Leishmania* LIFE CYCLE ............................................................... 23
FIG 5. CUTANEOUS LEISHMANIASIS DERMAL LESION ............................. 25
FIG 6. POSITIVE *T. cruzi* AND *Leishmania spp.* CASES ............................ 35
INTRODUCTION

Recognized by the World Health Organization (WHO) as two of the world’s thirteen most neglected tropical diseases (NTD) [Hotez et al. 2007], both Chaga’s disease and Leishmaniasis, continue to rank among the most important public health problems in Central and South America. Trypanosoma cruzi and Leishmania spp. are the causative agents of Chagas’s disease and Leishmaniasis respectively. Together Leishmaniasis and Chagas affect 300 million people (WHO, 2015).

Chagas disease is a chronic, systemic, parasitic infection which is responsible for a greater disease burden than any other parasitic disease in the New World [Bern & Montgomery, 2009]. Leishmaniasis is caused by different species of the protozoan parasite Leishmania. Current estimates show an annual global incidence of 0.2-0.4 million cases of visceral leishmaniasis (VL) and 0.7-1.2 million cases of cutaneous leishmaniasis (CL). [Okwor & Uzonna 2016]

In the last decade, there has been studies aimed to the identification of these disease-causing parasites in the border region of El Paso, Texas. Studies included the tissues of canines, cats, and other sylvatic mammals within the region [Gonzalez, 2015, Kipp et al. 2016, Matamoros, 2016]. Studying the roles of wild animals as reservoirs of leishmaniasis and Chagas disease is becoming increasingly important as we further encroach on their territories, and as their lifestyles become more urban as they adapt to ours [Parsons, 2021]. Wildlife reservoirs play an important role in the maintenance and transmission of parasites in sylvatic transmission cycles [Hodo et al. 2016]. For this reason, it is the aim of this study to explore the possibility that bats could be reservoirs for these parasites since bats have been documented to be novel reservoirs for Leishmania spp. and known reservoirs for T. cruzi in South America.
BACKGROUND AND SIGNIFICANCE

1. Chagas Disease

Chagas disease also known as American trypanosomiasis, is a tropical parasitic disease caused by *Trypanosoma cruzi* [Hotez et al. 2007]. It is spread mostly by insects known as Triatominae, or “kissing bugs” [Gonzalez, 2015, WHO, 2020]. Infection occurs when the triatomine vector (Kissing bug) defecates during its blood meal and fecal material containing the parasite is inoculated through the bite wound or mucous membranes [Mcguire et al. 2004].

*T. cruzi* is a genotypically heterogeneous species that has been divided into six discrete typing units (DTUs), Tcl – TcVI [Zingales et al, 2012] and a seventh recently discovered bat associated type TcBat [Lima et al. 2015, Marcili et al 2009].

1.1 Chagas Disease and T. cruzi Worldwide

In 2017, an estimated 6.2 million people worldwide had Chagas disease, with approximately 162,000 new infections and 7,900 deaths each year [GBD, 2017, 2018].

Chagas is endemic to 21 countries in continental Latin America: Argentina, Belize, Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, El Salvador, French Guiana, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Suriname, Uruguay, and Venezuela [Perez & Molina, 2018]. Vector-borne transmission occurs only in the Americas, where an estimated 8 million people are currently infected with *T. cruzi* [OMS, 2016].

Historically, transmission was concentrated only in rural Latin America, but successful vector-control programs have greatly decreased transmission in areas where the disease was formerly endemic. The United States cannot be classified as an area of nonendemicity for Chagas
disease in the same sense as Europe and Asia. The southern states have enzootic *T. cruzi* transmission that involves at least 11 triatomine species and hosts such as racoons, opossums, and domestic dogs [Bern & Montgomery, 2009].

Chagas disease causes the highest burden of any parasitic disease in the Western hemisphere. It is estimated that applying published seroprevalence figures to immigrant populations, 300,167 individuals with *Trypanosoma cruzi* infection live in the United States, with 30,000 – 45,000 cardiomyopathy cases and 63 – 315 congenital infections annually [Bern & Montgomery, 2009].

The vast majority of *T. cruzi* infected individuals are immigrants from areas of endemcity in Latin America. Only 7 autochthonous vector-borne cases of infection (4 in Texas and 1 each in California, Tennessee and Louisiana) have been reported in the United States since 1955 [Bern & Montgomery, 2009].

Early identification of clinical cases is critical, as the presence of irreversible tissue damage is inversely proportional to drug efficacy and survival [Bern & Montgomery, 2009].

**1.2 Chagas Disease and T. cruzi in Texas**

In Texas, cases of Chagas disease are reported to the Texas Department of State Health Services so that the numbers can be tracked. From the years 2013-2016, 91 cases of Chagas disease were reported in Texas. Of those cases, 20 people were infected while in Texas, and the other cases were probably acquired outside of Texas or the U.S. While there are not very many cases, many people may be living with the disease and not know it [NIH, 2021].

A study tested a patient cohort of 1,196 in Starr County, Texas residents using the Hemagen Chagas ELISA Kit as a preliminary screening assay. Samples testing positive using the Hemagen
test were subjected to additional confirmatory tests. Two patients (0.17%) without previous Chagas disease diagnosis were identified; both had evidence of acquiring disease in the United States or along the Texas-Mexico border [Nolan et al. 2018].

Human exposure to *T. cruzi* from triatomine bites date back to 1935 in Texas. Disease burden from Chagas disease has been consistently documented over the past 70 years in the state of Texas [Garcia et al. 2015].

**1.3 Chagas Disease and *T. cruzi* in El Paso, Texas.**

In 2006, three out of 10,189 people who had donated blood, tested positive for Chagas disease (0.03%), with no additional demographic or clinical information available; Two of the three positive samples were believed to be from the same donor [Tobler et al, 2007]. From January 2007 to December 2009; El Paso, Tx; McAllen, Tx; Lubbock, Tx, Fifteen out of 179,540 (0.01%) blood donors confirmed positive for *T. cruzi* infection, 0.01% belonged to El Paso, Tx [Custer et al, 2012].

In 2013, there were 10 PCR confirmed samples infected with *T. cruzi* from El Paso, Texas in stray dogs [Mariscal, 2013]. In 2015 a similar study was conducted in a total of 155 stray cats, no samples produced evident PCR bands, resulting in negative results in every cat [Gonzalez, 2015].

**1.4 *T. cruzi* in Bats**

Bats are associated with several zoonotic pathogens. Their reservoir potential may be heightened due to their ability to fly, highly gregarious social structures and long life spans [Hayman et al, 2013]. In contrast to other mammalian reservoirs, many bat species migrate long distances and have the potential to introduce exotic pathogens to new areas [Hodo, 2016].
*T. cruzi* is a genotypically heterogeneous species that has been divided into six discrete typing units (DTUs), TcI – TcVI [Zingales et al, 2012] and a seventh recently discovered bat associated type TcBat [Lima et al. 2015, Marcili et al 2009].

In 2016, Hodo et al., tested 593 bat samples. Detection of trypanosomes was done using both nested PCR and qPCR, a single male *N. humeralis* bat was positive for *T. cruzi* on both qPCR and nested PCR, 9 peridomestic bats were positive for *T. dionisii* via nested PCR and 4 peridomestic bats (3 *T. brasiliensis* and 1 *N. humeralis*). The *T. cruzi* positive bat was from Hidalgo county; *T. dionisii* positive bats were from Hidalgo, El Paso and Webb counties [Hodo et al. 2016].

Texas is home to 32 species of bats and at least seven species of triatomines and all have the potential to transmit *T. cruzi* [Ammerman et al., 2012; Curtis-Robles et al, 2015]. Biologists have identified 14 species of bats living in the El Paso area during the month of May to October [El Paso Zoo, 2020] when they migrate back to south of Mexico. Bats travel an estimated 35 to 50 miles southwest of El Paso from what is believed to be a summer home of a colony of Mexican free-tailed bats that head south in the winter [El Paso Zoo, 2020]. The *Tadarida brasiliensis* or Mexican Fre-tail bat is the most common bats found in El Paso, Texas. [Harris, 2000].

1.5 Vector for Transmission of *T. cruzi*

Chagas disease, also known as American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan parasite *Trypanosoma cruzi* (WHO, 2020). The causative agent of Chagas disease, *Trypanosoma cruzi*, is transmitted by triatomine vectors. The insect is endemic in the Americas, including the United States, where epidemiological studies are limited, particularly in the Southwestern region [Rodriguez et al. 2021]. There are 141 currently recognized triatomines species in the Americas, many of which can be infected by and transmit *T. cruzi* [Shofield &
Galvao, 2009]. Of those, 11 species are native to the United States, distributed across the southern half of the country from East to West [Bern, 2011]. Seven of these species have been collected in Texas and all have the potential to transmit *T. cruzi* [Sjo et al, 2009].

Transmission to mammals occurs after the introduction of infected triatomine fecal material into a wound or mucous membrane, as well as by the oral (consumption of foods and juices contaminated with *T. cruzi*-infected kissing bugs or their feces), congenital, and/or transfusion/transplantation routes [Cuora & Dias, 2009]. The nocturnal triatomine vector, also known as “kissing bug”, serves as the main mode of transmission, particular in established sylvatic and domestic transmission cycles [Lazzari et al. 2013].

### 1.6 *T. cruzi* Life Cycle

The life cycle of *T. cruzi* as described by the Centers for Disease Control and Prevention (2015), begins with an infected triatomine vector taking a blood meal and releasing trypomastigotes in its feces near the bite site. Trypanosomes enter the host via mucosal membranes or through the bite site. Once the trypomastigotes are inside the host, they use the cells near the wound site to differentiate into intracellular amastigotes, multiplying by binary fission. Once they differentiate into trypomastigotes, they spread through the blood system. Trypomastigotes infect the cells throughout the body of the host and transform into intracellular amastigotes in new sites. Replication can only take place when parasite enters another cell or are ingested by a new vector. These ingested trypomastigotes by the vector are transformed into epimastigotes inside their midgut, where they multiply. In the hindgut, they differentiate into infective metacyclic trypomastigotes. Figure 1. *T. Cruzi* life cycle.
Figure 1. Trypanosoma cruzi life cycle.

Figure 2. T. cruzi vector: Kissing Bug
1.7 Clinical Manifestations of Chagas Disease

Chagas disease has two successive phases: an acute phase and a chronic phase. Most acute phases are asymptomatic or have non-specific symptoms. During the chronic phase patients may also be symptom-free but some may progress to clinical forms of the disease (cardiac, digestive and/or neurological), which can be life threatening if left undiagnosed and untreated (WHO, 2020).

The initial acute phase lasts for about two months after infection. During this phase, a high number of parasites circulate in the blood. In most cases, symptoms are absent or mild, but may include fever, headache, enlarged lymph glands, pallor, muscle pain, cough, difficulty in breathing, liver enlargement, generalized body swelling, diarrhea, heart inflammation (with chest pain and even heart failure) and, less frequently, meningoencephalitis (with seizures and even paralysis).

In less than 50% of people bitten by a triatomine bug, characteristic first visible signs can be a skin lesion (chagoma) or a purplish swelling of the lids of one eye (Romaña sign). The acute phase can occur at any age but is frequently more severe in children aged <2 years. The acute phase is followed by the chronic phase, during which parasites are hidden mainly in the heart and digestive muscle.

Different clinical forms may be observed: The indeterminate form, the most frequent form, asymptomatic and without apparent signs of disease, is typically found immediately after the acute phase and is life-long in most patients; the cardiac form occurs in up to a third of patients, affecting the heart’s electrical conduction system, causing arrhythmia, heart muscle disorder, heart failure and embolisms. The digestive form (generally enlargement of the esophagus and/or the colon), observed in the South of the Amazon basin, or a mixed form that affects the heart, the digestive system and the autonomic nervous system occurs in ≤15% of patients.
Depending on cardiac damage, the mortality rate in a period of 10 years may range from 9 to 85%. Patients usually die, by frequency order, from sudden death caused by arrhythmias, heart failure and vascular cerebral accident, often in early adult life. It is estimated that over 10 000 people die every year from clinical manifestations of Chagas disease, and more than 25 million people risk acquiring the disease (WHO, 2020).

Studying the roles of wild animals as reservoirs of leishmaniasis disease is becoming increasingly important as we further encroach on their territories, and as their lifestyles become more urban as they adapt to ours [Parsons, 2021], plus as mentioned before comes the important issue of climate change since these ectothermal arthropods will become more active within warmer regions.

Figure 3. CDC: Child with Romana’s Sign
2. Leishmaniasis

Leishmaniasis is one of the neglected tropical diseases caused by different species of the protozoan parasite *Leishmania spp.* which are an obligate intracellular protozoan parasite that is transmitted by the bite of infected female sandflies. Over 20 species of the parasite cause disease in both human and animals [Schroeder et Aebischer, 2011]. There are approximately 2.3 million cases per year, 300,000 of them being Visceral Leishmaniasis (VL) [WHO, 2020].

2.1 Leishmaniasis Worldwide

Leishmaniasis continues to pose a major public health problem worldwide [Okwor & Uzonna, 2016]. Current estimates show an annual global incidence of 0.2-0.4 million cases of visceral leishmaniasis (VL) and 0.7-1.2 million cases of cutaneous leishmaniasis (CL). Over 90% of VL occur in poor rural and suburban areas in six countries: Bangladesh, Ethiopia, Brazil, India, Sudan and South Sudan [Alvar et al. 2012]. Unlike VL, CL is more widespread and occurs in the Americas, the Mediterranean and the Western Asia. Globally, 70 – 75 % of CL cases occur in ten countries: Afghanistan, Algeria, Brazil, Iran, Peru, Ethiopia, North Sudan, Costa Rica, Colombia and Syria [Alvar et al, 2012].

Leishmaniasis has been so well documented in various American animal species that zoologist and parasitologists accept North America endemicity of leishmaniasis as a universal reality [McIlwee et al, 2018].

2.2 Leishmaniasis in Texas

In Texas, Leishmaniasis has become so prevalent that it is now reportable to the Texas DSHS. In 2018, McIlwee et al collected histopathologically confirmed cases from defined data sources resulted in the identification of 69 novel cases of leishmaniasis, 41 (59%) were
autochthonous, occurring in patients with no history of travel outside of the US. All identified cases of endemic leishmaniasis were from Texas, of these cases only 14 (20%) were reported to the Texas DSHS as required by law.

2.3 Leishmaniasis in El Paso, Texas

Mariscal & Armijos (2013) did a study where they tested spleen, skin and heart tissue from stray dogs and sylvatic animals through Polymerase Chain Reaction (PCR) and found 2 positive cases among 96 canines and 12 positive cases among 20 sylvatic animals. In 2015, Gonzalez et al collected tissues from 155 stary cats, 10 (12%) of those tested positive for *Leishmania spp.* In 2016, Matamoros worked with sylvatic animals and found that from 146 collected samples, 18 were positive for *Leishmania spp.* Until this date, no registered cases of leishmania in humans exists.

2.4 Leishmania spp. in Bats

Bats are associated with several zoonotic pathogens. Their reservoir potential may be heightened due to their ability to fly, highly gregarious social structures and long life spans [Hayman et al, 2013]. In contrast to other mammalian reservoirs, many bat species migrate long distances and have the potential to introduce exotic pathogens to new areas [Hodo, 2016].

Natural infections by various *Leishmania spp.* species have been repeatedly reported in domestic, peridomestic and wild animals, which dogs and rodents being the most commonly investigated animals and traditionally considered reservoirs [Aysheshm et al, 2015]. However recent investigation of *Leishmania* parasites in animals including hares [Jimenez et al, 2013] and marsupials have diverted attention to other possible sylvatic reservoir hosts in endemic leishmaniasis foci. Bats were also suggested as natural possible blood source for sandflies and
known to host several trypanosomes transmitted by sandflies. Importantly, being cave dwelling organisms, bats and sandflies frequently share living habitats where opportunity presents for sandflies to feed on bats [Aysheshm et al, 2015]. Several studies have been performed in the last decade with findings of Leishmania positive bats in Ethiopia, Mexico, Brazil and French Guiana.

Studying the roles of wild animals as reservoirs of leishmaniasis disease is becoming increasingly important as we further encroach on their territories, and as their lifestyles become more urban as they adapt to ours [Parsons, 2021], plus as mentioned before comes the important issue of climate change since these ectothermal arthropods will become more active within warmer regions.

2.4 Vector for Transmission of Leishmaniasis.

The parasite is transmitted to humans through the bite of infected female sandflies, Phlebotomus in the old world and Lutzomya in the new world. On average, the sand flies that transmit the parasite are only about one fourth the size of mosquitoes or even smaller, its blood meal is visible in its distended transparent abdomen [CDC, 2020].

Data on the biting rates of sand flies affecting humans and animals are scarce. Since adult females are the only hematophagous stage, biting rates in a particular area would be strongly correlated to the abundance of adult females. Sand flies require a minimum of six weeks to complete a life cycle, with adult activity and, consequently, parasite transmission being mostly nocturnal and typically seasonal [Alten et al, 2016]. The active season for adults in Europe spans from April to November depending on the latitude, with warmer regions having longer seasons and up to three generations between May and September [Alten et al, 2016]. Areas with longer activity periods have a greater incidence of sand fly-borne pathogens. Sand-fly-endemic areas
where a suitable vertebrate reservoir host species of the parasite is absent or rare may have high vector density and biting rates but may not sustain Leishmania transmission.

*Phlebotomus* spp. occur predominantly in warm, humid, tropical climates and semi-desert vegetation habitats, although a few species occur in temperate zones. They are able to colonize rural, peri-urban and urban areas. Sand flies require a humid microclimate for their eggs to develop and larvae need a cool, moist habitat with decaying debris. Adult sand flies often inhabit rock crevices, caves, and rodent burrows, and in peri-domestic settings rest in cool, dark and humid corners of animal shelters or human dwellings. Both rodent burrows and peri-domestic areas provide ready access to blood meals in addition to shelter from the elements [ECDC, 2020].

**LEISHMANIA LIFE CYCLE**

**Life Cycle**

![Leishmania spp. Life Cycle](image)

*Figure 4. Leishmania spp. Life Cycle*
2.5 Leishmania spp. Life Cycle

The Leishmania life cycle consists of two stages. It starts when the sandfly takes a blood meal and injects the promastigotes into the skin [CDC, 2020]. These promastigotes are phagocytized by the macrophages, where they transform from promastigotes to amastigotes [CDC, 2015]. They then multiply and infect the host’s cells and tissues. The sandfly then takes another blood meal and ingests infected macrophages. In the midgut of the insect, the amastigotes transform into promastigotes, divide and migrate to the proboscis of the sandfly to be ready to be injected into a mammal once again [CDC, 2015].

2.6 Leishmaniasis Clinical Manifestations

Clinical manifestations of VL cases include at least two of the following symptoms: persistent fever of more than 38 °C, hepatosplenomegaly, substantial weight loss, anemia, leukopenia, and lymph node enlargement. In order to confirm VL, two of three tests need to be confirmed which include: serology, demonstration of parasite by smear in tissue samples, and/or molecular techniques (Georgiadou et al., 2015). Pentavalent antimonials are the main treatment for CL in the New World. The dose range is 10-20 mg/kg/day for a minimum of 20 days. Due to the higher toxicity of Amphotericin B and 10 pentamidine, these are used only in cases with a contraindication, intolerance or resistance to antimonials (Pech-May et al., 2013).

Figure 5. Cutaneous Leishmaniasis Dermal Lesion
3. Environmental Factors

3.1 Climate Change

Globally, vector-borne diseases pose a serious and increasing problem to public health. Today, almost one third of the emerging cases of infectious diseases are vector-borne [Fischer et al. 2011]. Most of the disease vectors are ectothermal arthropods which cannot regulate their body temperature themselves. Therefore, climate change may be associated with spatio-temporal variations in occurrences of vector-borne diseases [Fischer et al. 2011]. Climate models predict that the number of citizens exposed to vector-borne diseases will double by 2080 [McIlwee, et al. 2018].

3.2 Social and Economic Burden of Leishmaniasis and Chagas Disease

Leishmaniasis affects mostly people living in the most impoverished parts of developing countries and places for their economic stress on already strained meagre financial resources [Okwor & Uzonna, 2016]. Compared to other diseases, treatment of leishmaniasis is expensive ranging from 30 to 1,500 dollars for the drugs alone [Alvar et al. 2006]. The average cost of treatment of an episode of Visceral Leishmaniasis (VL) was shown to be 17.5% while the average total cost for the entire duration of the disease was 44% of the average household income [Adhikari et al. 2009].

The connection between poverty and the risk of developing leishmaniasis is very strong and mediated through some factors: ecological factors such as poor housing conditions, including cracked walls that provide resting places for the sandfly, damp earthen floors that enhance vector survival and improper doors that allow sandfly entry [Okwor & Uzonna, 2016]. Poor sanitation and irregular garbage collection, provide sandfly breeding grounds. Although insecticides bed nets
have shown to be effective in the control of leishmaniasis, as evident in a study conducted in Afghanistan, 78% of respondents reported that they cannot afford bed nets [Reithinger et al, 2005].

Leishmaniasis also negatively impacts on the psychological and social status of women [Okwor & Uzonna, 2016]. The disfiguring scars lead to social stigmatization, exclusion from community activities precipitating psychological problems.

Chagas disease is responsible for the greater disease burden than any other parasitic disease in the new world. Rural populations that live in areas with ecology that is hospitable to vector infestation have a disease prevalence that is many times that fund among urban populations [Okwor & Uzonna, 2016]. Also, immigrant populations who come from specific regions may have a higher or lower risk of T. cruzi infection, compared to the national population.
STUDY RATIONALE

Based on a previous publication by Mariscal et al. (2013), 20 sylvatic animals were collected in the El Paso del Norte Area to test their tissue samples through PCR. From these animals, 13 (65%) reported to be positive for Chagas’ disease and 1 (5%) where positive for *L. mexicana*.

*T. cruzi* is also endemic to Texas and wild animals, such as mice and woodrats from the region have been found to be hosts of both *T. cruzi* and *Leishmania spp*. It has been known for *Leishmania spp.* to be autochthonous in Texas. In recent years it has also been found in northern states of the United States. As previously mentioned, infections caused by these parasites are neglected diseases that often occur in low resource countries. As public health professionals, it is important to determine if *Leishmania spp.* and *T. cruzi* are present in El Paso County area to promote awareness to inform the community that these diseases may no longer be recognize as a tropical or travelers’ diseases. For this reason, it is important to educate the population on how to prevent them and be on the lookout for these parasites, their vectors and hosts. It is also important to identify the reservoirs to be able to respond and most importantly, prevent these diseases.

**Problem Statement**

In the last decade, three studies have been conducted with the intention to identify *Leishmania spp.* and *T. cruzi* in dogs, cats and sylvatic animals. These studies have provided evidence that these parasites are found in this border region and that dogs, cats and sylvatic animals are reservoirs for these parasites. However, bats have not been studied in this region even though bats are mammals that migrate every year from South America where bats have been documented to be novel reservoirs for *Leishmania spp.* and known reservoirs for *T. cruzi*. In the city of El Paso,
there are no routine surveillance studies being conducted in the city of El Paso, Tx. to monitor either Leishmaniasis or Chagas disease.

Research Question

What is the frequency of sylvatic bats infected with *Leishmania* spp. and *Trypanosoma cruzi* parasites in the region of El Paso, Texas?

Overall Aim of the Research

The goal of this study is to determine if sylvatic bats are infected with *T. cruzi* and *Leishmania* spp. parasites in the El Paso, Texas region.

Objectives

1. To determine the presence of *Leishmania* spp. and *T. cruzi* parasites in the El Paso region by testing the heart, spleen and if dermal lesions observed skin tissue from bats captured in the region.

Expected results

Based on a previous study by Matamoros, 2016, it is expected to identify 65% or more of the collected animals to be positive for *Trypanosoma cruzi* and 10% or more to be positive for *Leishmania* spp.
METHODOLOGY

Study Design

This study is a cross sectional, observational study collecting tissue samples of wild bats for a period of 5 months. These samples were collected to represent the bat population in El Paso, Texas region.

This study was approved by the UTEP Institutional Biosafety Committee Protocol# 1659945-1

Sample population

Bats were donated and delivered in conical tubes by TDSHS, Zoonosis Control, Animal Services. Mentioned bats will previously undergo a rabies test performed by Zoonosis control and the ones that are negative will be donated for this study. Tissue samples were obtained from the heart, spleen and skin of each bat to measure the frequency of DNA testing positive for Leishmania spp. and/or Trypanosoma cruzi. Samples were collected for a period of 5 to 6 months These tissues have been chosen because of its relationship to Trypanosoma cruzi and Leishmania spp. T. cruzi can infect all the tissues of its mammalian host mainly affecting the heart and skin in a chronic manner (Noireau et al, 2009). The spleen plays an important role in the immune system by producing white blood cells which help fighting infections and synthesize antibodies. Because of this, a symptom of individuals with Leishmaniasis is having enlarged lymph nodes, given that white blood cells are fighting the disease. Given that CL develops skin lesions, bats were visually inspected for dermal lesions; tissue samples were also collected from the area the lesion or abnormality is present. Approval from the Institutional Biosafety/Recombinant DNA committee from the University of Texas at El Paso was obtained to comply with university policies and be able to work with tissue that could possibly be infected with T. cruzi and/or Leishmania spp.
Materials

Data Sheet

A data collecting sheet was created to track the bats that were dissected and to document their characteristics such as species and any external or internal abnormalities found as well as their ID number to later request TDSHS for the coordinates or location where the bat was captured from.

<table>
<thead>
<tr>
<th>DATE</th>
<th>ID #</th>
<th>SPECIES</th>
<th>WEIGHT</th>
<th>COMMENTS</th>
</tr>
</thead>
</table>

Equipment

The following materials were used during DNA extraction, PCR technique and electrophoresis procedure:

- Laminar flow hood
- Dissection kit
- Zymo Quick DNA tissue/insect 96 kit for DNA extraction
- Micropipettes with sterile tips with filter
- GentleMACS M – Tubes
- GentleMACS tissue dissociator
- Microtubes
- Centrifuge for microtubes and 96 well plate
- VortexGenie
- Nanodrop ND-1000
- Bio-Rad Thermal Cycler
- Agarose
- Electrophoresis chambers
- iBrightFL1000 Invitrogen imaging system.

The following organs were dissected from each bat under a laminar flow safety hood: the heart, spleen and if any dermal lesions and/or abnormalities found in the skin, a sample was taken directly from the lesion. The organs were weighted and placed in a labeled M-tube with Tissue Lysis Buffer (TLB) 200 µL per 50 mg, after this it was homogenized in the gentle MACS tissue dissociator and placed in -80º C freezer. The bat corpse was kept in a -20 º C freezer until the end of this study. All tissues were incinerated.

Once enough tissue samples were collected, I utilized Zymo Quick DNA tissue/insect 96 kit for DNA extraction and followed the protocol. Once DNA was extracted, NanoDrop® ND-1000 was used to determine the DNA concentration.

Bio-Rad® Thermal Cycler was used to run the PCRs and primers were ordered from Integrated DNA Technologies (IDT) and finally electrophoresis was performed in chambers available at the biology laboratory.
**DNA Extraction**

Genomic DNA was extracted from the tissue samples collected from each bat corpse. Each spleen, heart and skin samples were placed in an M tube with Tissue Lysis Buffer (TLB) 200 µL per 50 mg. After this, samples were homogenized in the gentle MACS tissue dissociator and stored at -80 C. Genomic DNA was extracted following the Zymo DNAKit recommendations. Briefly, 500 µL of 2-mercaptoethanol were added to the Genomic Lysis Buffer (GLB). Then 200 µL of tissue sample were taken from the M tube homogenized sample and placed in a microtube. 360 µL of GLB were then added and vortexed followed by a centrifugation at 10,000 g for 5 minutes. 600 µL were then placed in a silicon plate for 66 samples accordingly and centrifuged to 2,500 g for 5 minutes. After centrifugation, 200 µL of DNA prewash buffer was added to every sample well, and the plate was centrifuged at 2,500 g for 5 minutes. Then, 300 µL of DNA wash were added and centrifuge once. Lastly 30 µL of DNA elution buffer were added to elution plate and incubated for 5 min. All wells with DNA product were transferred to a previously labeled microtube.

DNA concentrations, quality and purity were measured using NanoDrop® ND-1000. Samples were diluted with nuclease free water to a final concentration of 100 ng/µL. Samples were then ready to be used as templates for PCR or store at 4 C until use.

**Primers and PCR Protocols**

PCR reactions included 12.5 µL of PCR master mix (Promega), 1 µL of reverse primer and 1 µL of forward primer, 8.5 µL of nuclease free water and 2 µL of DNA template to have a final volume of 25 µL per tube. Primers 121 and 122 were used to amplify 330 bp of the kinetoplast minicircle DNA from *T. cruzi*. PCR conditions were as follow: initial denaturation at 94° C for 3 min, 40 cycles of denaturation at 94° C for 30 sec, annealing at 57° C for 30 sec, and extension at 72° C for
40 sec. Extension at 72°C for 7 min and end with 4°C. *T. cruzi* (dm28c) DNA was used as positive control and nuclease free water as negative control. Controls were included with every run. Primers LITSR and L5.8S were used to amplify 320 bp of the ITS1 region of any *Leishmania spp.* DNA. PCR conditions were the following: Initial denaturation at 95°C for 2 min, 34 cycles of denaturation at 95°C for 20 sec, annealing at 53°C for 30 sec, and extension at 72°C for 1 min, extension at 72°C for 6 min and end with 4º C. *L. donovani* DNA was used as a template for the positive control and nuclease free water for the negative control. Controls were included with every run.

PCR products were ran in a 2.0 % agarose with DNA dye at 90 volts for 120 minutes and bands were visualized with iBrightFL1000 Invitrogen imaging system.

**Table 2. Gene and Primers utilized for PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Parachute</th>
<th>Primer</th>
<th>Sequence</th>
<th>Product Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>kDNA</td>
<td><em>T. cruzi</em> (dm28c)</td>
<td>121</td>
<td>5’-AAATAATGTACGGGGGAGATGCATGA-3’</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>122</td>
<td>5’-GGTTCGATTGGGGGTGGTTGTAATATA-3’</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>330</td>
</tr>
<tr>
<td>ITS1</td>
<td><em>Leishmania spp.</em> (L. donovani)</td>
<td>LITSR</td>
<td>5’-CTGGGATCATIITCCGATG-3’</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L5.8S</td>
<td>5’-TGATACCATATTATCGACIT-3’</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>320</td>
</tr>
</tbody>
</table>

Samples that tested positive through PCR were not subjected to sequencing due to the limitation in time secondary to COVID-19 pandemic.
RESULTS

T. cruzi PCR Results

Tissues collected from the 32 bats were all tested for *T. cruzi*. Out of those tested, 19 (59%) were cases found to be infected with *T. cruzi* in the El Paso region.

Leishmania spp. PCR Results

Tissues collected from the 32 bats were all tested for Leishmania spp. Out of those tested 5 (16%) were cases found to be infected with Leishmania spp. in the El Paso region.

<table>
<thead>
<tr>
<th>Species</th>
<th><em>T. cruzi</em> Status P/T (%)</th>
<th><em>L. spp</em> Status P/T (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Yellow Bat (<em>Lasiurus xanthinus</em>)</td>
<td>7/13 (54)</td>
<td>1/13 (8)</td>
</tr>
<tr>
<td>Pallid Bat (<em>Antrozous pallidus</em>)</td>
<td>2/3 (67)</td>
<td>0/3 (0)</td>
</tr>
<tr>
<td>Hoary Bat (<em>Lasiurus cinereus</em>)</td>
<td>2/4 (50)</td>
<td>0/4 (0)</td>
</tr>
<tr>
<td>Canyon Bat (<em>Parastrellus hesperus</em>)</td>
<td>2/3 (67)</td>
<td>2/3 (67)</td>
</tr>
<tr>
<td>Western Red Bat (<em>Lasiurus blossevillii</em>)</td>
<td>1/2 (50)</td>
<td>0/2 (0)</td>
</tr>
<tr>
<td>Mexican Free Tail Bat (<em>Tadarida brasiliensis</em>)</td>
<td>1/5 (20)</td>
<td>1/5 (20)</td>
</tr>
<tr>
<td>Silver Haired Bat (<em>Lasionycteris noctivagans</em>)</td>
<td>4/4 (100)</td>
<td>1/4 (25)</td>
</tr>
</tbody>
</table>

**TOTAL** 19/32 (60%) 5/32 (16%)

* Positive/Total of bats available.
Location of the bats

Spatial distribution of the bats was not significant, there is no obvious pattern. We must also note that the bats were collected randomly among the city of El Paso, Texas and were also picked up from residences that had reported bats in their homes.

Bats infected with *T. cruzi* were found on zip codes: 79932, 79903, 79938, 79932, 79936, 79905, 79912, 79902, 79934, 79907.

Bats infected with *Leishmania spp.* were found on zip codes: 79938, 79912, 79922.

3 bats tested positive for both parasites. They were found on zip codes: 79938 and 79912.

Figure 6. Positive cases of *T. cruzi* and *Leishmania spp.*
DISCUSSION

Based on PCR results, the expected results were different from the final results. The percentage of positive results for *T. cruzi* was 60% compared to the expected results of 65%. Leishmania spp. results were also different and much higher than expected with 16% of the samples being positive, compared to the expected 10%. There are 14 different bat species in the city of El Paso but only seven bat species were studied in this research. Our of 32 bats, 24 (75%) were identified as reservoirs for *T. cruzi* and/or Leishmania spp. via PCR.

The prevalence of *T. cruzi* infection in humans and animals in the United States has been studied more recently because of its possible emergent public health threat in the southeast and southwest regions (Garcia et al. 2014). Most recently, Rodriguez et al. (2021) demonstrated that El Paso County and surrounding communities are high risk areas for *T. cruzi* transmission to humans, feral cats and dogs and wild animals (Rodriguez et al. 2021) due to the active presence of the triatomine insects in our region. These results were expected to have a high prevalence due to this last fact and that the bats in this research feed of insects, live in the same environment as the kissing bug as well.

Annually in April, bats migrate to El Paso, Texas from South America. These bats feed, reproduce, and stay all through summer leaving by October. As climate change takes more and more effect, this period may extend. Almost one third of the emerging cases of infectious diseases are vector-borne [Fischer et al. 2011]. Most of the disease vectors are ectothermal arthropods which cannot regulate their body temperature themselves. Therefore, climate change may be associated with spatio-temporal variations in occurrences of vector-borne diseases [Fischer et al. 2011]. Climate models predict that the number of citizens exposed to vector-borne diseases will double by 2080 [McIlwee, et al. 2018]. Today we are witness to a more extended raining seasons
creating floods and hence a humid environment giving the perfect conditions for ectothermal arthropods and other vectors to sustain life.

Thirteen bats were identified as Western Yellow Bat (Lasiurus xanthinus), from these, 7 tested positive to T. cruzi infection and 1 tested positive for Leishmania spp. infection. Canyon Bat (Parastrellus Hesperus) reported 2 positive cases for both parasites, the canyon bat is known to be the smallest bat species, they feed during daylight and nighttime according to their necessities. The pallid Bat (Antrozous pallidus) can hear its next meal’s footsteps on the ground, known to feed of scorpions and sometimes snakes and desert mice, we had 3 specimens and 2 tested positive for T. cruzi, pallid bats are prey for dogs, owls, skunks, foxes and coyotes. There were 4 Silver haired bats (Lasionycteris noctivagans), one of the most abundant bats in forested areas of the northern United States and Canada, all 4 of them tested positive for T. cruzi and 1 tested positive for both parasites. Mexican Free Tail Bat (Tadarida brasiliensis) we received 5 specimens out of which one was positive for T. cruzi and another one for Leishmania spp., this bat is known to migrate from South America and has been presented as Leishmania spp. reservoir in South America.

Although this study presents clear evidence that these bats are potential reservoirs for Leishmania spp. and T. cruzi parasites and it may serve as a platform for continued research and surveillance for both parasites in this border area and complete. DNA sequencing which was missing from this study due to some limitations (COVID-19 pandemic, time, material present in the laboratory and convenient sampling).
CONCLUSIONS

Annually in April, bats migrate to El Paso, Texas from South America. These bats feed, reproduce, and stay all through summer leaving by October. This period may extend due to climate change.

It is essential to teach the El Paso population on the signs and symptoms of these diseases. Having health care providers actively looking for symptoms for leishmaniasis or Chagas’ disease is important given that some of the symptoms can be easily confused with other diseases.

It is also important to teach the population via vector borne campaigns about these parasitical diseases for them to be able to recognize the threat and respond in the event of presence of symptoms, recognize a kissing bug or apply preventable measures such as fumigation services by experienced exterminators in their home and to keep their pets clean, acquire good veterinarian healthcare for their pets especially if they have an exotic pet like a racoon, a fox or owls, all predators of bats. The public knows bats may transmit rabies virus, now they need to be educated on the fact that bats may serve as reservoirs for parasites as well, and that even though humans rarely become in close contact with them, bats can be in contact with other mammals close to humans such as dogs.

This study presents clear evidence that these bats are potential reservoirs for *Leishmania spp.* and *T. cruzi* parasites. Surveillance and awareness must be promoted in our border region as leishmaniasis and Chagas disease may no longer be considered only tropical diseases or travelers’ diseases. This study may serve as a platform from which to continue research and surveillance for both parasites.
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CURRICULUM VITA

Edith Sandoval was born in El Paso, Tx and like many others in our border city, was raised in Cd. Juarez, Chih. Daughter to an Industrial Engineer and a Soldier for the US ARMY, she grew up with the best of both worlds. She exhibited interest for biological sciences from a young age and volunteered at the Mexican Red Cross as a paramedic until the age of 18 when she was admitted to Medical School where she was awarded for two cardiac physiology studies. At age 26 she received her Medical Doctor degree by the Universidad Autonoma de Ciudad Juarez (UACJ) and soon after accomplished her dream of giving back to her Alma Mater by becoming a Human Physiology Professor. She came back to El Paso, Texas to pursue higher education by applying to the University of Texas at El Paso (UTEP) Master of Public Health program given her passion to make a difference in the border community’s health.

She wishes to continue her medical career focusing on Infectious Disease Epidemiology.