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Identification of Leishmania spp. and Trypanosoma Cruzi in Sylvatic Animals in El Paso County, Texas

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IDENTIFICATION OF *LEISHMANIA SPP.* AND *TRYPANOSOMA CRUZI* IN SYLVATIC
ANIMALS IN EL PASO COUNTY, TEXAS

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IDENTIFICATION OF *LEISHMANIA SPP.* AND *TRYPANOSOMA CRUZI* IN SYLVATIC
ANIMALS IN EL PASO COUNTY, TEXAS

by

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THESIS

Presented to the Faculty of the Graduate School of
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Abstract

Background Leishmaniasis and Chagas's Disease are two of the seventeen diseases considered as Neglected Tropical Diseases (NTD) by the CDC. Ten million people worldwide are at risk of being infected by Leishmaniasis and Chagas' disease affects six million people in the world; however, these are mostly cases in Latin America. The vector for Chagas' disease, triatomine, has been identified near El Paso, Texas region to be positive for *Trypanosoma cruzi*. Recent studies have also shown positive results for Chagas' disease and Leishmaniasis in sylvatic animals in El Paso.

Objective To determine the prevalence of *Trypanosoma cruzi* and/or *Leishmania spp.* infection in collected tissue samples from sylvatic animals, a study was conducted in the El Paso County Region to 1) identify DNA of *Leishmania spp.* and *T. cruzi* in tissue samples from sylvatic animals such as foxes, coyotes, skunks and raccoons, and 2) identify and locate positive cases on a El Paso county map to determine the geographical areas where the animals were captured as a mean to identify potential locations for the presence of the vector insect.

Methods This study is a cross sectional study analyzing extracted DNA collected from tissue samples from spleen, heart and skin of wild animals captured in El Paso County, Texas region during an 18-month time period. Polymerase Chain Reaction (PCR) was used as the method of identification using TCZ primers for *T. cruzi*, ITS primers for *Leishmania spp.*, and IRBP primers to identify mammalian cells in the extracted DNA samples. PCR samples were run in 1.8% agarose gels.

Results Out of the 146 collected samples, 114 were considered as viable samples given that thirty were negative for mammalian DNA. Of these, the total number of positive samples

was thirty-three (40.24 prevalence) for *T. cruzi* and eighteen (21.95) for *Leishmania spp.* 9 (10.98) samples were identified as positive for both parasites. Regarding species, three striped skunks (12.50) tested positive for *T. cruzi* and none of samples tested positive for *Leishmania spp.*; eighteen gray fox samples (48.65) tested positive for *T. cruzi*, four (10.81) for *Leishmania spp.*, and of those four (10.81) had both diseases; five raccoon (35.71) samples tested positive for *T. cruzi*, ten (71.43) for *Leishmania spp.*, and five (35.71) for both; lastly, seven coyote (100.00) samples tested positive for *T. cruzi*, four (57.17) for *Leishmania spp.*, and four (57.17) tissues samples for both.

Conclusions As seen in previous studies, the prevalence of Chagas' diseases in sylvatic animals in El Paso is higher than that of Leishmaniasis. However, the prevalence found for *Leishmania spp.* is higher than was reported in previous studies for the area of El Paso, Texas. The distribution map showed that positive samples for *Leishmania spp.* and *T. cruzi* were mostly found in suburban areas with low population density. Furthermore, active surveillance for these diseases is needed. Also, it is necessary to educate the El Paso community on how to prevent infections and what to do in case any of these symptoms are noticed. Health care providers should consider symptoms for other common diseases such as cardiomyopathy and lymphadenopathy as also a symptom for Chagas' disease or Leishmaniasis.

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Introduction

According to the Centers for Disease Control and Prevention (CDC) (2013), there are seventeen diseases considered as Neglected Tropical Diseases (NTD). These are diseases that are not present in developed countries, but are continuously seen poorer countries. These parasitic and bacterial diseases are presented in every low income countries and are affected by at least five of these NTD. A hundred and forty-nine counties are affected by at least one NTD and individuals are often affected by more than one of these diseases. NTD affects more than one billion people and kills half a million people around the world every year. Only six out of the seventeen diseases may be controlled or even eliminated through medicine. These diseases not only have physical effects, but also create a social stigma. They are disfiguring, debilitating, and sometimes deadly making it difficult to work and keeping the poor in a poverty cycle (Centers for Disease Control and Prevention, Neglected Tropical Diseases, 2013).

The World Health Organization (WHO) (2013), estimates three hundred and ten million people at risk of being infected with leishmaniasis, one million cases of cutaneous leishmaniasis reported, and three hundred thousand cases of visceral leishmaniasis with twenty thousand deaths annually around the world. Chagas' disease affects between six and seven million people worldwide and are mostly cases in Latin America (WHO, 2013). There have been studies done around the El Paso County where the vector for Chagas' disease has been found testing positive for *Trypanosoma cruzi* (Buhaya, Galvan, & Maldonado, 2015). This study is based on previous research by Mariscal et al. (2013) that show positive identification for leishmaniasis and Chagas' disease in animals. The aim of this study is to identify *Leishmania spp.* and *T. cruzi* in tissue

samples of sylvatic animals. Based on the previous studies mentioned it is expected to find positive results for these diseases though DNA amplification methods.

Background and Significance

1. Leishmaniasis

Leishmaniasis is considered by the CDC as one of the Neglected Tropical Diseases. There are about 20 different Leishmaniasis diseases caused by parasites transmitted through the bite of the female sandflies of the genus *Phlebotomus*, in the Old World, and *Lutzomia*, in the New World. The major forms of Leishmaniasis include visceral (VL), cutaneous (CL), and mucocutaneous (MCL). VL is the most severe form of Leishmaniasis that almost always fatal if untreated, and some symptoms include fever, weight loss, splenomegaly, hepatomegaly and anemia. CL, although not deadly, develops into skins lesions leaving disfiguring scars around the body, including the face, creating a stigma. Some forms of CL convert into diffused cutaneous leishmaniasis (DCL) creating several lesions throughout the body (Desdeux, 1996).

According to Alvar et al. (2012) due to the intricate epidemiology and ecology of the *Leishmania spp.*, Leishmaniasis is the ninth infectious disease causing the most burden, and yet it is greatly ignored as a priority of tropical diseases. Also, there are not many tools to easily manage cases or to keep track of the incidence data. An additional complication is the poor ability of policy makers to recognize the burden Leishmaniasis causes in individuals and their communities (Alvar et al., 2012)

1.1 Leishmaniasis Worldwide

Estimates collected through the World Health Organization (WHO) between the years 2007 and 2011, estimated 0.2 to 0.4 million cases of VL, and 0.7 to 1.2 million cases of CL (Alvar et al., 2012). VL is endemic in 88 countries. It is also the most severe form of

leishmaniasis and it is listed as one of the most important diseases by the WHO, Tropical Disease Research. VL is caused by *L. infantum*, *L. donovani* and *L. chagasi*. (Sharma & Singh, 2008). About 90% of VL cases in the world occur in the countries of India, Bangladesh, Sudan, South Sudan, Ethiopia, and Brazil (Alvar et al., 2012). Worldwide endemicity of VL can be seen in Figure 1: Geographical Distribution of Visceral Leishmaniasis (WHO, 2013).

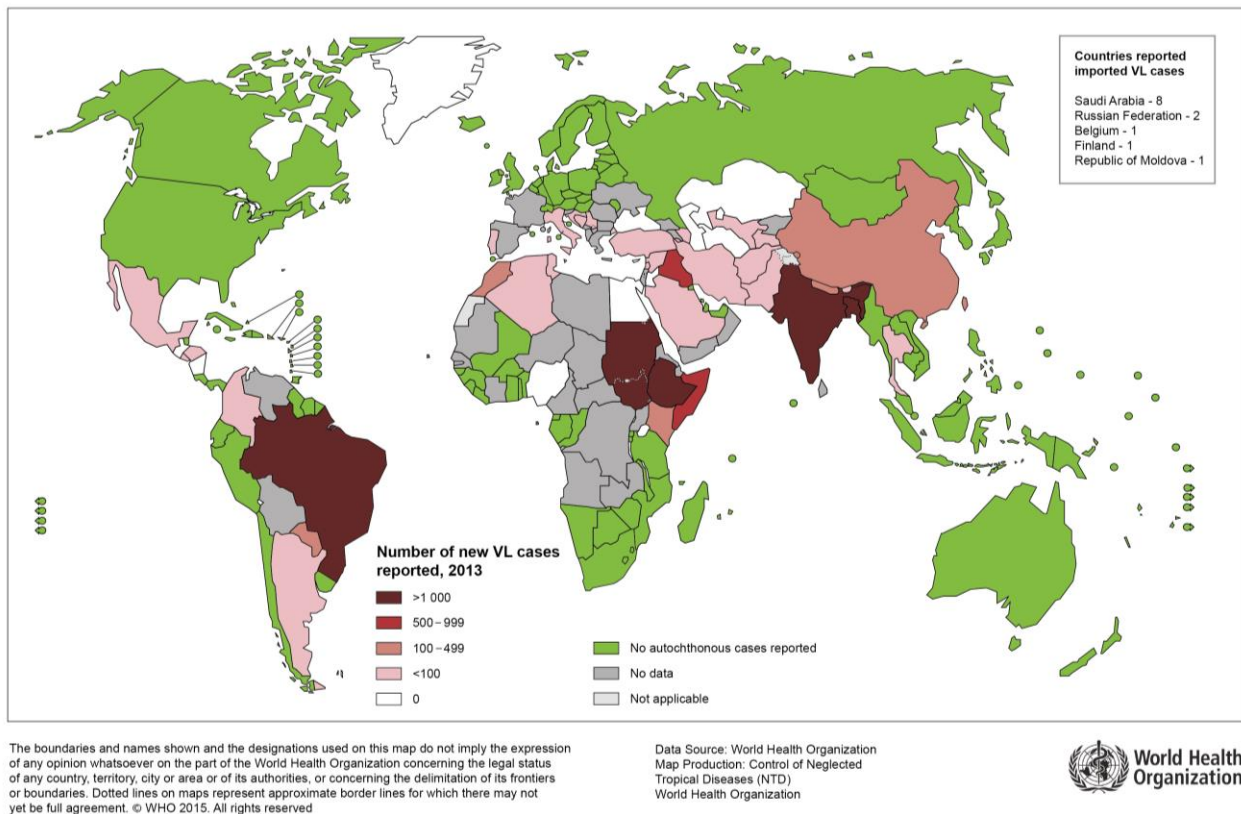


Figure 1: Geographical Distribution of Visceral Leishmaniasis (WHO, 2013).

CL cases are found in more areas of the world, and it is estimated that more than 70% of the cases in the world are found in Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, North Sudan, Costa Rica and Peru (Alvar et al., 2012). Data on mortality is scarce and the data available are mostly of cases reported from hospitals (Alvar et al., 2012). *Leishmania mexicana*, a form of CL, can be found in South America, Mexico and the United States (Reithinger et al.,

2007). Of the *Leishmania* spp., 21 have been identified as pathogenic to humans. CL is caused by *L. major*, *L. tropica*, *L. mexicana*, and *L. amazonensis* (Sharma & Singh, 2008). Leishmaniasis has 10% case-fatality rate, estimating 20,000 to 40,000 deaths a year (Alvar et al., 2012). Figure 2 shows the Geographical distribution and endemicity of CL (WHO, 2013).

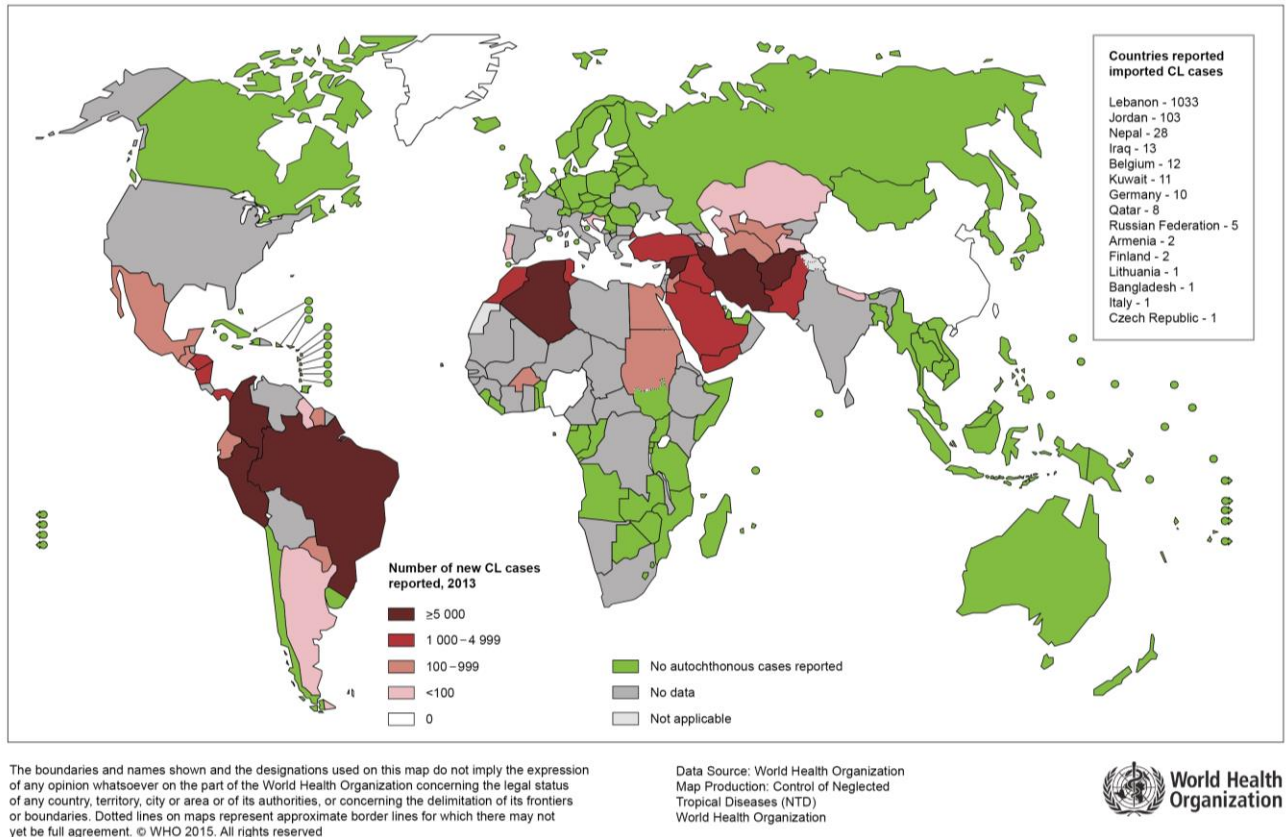


Figure 2: Geographical Distribution of Cutaneous Leishmaniasis (WHO, 2013)

1.2 Leishmaniasis in the United States and in Texas

Canine cases have been reported in the United States in the states of Texas, Oklahoma, and Ohio (Kerr, McHugh, & Merkelz, 1999). Woodrats, *Neotoma albigula*, *L. mexicana* positive results using PCR were reported in Arizona (Kerr et al., 1999). *Leishmania infantum* has also

been found in foxhounds that have been naturally infected in parts of Canada and in Virginia in the U.S. (Rosypal et al., 2003).

Although leishmaniasis is seen every year in the United States among foreign travelers, immigrants, and in members of the military, it is not seen in people with no travel history outside the country. However, Texas is considered an endemic area since there have been reports of 30 autochthonous cases in the United States and they are all cases from Texas throughout the south central area. Nonetheless, in 2010, there were reports of nine autochthonous cases of CL in northern Texas of patients with no travel history to endemic areas (Wright, Davis, Aftergut, Parrish, & Cockerell, 2008).

In the years of 1988 and 1992, there were two cases reported in Brown County in Central Texas. Albany, Shackelford County had another case in November 1994 (Wright et al., 2008). Texas has had documented nine cases of CL between 2005 and 2007 as shown in Figure 3. The black circles in the map represent the 30 indigenous cases of cutaneous leishmaniasis (CL) reported in south-central Texas since 1903. Red circles are 9 cases of CL concentrated in Dallas-Fort Worth metroplex and surrounding counties (McHugh, Melby, & LaFon, 1996).

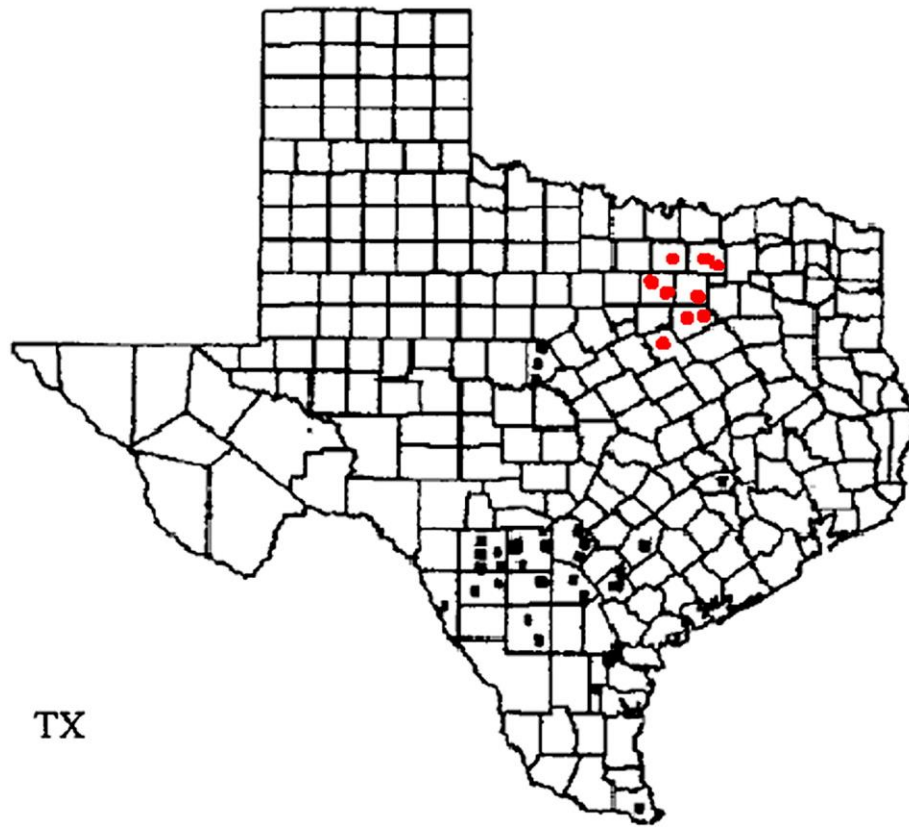


Figure 3: Geographic distribution of Cutaneous Leishmaniasis cases in Texas

1.3 Life cycle of *Leishmania*

The *Leishmania* parasites are found in two developmental stages, promastigote and amastigote. The promastigote is the proliferative stage and it is found in the mid gut of the female sandfly. The amastigote is the proliferation stage and it is found inside of mammalian host cells (Teixeira et al., 2013).

(Teixeira et al., 2013) describe the life cycle of *Leishmania spp.* beginning by an infected mammal being bitten by a female sandfly during blood meal and then becomes infected with the amastigote form of *Leishmania*. The amastigote is then transformed into procyclic promastigotes that then multiply in the midgut of the sandfly. Promastigotes transfer to the anterior midgut into the stomodea valve and cell division is reinitiated transforming into metacyclic promastigotes.

The female sandfly will then release the promastigote into a new mammalian host during a blood meal. These metacyclic promastigotes will infect macrophages to later be transformed into amastigotes attaching to the parasitophorous vacuole and begin multiplication inside the vacuole. The large multiplication of amastigotes will cause the cell to burst out and the cycle of multiplication will repeat (Teixeira et al., 2013). Figure 4 is a graphic representation by the CDC of the life cycle of the *Leishmania spp.*

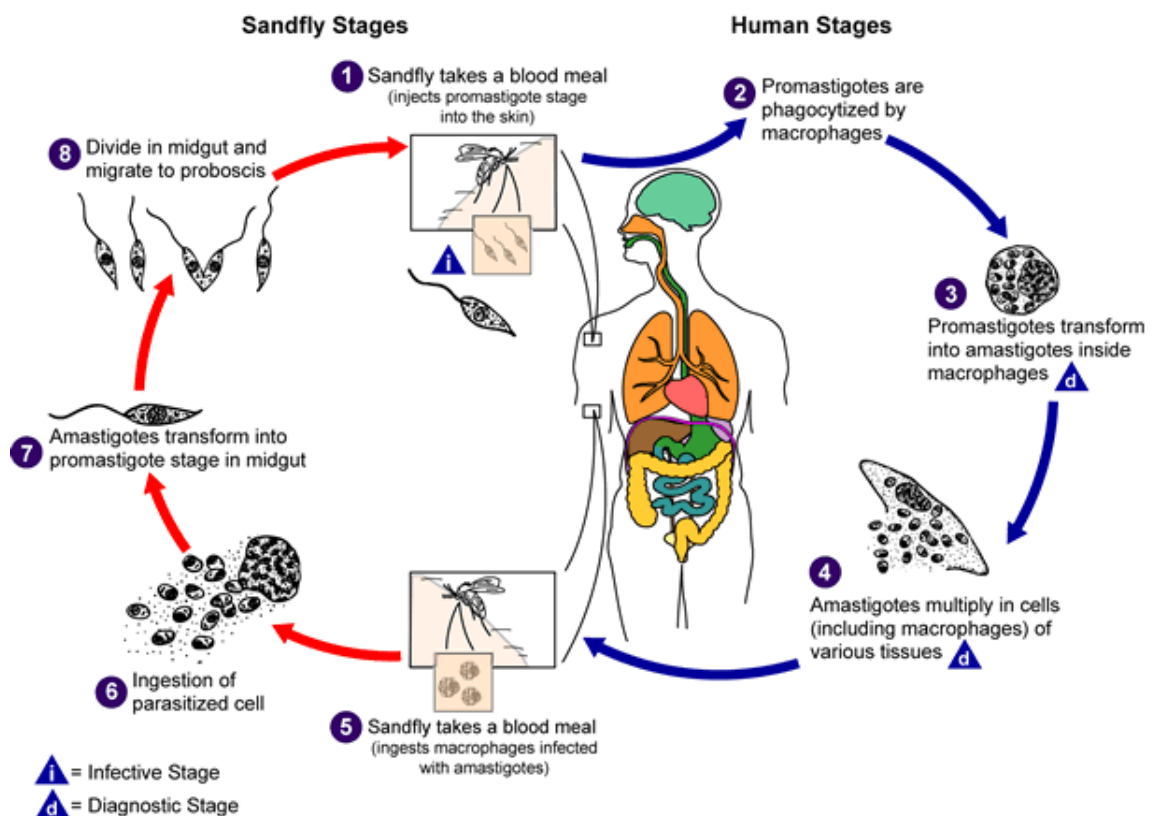


Figure 4: *Leishmania spp.* Life Cycle

1.4 Vectors and Reservoirs

Leishmaniasis is usually spread through the bite of phlebotomine sandflies. Specie genetic variation and population separation in sandflies is due to several factors. These include climate, distance, differences in latitude or altitude, habitat modification such as domestic, peri-

domestic, or sylvatic, and other geographical barriers (Pech-May et al., 2013). *Lutzomia diabolica* is known to transmit Leishmaniasis to mammals including rodents and humans and it has been found in south Texas (McHugh, 1991). *Lutzomia cruciata* ranges from Panama and Mexico to Texas, Georgia, and Florida in the United States (Pech-May et al., 2013). Sand flies have a limited flight range of 6 to 10 meters, which allows them to fly on average 100 meters, but cannot travel more than 1000 meters from their breeding site (Pech-May et al., 2013). *Lutzomia olmeca olmeca* has also been identified as a vector for *L. mexicana* in the U.S. (Sharma & Singh, 2008).

Neotoma species including, *N. micropus*, *N. albigula*, and *N. floridiana* are known to be hosts of *L. mexicana* (McHugh et al., 2003). Predictions by McHugh et al. (1996) reports that *L. mexicana* could be found in most of the southern United States, ranging from southern California to Florida, including the southern regions of Colorado, Utah, Nebraska and North Carolina (McHugh et al., 1996).

1.5 Clinical aspects of Leishmaniasis and treatment

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

pentamidine, these are used only in cases with a contraindication, intolerance or resistance to antimonials (Pech-May et al., 2013).

2. Chagas' disease

Dr. Joseph Reinhardt Cooper first described the epidemiology and clinical presentation of the disease known in Portuguese as “mal de engasgo” or “evil of choking” in the 1850s. He worked in the cities of Limeria and Campinies, in Sao Paulo where he closely studied cases of this disease (Meneghelli, De Rezende, Troncon, Madrid, & de Moura, 1997). Carlos Chagas, a physician in Brazil, discovered the cause of this disease in 1909, when the protozoan parasite, *Trypanosoma cruzi*, was identified in a Brazilian child's blood. This parasite had been previously reported by Carlos Chagas in the intestine of a triatomine insect in 1908 (Woody & Woody, 1955). The discovery of Chagas disease was due to the malaria outbreak among workers of the Central Railway of Brazil. After Dr. Chagas had spent over a year collecting data even from cases in mountainous regions where malaria is not possible to be found, in the town of Piropora, he was introduced to the kissing bug. He later dissected this insect finding *T. cruzi* in the posterior of the intestine (Chagas, 1922).

2.1 Chagas' Disease Worldwide

Chagas' disease is an important public health problem affecting large population sectors predominantly rural and suburban in Latin America. It is a chronic infection difficult to diagnose, manage and treat, and plays a major role in the morbidity, mortality, and disability for endemic regions (Jannin & Salvatella, 2006). Chagas' disease is transmitted through vectors, during transplants and transfusions. Endemic growth is also due to low socioeconomic and cultural levels having a large impact over the health, well-being, and economy of Latin-American countries (Jannin & Salvatella, 2006). An estimated 12 million Latin Americans are positive for

Chagas' disease, and approximately of 30% of the cases develop cardiomyopathy, which can be life-threatening (Doyle, Zhou, et al., 2007).

2.2 Chagas' Disease in the U.S. and in Texas

Chagas disease serious public health concerns given that its vector and reservoirs have been reported throughout the state of Texas (Sarkar et al., 2010). Having said this, it is a reportable disease for the states of Arizona and Massachusetts, however it is not for Texas (Sarkar et al., 2010). Based on Chagas' seroprevalence and U.S. immigrants, 300,167 people are estimated to be infected in the United States. Annually, about 30,000 – 45,000 are cardiomyopathy cases and between 63 and 315 have congenital infections (Rassi & Marin-Neto, 2010). Chagas' disease is known to be found in countries south of the United States (Jannin & Salvatella, 2006). Chagas disease is mostly found in the U.S. as a zoonotic disease (Beard et al., 2003).

The first reported Chagas disease case in the U.S. was in Corpus Christi, Texas in 1955. This case involved a 10 month old child, who had never left the state since birth and her parents had not previously been diagnosed with any disease. However, opossums had been seen around the house and the family had also been having trouble with getting rid of triatomid insects inside the house (Woody & Woody, 1955). Since then, there have been six other autochthonous human cases in the U.S. (Buhaya et al., 2015). Figure 5: Geographic distribution of triatomid species, infected vectors and hosts, and human cases (Hanford, Zhan, Lu, & Giordano, 2007)

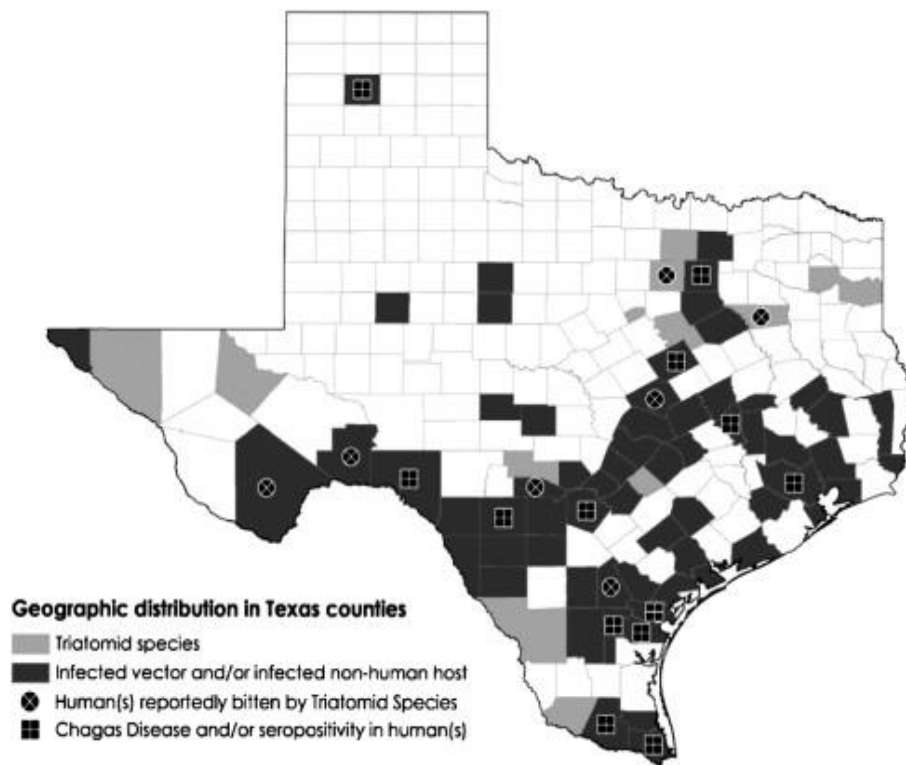


Figure 5: Geographic distribution of triatomid species, infected vectors and hosts, and human cases (Hanford, Zhan, Lu, & Giordano, 2007)

Based on the prevalence of Chagas' disease of immigrant's country of origin, it is estimated that 300,000 people living in the U.S. are positive for *T. cruzi*. However, no large studies have been made in Latin American populations. Of the few smaller studies made, 985 Latin American immigrant from Los Angeles County were tested and 10 (1%) had positive serological results. Patients with symptoms with this disease are known to be present, yet they are not diagnosed with Chagas' by health care facilities and hospitals in the U.S. Other targeted studies in hospitals of Los Angeles, provided 15 (16%) positive serological tests for *T. cruzi* among 93 patients from Latin America that have been diagnosed with idiopathic cardiomyopathy (Bern, Kjos, Yabsley, & Montgomery, 2011).

2.3 Life cycle of *T. cruzi*

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. Trypanosomas 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 spreading 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 6 shows the graphical description of Chagas' disease life cycle (CDC, 2015).

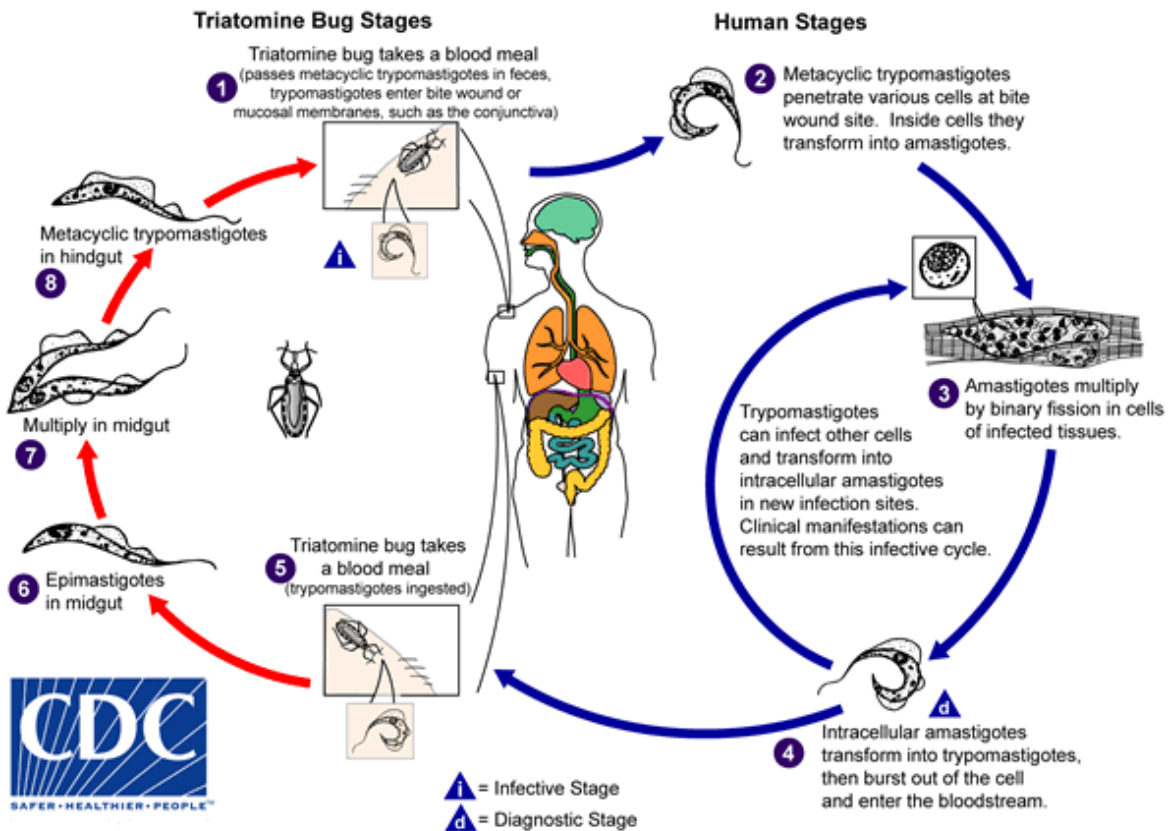


Figure 6: *Trypanosoma cruzi* Life Cycle

2.4 Vector

There is a total of 21 endemic countries for Chagas' disease caused by the flagellated protozoan *Trypanosoma cruzi*, which can be transmitted via vectors, transfusions, congenic, and several other modes of transmission such as digestive, transplant and laboratory accidents. However, vector mediated is the main mode of transmission, and it is spread by an infected triatomine insect also known as "kissing bug". Table 1 shows the triatomine insects found in each region in America (Jannin & Salvatella, 2006).

Table 1

Main vectors for Chagas' disease by sub-region

Sub-region	Vectors
South America	<i>Triatoma infestans</i>
Central America	<i>Rhodnius prolixus</i> , <i>Triatoma dimidiata</i> , <i>Rhodnius pallescens</i>
Andean Countries	<i>Rhodnius prolixus</i> , <i>Triatoma dimidiata</i> , <i>Rhodnius eduardi</i>
Amazonia	<i>Rhodnius robustus</i> , <i>Rhodnius stali</i> , <i>Rhodnius brethesi</i> , <i>Rhodnius neglectus</i> , <i>Rhodnius pictipes</i>
Mexico	<i>Triatoma barberi</i> , <i>Triatoma dimidiata</i> , <i>Triatoma pallidipennis</i> , <i>Triatoma phyllosoma</i> , <i>Triatoma longipennis</i> , <i>Triatoma mazzottii</i> , <i>Triatoma picturata</i> , <i>Triatoma mexicana</i> , <i>Triatoma gerstaeckeri</i>

Infected triatomines have been found throughout Arizona and California (Woody & Woody, 1955). Triatomine insects have also been found in 97 of the 254 counties in Texas, and in 48 counties *T. cruzi* infected triatomines have been found. *Triatoma gerstaeckeri* is the most common species found, followed by *T. sanguisuga* and *T. lecticularia* (Kjos, Snowden, & Olson, 2009). *T. gerstaeckeri* is a common species found among livestock and is also a pest of houses in rural areas of Texas. In the north of Mexico this *Triatoma* species is also considered an important vector for Chagas's disease in homes (Bern, Kjos, et al., 2011). *T. protracta*, *T. indictiva*, and *T. rubida* have been found in El Paso county, however only *T. rubida* has been found to be *T. cruzi* positive (Bern, Kjos, et al., 2011).

2.5 Clinical aspects of Chagas' disease and treatment

Clinical presentations of Chagas depends on the stage of the illness, immune response of the patient, and if cardiac system is affected (Berkowitz, Raibagkar, Pritt, & Mateen, 2015). Mild symptoms include skin lesions, fever, malaise, headaches, myalgia, and lymphadenopathy. Severe symptoms include difficulty when breathing. It has been reported that 10% of the patients in the acute phase present neurologic complications which include meningoencephalitis, and neuropathy (Berkowitz et al., 2015). Immunocompromised patients and children are at a higher risk of developing severe neurologic symptoms. Maternal infection, congenital Chagas, is characterized by newborn meningoencephalitis, microcephaly, and brain calcifications (Berkowitz et al., 2015)

Treatment for Chagas' disease includes benznidazole and nifurimox, which are the only antitrypanosomal drug treatment approved and that has shown results (Bern, Martin, & Gilman, 2011). During early stages of the Chagas, both drug treatments are able to reduce the symptoms and shorten the duration of the disease. About 60 to 85% of the patients treated are cured (Bern, Martin, et al., 2011).

3. 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. Of 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*. 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 these parasites are the causative agents amongst the most burden causing diseases. As public health professionals, it is important to determine if *Leishmania spp.* and *T. cruzi* is present in El Paso County area in order for people to be aware of the diseases being in this area and be in the alert when the symptoms to these diseases are present in a patient. For this reason it is important to educate the population on how to prevent acquiring the diseases and be in the lookout for these parasites and their vectors and hosts. It is also important to identify the reservoirs in order to know how to respond in order to prevent these diseases.

3.1 Specific Aims and Objectives

The aim of this study is to determine if sylvatic animals from El Paso County could be reservoirs for *Leishmania spp.* and *Trypanosoma cruzi*.

The objectives of this study are 1) to identify DNA of *Leishmania spp.* and *T. cruzi* in tissue samples from sylvatic animals such as gray foxes, coyotes, striped skunks, and raccoons from El Paso County if they are carriers for *Trypanosoma cruzi* and/or *Leishmania spp.* which

will create a positive identification of carrier. 2) Map positive results of DNA samples for *Trypanosoma cruzi* and/or *Leishmania spp.* to determine the areas where these parasites are found.

3.2 Research Question

To determine the prevalence of collected tissue samples from sylvatic animals to be identified as positive for *Trypanosoma cruzi* and/or *Leishmania spp.*.

4. Methodology

4.1 Study Design

This study is a cross sectional study collecting tissue samples of wild animals for a time period of 18 months. These samples were collected in an effort to represent the sylvatic animal population in the County of El Paso.

4.2 Sampling

A convenience sample of sylvatic animals was obtained to measure the frequency of DNA testing positive for *Leishmania spp.* and/or *Trypanosoma cruzi*. Samples were collected for a period of 18 months. Heart, spleen, and skin tissue samples were collected from sylvatic animals around the El Paso County area by the Texas Department of State Health Services (DSHS), Zoonosis Control and the Animal Services. These tissues have been chosen because of its relationship to *Trypanosoma cruzi* and *Leishmania spp.* *T. cruzi* can infect all of the tissues of its mammalian host mainly affecting the heart and digestive system in a chronic manner (Noireau, Diosque, Jansen, 2009). The spleen plays an important role in the immune system by producing white blood cells which help fighting infections and also 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, animals will be visually inspected for skin lesions; tissue samples will also be collected from the area the lesions are present and noted in Table 2: Sample Data sheet.

Table 2: Sample Data sheet

Reference #	Species	Sex	GPS Coordinates	Comments

4.3 Materials

The following materials were used during the DNA extraction: SNET composed of 10 mM Tris pH 8, 0.1 M EDTA, and 0.5% SDS. SteadyShake 757 Bench top Incubator Shaker by Amarex Instruments Inc. ® is used during incubation time of the DNA extraction. Vortex-Genie® is used to evenly mix samples. Centrifuge used is the Beckman Coulter® Allegra X-15R Refrigerated Bench top Centrifuge. Bench top centrifuge used is the Eppendorf® Centrifuge 5415D with 1.5 ml Eppendorf® tubes. NanoDrop® ND-1000 will be used to determine the DNA concentration.

Bio-Rad® Thermal Cycler will be used to run the PCRs and primers ordered from New England Biolabs. Positive samples will be sequenced for validation in the University of Texas at El Paso Biological Sequencing Laboratory.

4.4 Procedure

4.4.1 Sample Collection

Heart, spleen and skin tissue samples were collected and provided by the Animal Services of El Paso, through the Texas Department of State Health Services, of the following sylvatic animals that were collected in El Paso County:

- Striped Skunk (*Mephitis mephitis*)
- Gray Fox (*Urocyon cinereoargenteus*)

- Raccoon (*Procyon lotor*)
- Coyote (*Canis latrans*)

This study focuses on the heart, skin, and spleen samples for each specimen along with the GPS coordinates from where the specimen will be collected in Table 2. Animals were also examined for any skin lesions and skin samples were taken from the area. All tissue samples will be preserved in DMSO/EDTA/Salt solution at room temperature.

4.4.2 DNA Extraction

DNA of tissue samples were extracted using 20 µl of proteinase K and 2 mL of SNET. Mixture was then be incubated overnight in a shaking plate at 55 °C. After incubation period, equal amount of Phenol:Chloroform:Isoamyl alcohol (25:24:1 v/v) will be added to the sample, shaken at room temperature for 30 minutes. After incubation period, samples were vortexed and then centrifuged for 5 minutes at 15,000. A 1:1 ratio of aqueous part extracted from centrifuged tube and ice cold isopropanol was mixed in 1.5 ml tube. Mixture was centrifuged for 15 minutes on bench top centrifuge. A pellet formed at the bottom of the tube and supernatant was disposed. 100 µl of 70% ethanol was added to the tube to wash the pellet to remove the residue of supernatant. The tube with the pellet was left to air dry for 30 minutes and later diluted to 100 ng/µl using nuclease free water.

4.4.3 Primers and PCR

For the PCR, each sample was ran with *Trypanosoma cruzi* specific primer (TCZ) (Braz et al., 2008)., *Leishmania spp* primer (LITS) (El Tai, Osman, El Fari, Presber, & Schönian, 2000) and IRBP primer to test quality of the DNA extracted (Ferreira, Gontijo, Cruz, Melo, & Silva,

2010). 12.5 µL PCR master mix, 1.5 µL DNA template, 1 µL reverse primer, 1 µL forward primer and 9 µL of nuclease free water to was mixed to end up with a final volume of 25 µL in each tube. Bio-Rad® Thermal Cycler was used to run the PCRs.

T. cruzi specific primer sequence used has 188 base pairs and is as follows: TCZ sense F2: 5' – TGCACTCGGCTGATCGTTTTTCGAG – 3' and TCZ anti-sense B3: 5'– AGGGTTGTTTGGTGTCCAGTGTGTG–3. Table 3 provides the temperatures and times used for denaturing, annealing and elongation of the DNA.

Table 3
TCZ PCR Protocol

Cycles	Temperature (°C)	Time
1 cycle	95°	5 min
40 cycles	95°	1 min
	55°	1 min
	72°	1 min
1 cycle	75°	5 min

The *Leishmania* spp. primers for ITS 1 segment has 320 base pairs and are the following: LITSR (5'- CTGGATVATTTTCCGATG-3') and LITSV (5'- ACACTCAGGTCTGTAAAAC-3'). Cycles, temperatures, and time used for these primers are as shown in .

Table 4.

Table 4

ITS 1 PCR Protocol

Cycles	Temperature (°C)	Time
1 cycle	95°	2 min
32 cycles	95°	20 sec
	53°	30 sec
	72°	1 min
1 cycle	72°	6 min

The following sequence was used as primer to test quality of DNA being extracted: IRBP

FW (5'-TCCAACACCACCACTGAGATCTGGAC-3') and IRBP RV (5'-GTGAGGAAGAAATCGGACTGGCC-3'). The temperatures shown in Table 5 Table 5Table 5

IRBP PCR Protocol

Cycles	Temperature (°C)	Time
1 cycle	94°	3 min
35 cycles	94°	30 sec
	57°	30 sec
	72°	1 min
1 cycle	72°	10 min

A 1.8% electrophoresis gel was run for 20 minutes at 100 Volts and gel was visualized under UV light. Figure 7 shows a sample agarose gel showing positive and negative controls for *L. genus*, *L. mexicana*, and *T. cruzi* (Mariscal, 2013). A bright band is seen under UV light if samples are positive.

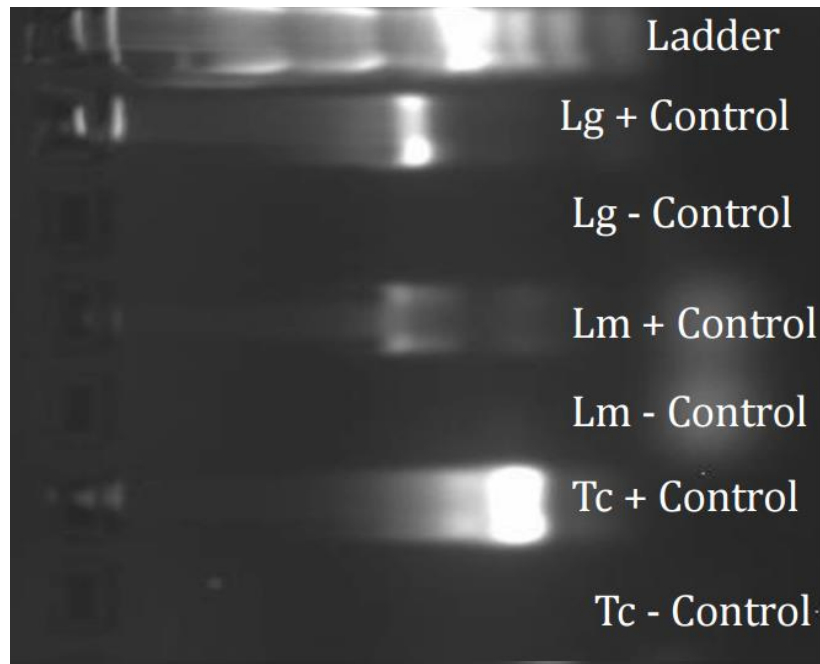


Figure 7: Sample agarose gel with PCRs of *L. genus*, *L. mexicana*, and *T. cruzi* positive and negative controls.

Samples showing positive results for *T. cruzi* using Tcz1/Tcz2 primers will be also be verified utilizing primers 121/122 following the protocol shown in Table 6. Primer 121 DNA sequence is 5'-AAATAATGTACGGGGGAGATGCATGA-3' and the sequence for 122 is 5'-GGTTCGATTGGGGTTGGTGTAATATA-3'. The fragment size for these primers has 330 base pairs (Fitzwater et al., 2008).

1.8% agarose gels will be run for these samples to compare results in samples that used the Tcz1/Tcz2 and 121/122 primers. Samples shown to be positive for both of these *T. cruzi* primers will be sequenced. PCR samples that show to be positive for *Leishmania spp.* based on the agarose gels outcomes will also be sequenced to confirm results.

Table 6
121/122 PCR Protocol

Cycles	Temperature (°C)	Time
1 cycle	94°	3 min
35 cycles	94°	30 sec
	57°	30 sec
	72°	30 sec
1 cycle	72°	7 min

4.5 Data Analysis

Samples with positive results were mapped based on the GPS coordinates of the location of where the animal was trapped. Maps will illustrate the distribution of leishmaniasis and Chagas' disease in sylvatic animals in El Paso region.

4.6 Project Approval

Approval from The University of Texas at El Paso Institutional Biosafety Committee (IBC) was required given that our study focused on two pathogens: *Leishmania spp.* and *T. cruzi* (IRBNet Identification Number: 807121-1). The University of Texas at El Paso Institutional Review Board (IRB) approval was not required since the wild animals for this study were not captured and killed specifically for this study.

5. Expected Results

Based on a previous study by Mariscal et al. (2013), it was expected to identify 65% of the collected animals to be positive for *Trypanosoma cruzi* and 5% to be positive for *Leishmania spp.*

6. Results

A total of 146 samples of skunks, gray foxes, raccoons, coyotes were collected between May 6, 2014 and November 9, 2015. Animals were catalogued by their species name followed by a number in the order of when they were collected such as first Gray Fox (*Urocyon cinereoargenteus*) UC1 followed by second fox UC2. Given that not all samples gave positive results for mammalian cells, 32 samples had to be discarded, leaving a total of 114 viable samples.

Of the 114 samples collected, the total number of positive samples for *T. cruzi* was 33 (40.24% prevalence), 18 (21.95%) for *Leishmania spp.* of which 9 (10.98%) samples had both of these diseases. The collected samples by the specific species is as follows: A total of 24 skunks samples were analyzed from which 3 (12.50%) were positive samples for *T. cruzi* and none for *Leishmania spp.*. Sample pool for skunks was made up of 15 females and 9 males with only 5 skunks not considered as adults. 37 samples of gray fox were collected from which 6 showed skin lesions, 12 were youth, and 19 were female. Gray fox had 18 (48.65%) positive samples for *T. %* and 4 (10.81%) had both diseases. There were 14 raccoon samples, where 8 were female, 5 had skin lesions, and 6 were youth. Raccoons had 5 (35.71%) samples positive for *T. cruzi*, 10 (7.43%) for *Leishmania spp.*, and 5 (35.71%) for both. There were 7 collected samples for coyotes, from which 6 where female, 1 had a skin lesion, and all were adults. Coyotes were positive in all 7 (100.00%) samples for *T. cruzi*, 4 (28.57%) for *Leishmania spp.*, with 4 (28.75%) samples positive for both diseases (Table 7).

Table 7: Sylvatic animals sample characteristics

	Total Samples	Total Samples	<i>Leishmania</i> <i>spp.</i> positive	<i>T. cruzi</i> positive	Both diseases	Skin lesion	Sex (Females)	Age< Adult
Striped Skunk	30	24	0 (0.00)	3 (12.50)	0 (0.00)	5	15	5
Gray Fox	40	37	4 (10.81)	18 (48.65)	4 (10.81)	6	19	12
Raccoon	23	14	10 (71.43)	5 (35.71)	5 (35.71)	5	8	6
Coyote	7	7	4 (57.17)	7 (100.00)	4 (57.17)	1	6	0
Total	144	114	25 (21.93)	39 (34.21)	12 (18.42)	17	48	23

() Prevalence shown in parenthesis.

6.1 PCR Results

Samples were considered to be viable based on the results of the IRBP primer specific for mammalian cells that would determine if the DNA extraction was successful and if what is being seen in the gel are actual samples of the animal tissue samples. Animal sample was considered viable if any of IRBP PCR showed positive results for at least one of the skin tissues collected, Figure 8. This gel was positive for *T. cruzi* in spleen. The 32 samples that were discarded was because no bands were visible for any of the IRBP PCRs of the tissue samples as shown on the right of Figure 8. Some gels like the one on the right side bellow were discarded although they showed positive results for both *T. cruzi* and *Leishmania spp.*

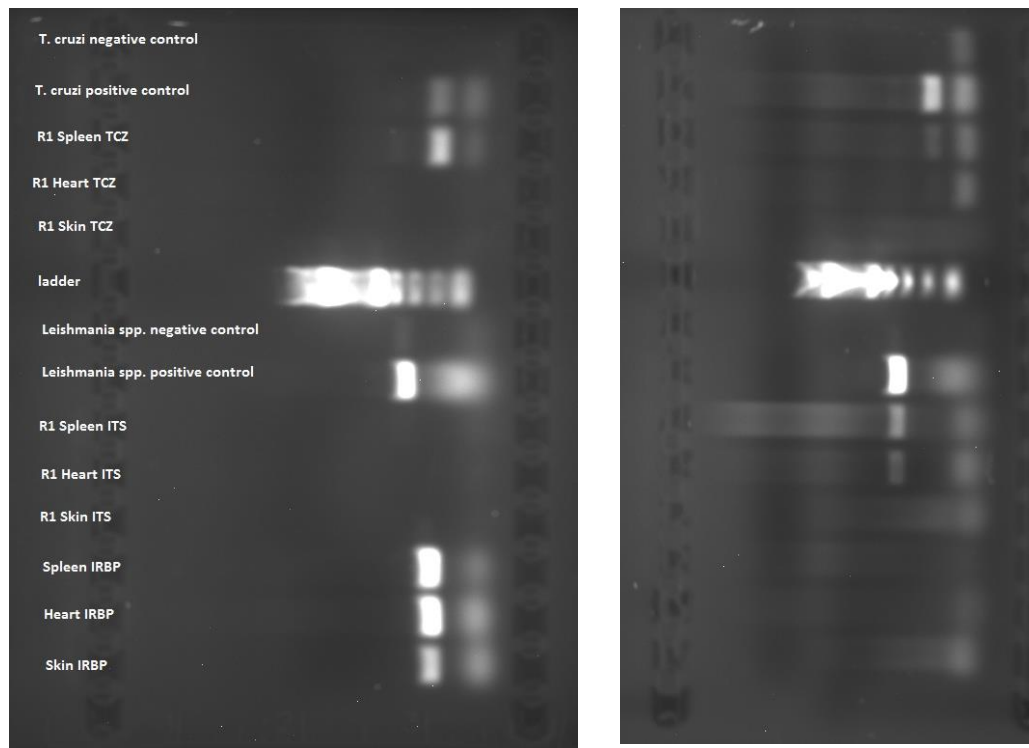


Figure 8: Sample positive and negative gel

PCR using primers 121/122 were used on the *T. cruzi* positive samples using the results of the TCZ 1/ TCZ2 primers. However, there was not enough *T. cruzi* DNA to run along with the samples to be able to compare where the positive bands should be appearing in the gel Figure 9: Gel using 121/122 primers

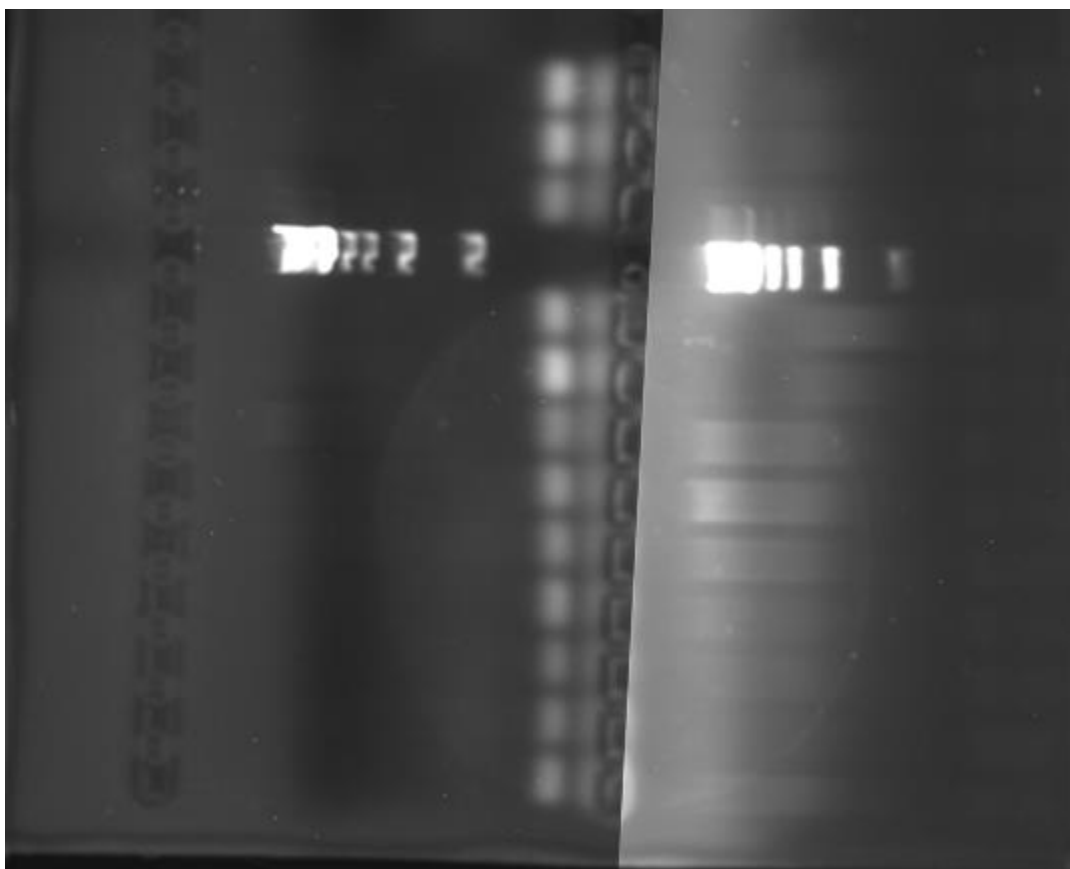


Figure 9: Gel using 121/122 primers

6.2 Geographic Distribution of Samples

Sylvatic animals were collected throughout El Paso County, and coordinates mapped using Epi Info 7.2 for samples positive for *Leishmania spp.* or *T. cruzi*. Mapped coordinates, show the distribution of animals, where it can be noted that animals were mostly captured in areas with low populations. Dots with number in map (Figure 10.) represent the number of samples collected in the area, and dots with no number are of only one animal collected.

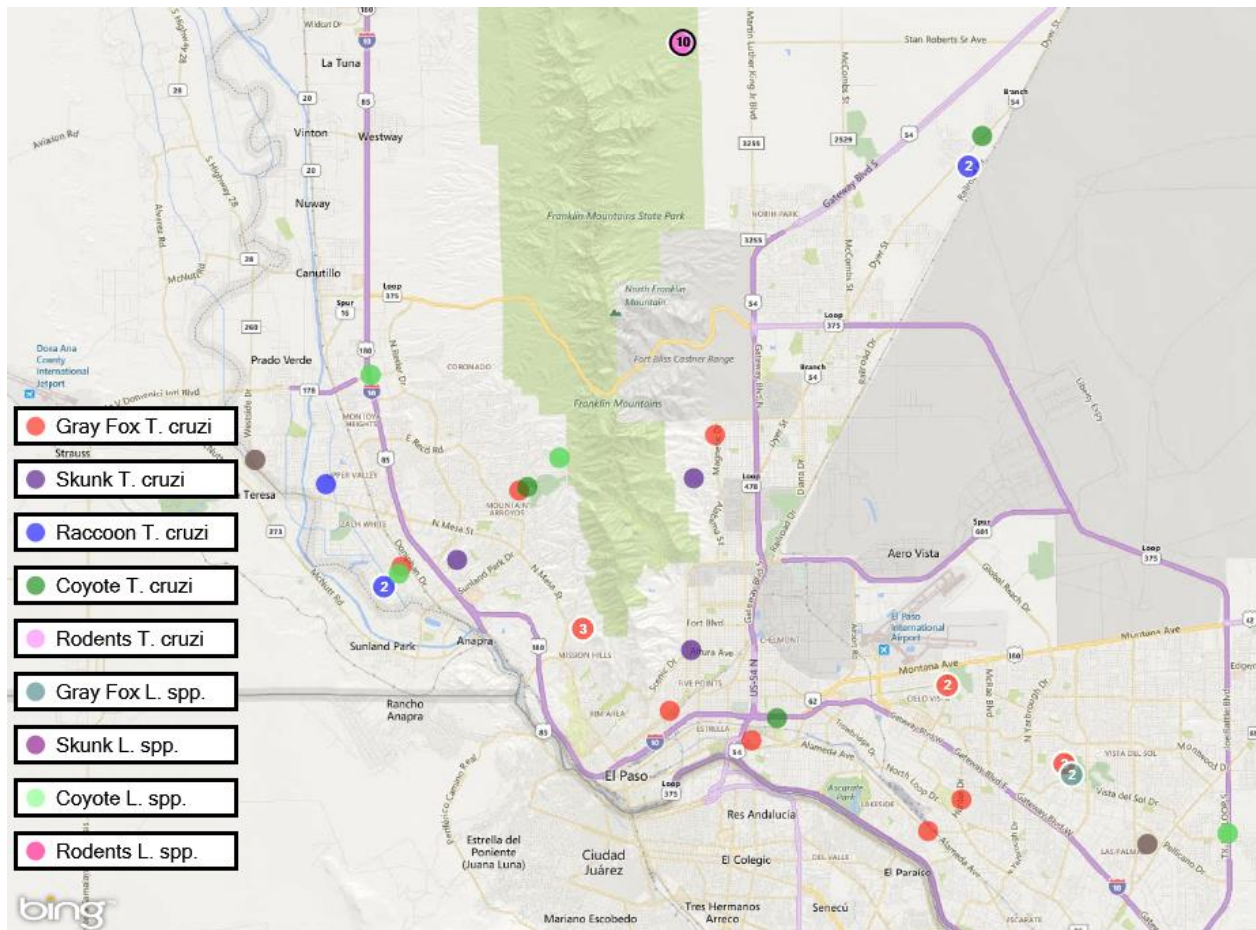


Figure 10: Distribution of captured sylvatic animals with positive identification for *Leishmania* spp. and/or *T. cruzi*

Figure 11 shows the geographic distribution of positive samples of *T. cruzi*. by sylvatic animal species. Although most of the positive samples were found in low populated areas, several gray fox samples shown to be positive were located in inner areas of the city (red dots) including the Downtown area. Samples of foxes collected in the same day were both positive for *T. cruzi*. Raccoons (blue dots), were found in the West side and far North East of the city. The raccoons found in the far North East where collected in June and July of 2014. The samples collected from the West side were from August, 2015. Coyotes (green dots), were mostly found

in the West side and in the out skirt of the city on the North East and far East side. There was a larger array of sylvatic animals on the West side, compared to the rest of the city.

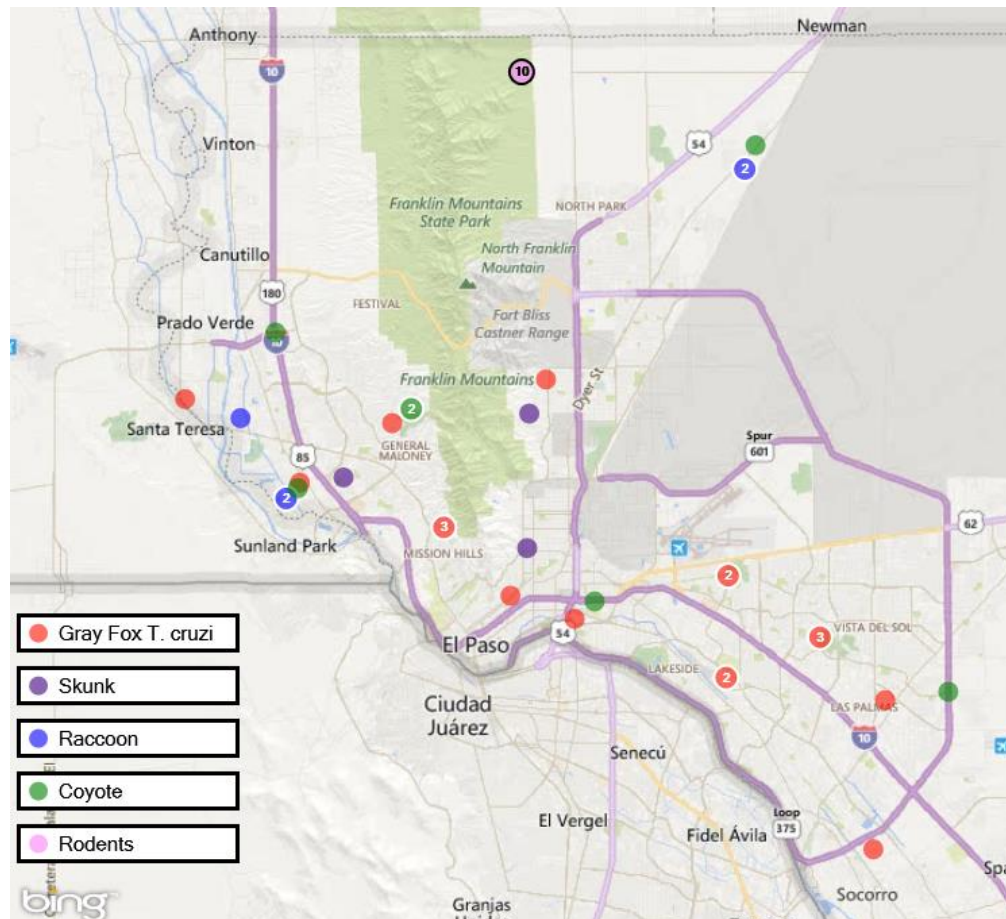


Figure 11: Geographic distribution of positive samples of *T. cruzi* by sylvatic animal species.

Figure 12 shows the distribution of gray fox comparing negative and positive PCR samples. Collection sites were evenly distributed throughout the city, showing that not all animals collected in the same area were positive of *T. cruzi*. Two samples, collected in two different days of June, 2015 in the same location were positive. A third sample from the same location collected in July was considered negative given that it showed negative results for IRBP, however, TCZ results were positive in both spleen and skin tissue samples.

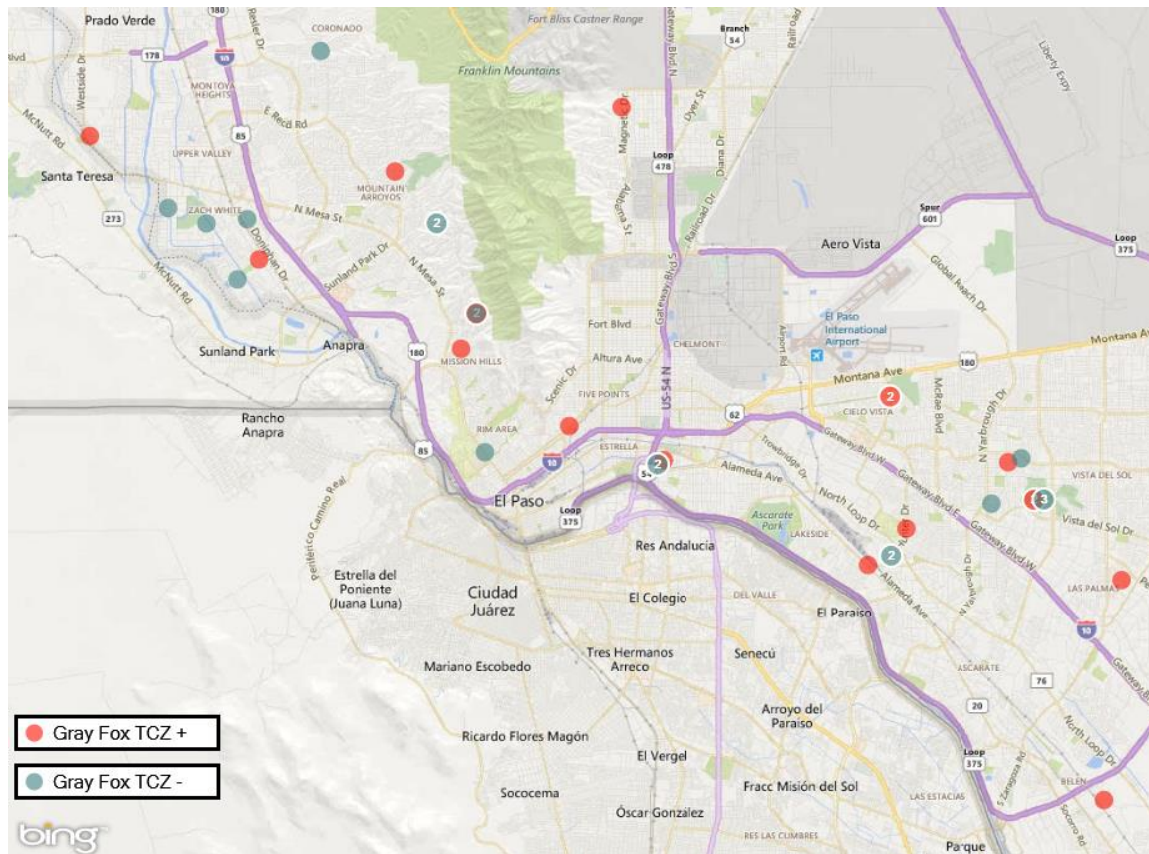


Figure 12: Geographic distribution of gray fox positive and negative PCR samples

Figure 13 shows the distribution of the collected skunks, they were mostly found on the west side of El Paso, however only one sample was positive in this side of town. Three samples were collected on the north east side of town near the Franklin Mountains, were two of those samples were positive for *T. cruzi*. Two skunks collected on July 24, 2015, near the intersection of Altura Ave. and Scenic Dr., were of the same litter and were about four to five months old; only one of these skunks was positive for *T. cruzi*. No skunks were collected on the East side of town.

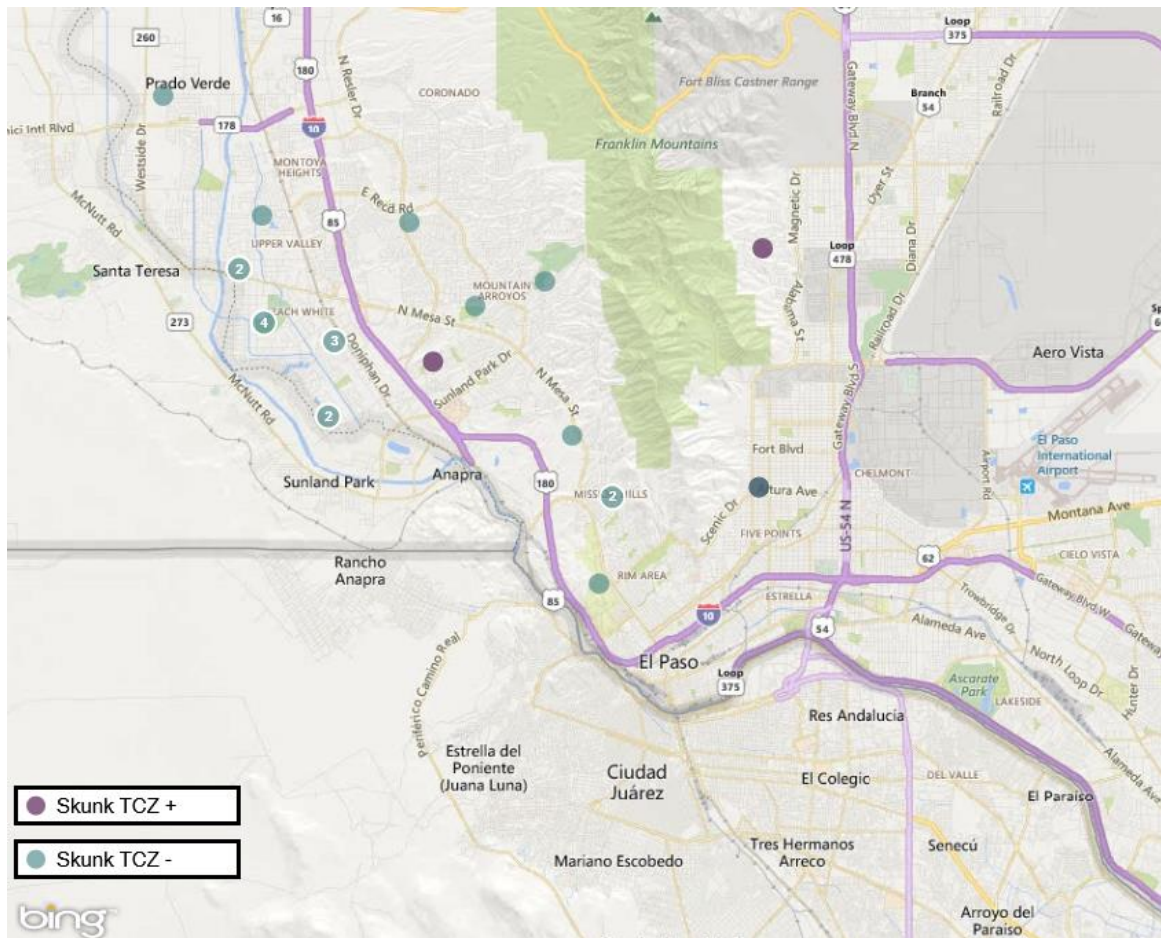


Figure 13: Geographical ditibution of skunks with positive and negative results for *T. cruzi*.

There were 7 coyotes collected and they all tested positive for *T. cruzi* via PCR. They were mostly found in areas where there is a low population density, except for the coyote collected near the intersection of I-10 and US 54 (Figure 14).

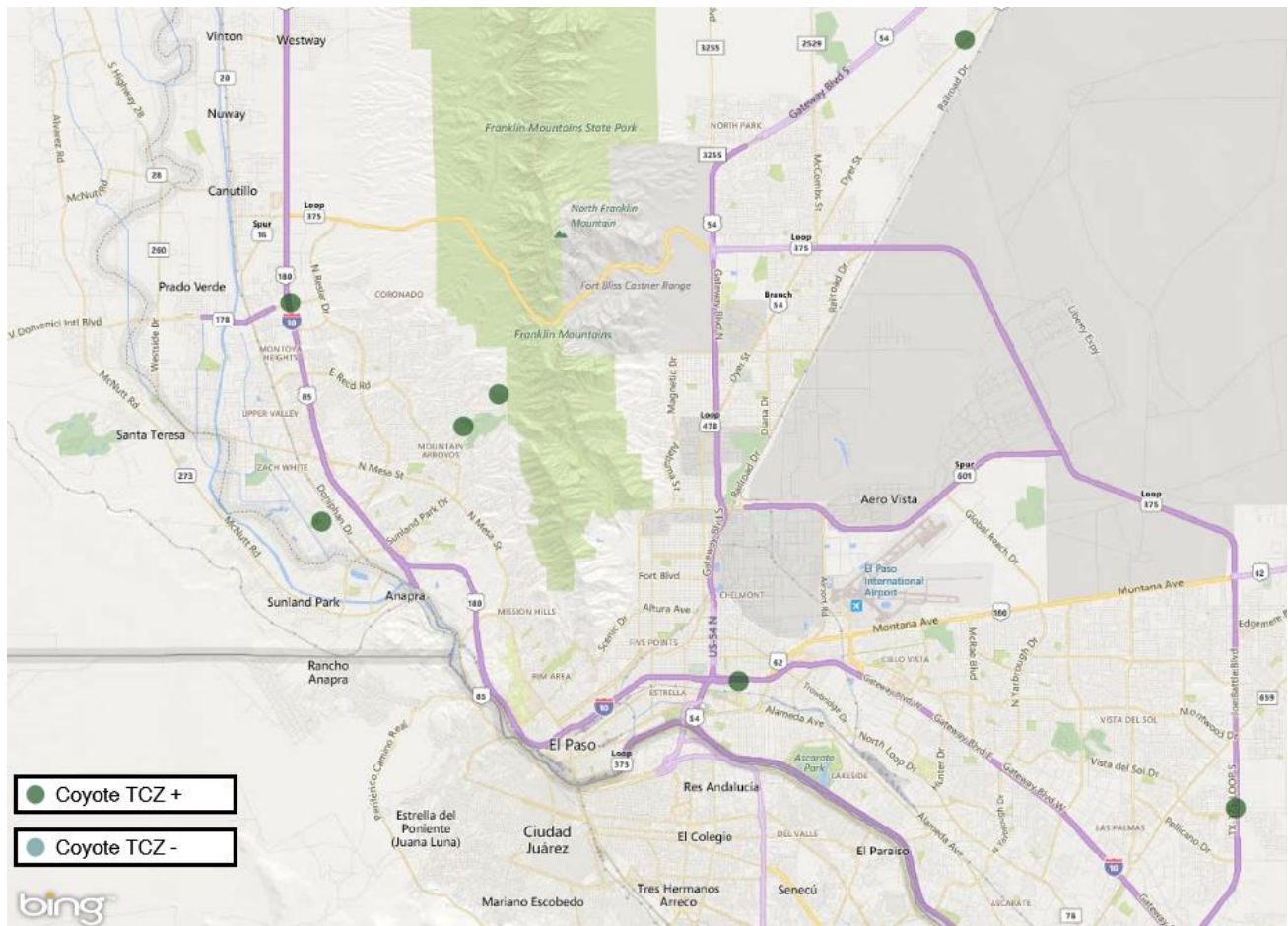


Figure 14: Geographic distribution of coyotes tested for *T. cruzi*.

Figure 15 shows the geographical distribution of the 21 sylvatic animals that tested positive for *Leishmania spp.* As it can be seen in the map, positive samples were mostly collected in the outskirts of the city, where there is a low population density. However, the gray fox, like in the *T. cruzi* samples, were also collected from inner parts of the city, where samples were positive for both diseases. Only the gray fox, had positive samples in the Far East side of the city. Raccoons were positive in both North East and in the West side.

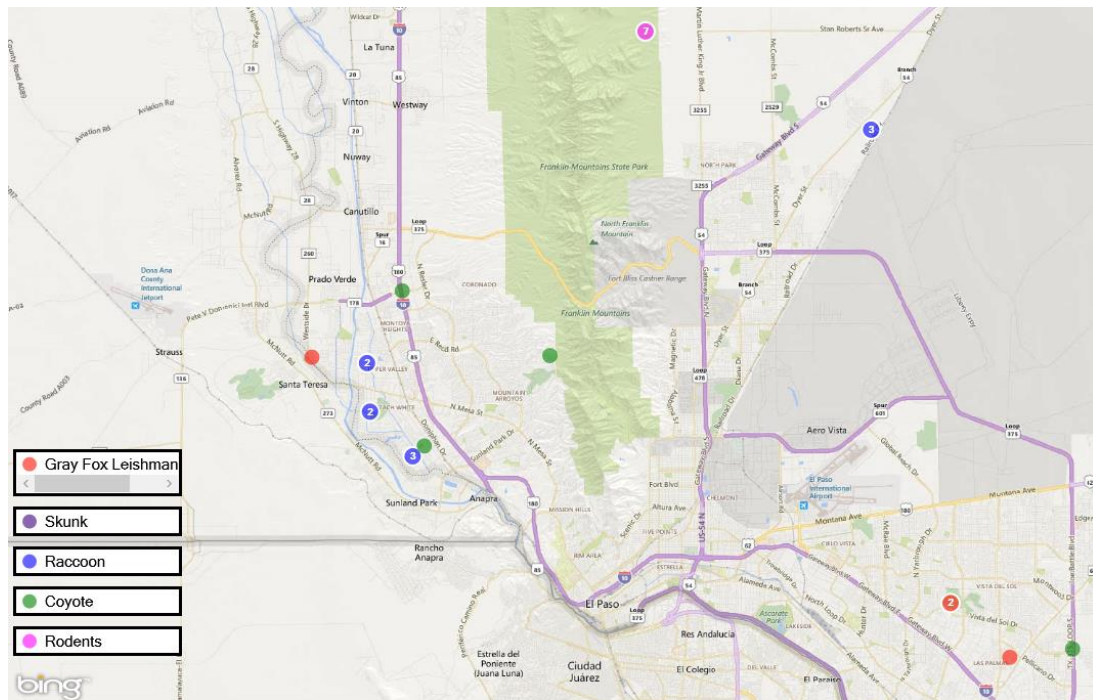


Figure 15: Geographic distribution of positive samples of *Leishmania spp.* by sylvatic animal species.

Gray foxes were collected throughout the city and although there were some found in central areas of El Paso, they were mostly found in low populated areas. Four out of the thirty seven samples collected were positive for *Leishmania spp.* (Figure 16).

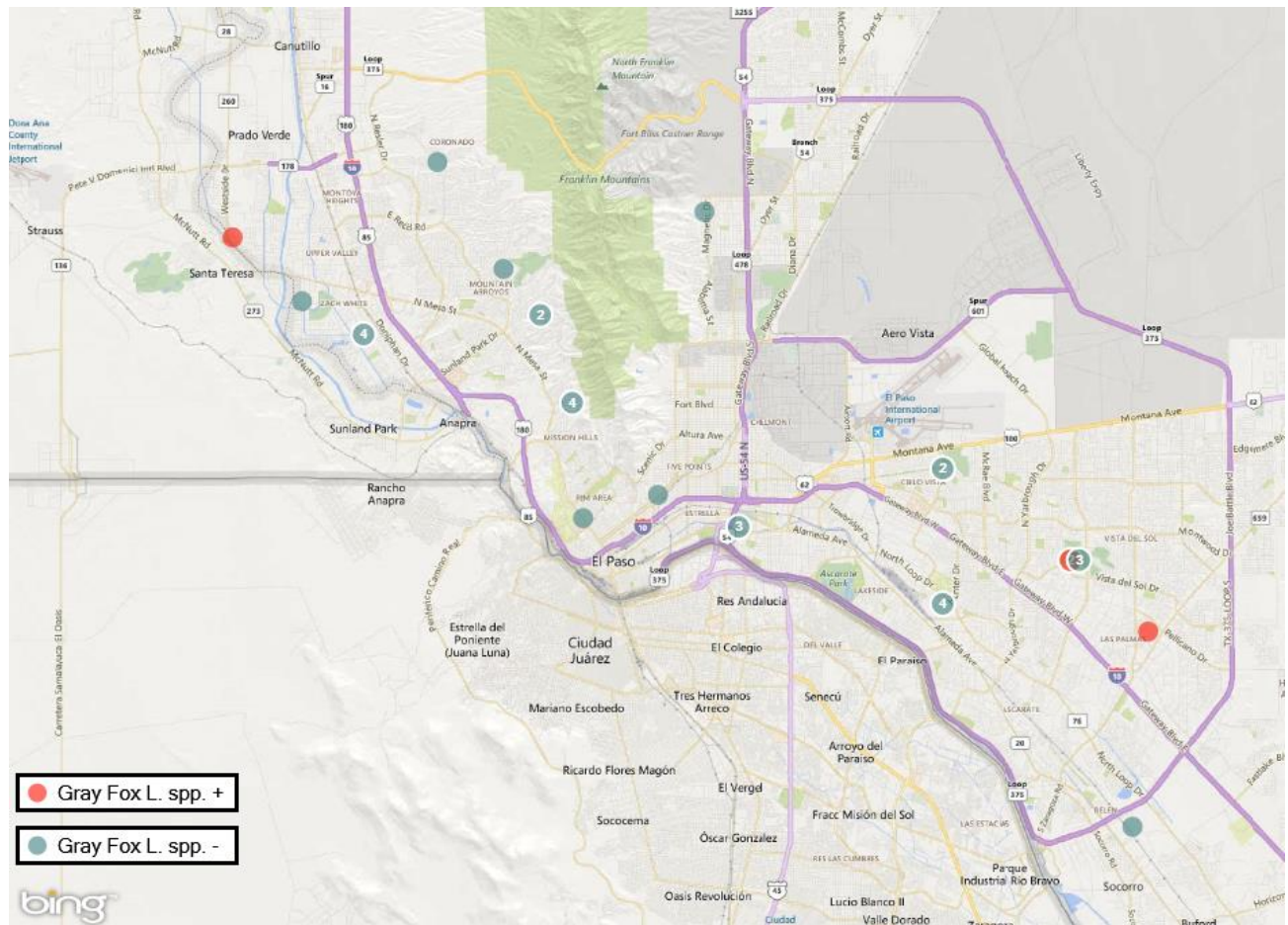


Figure 16: Geographic distribution of collected gray fox tested for *Leishmania spp.*

Figure 17 shows the location of where the raccoons were collected, and it can be seen that were mostly found in low populated areas. On the west side of the city they were collected near the Rio Grande. Samples were collected during the months of June through September of 2014 and 2015. Ten out of the twelve tested raccoons tested positive for *Leishmania spp.* via PCR. A total of three samples were collected near Zach White, on August 15, September 1st and September 15, 2015 from which two were considered as positive for *Leishmania spp.* The September 1st sample was not considered as positive sample given that it was negative for IRBP; however, the ITS primer for *Leishmania spp.* was positive on the skin. Skin lesion was found on the nipple of the raccoon from where DNA was extracted for PCR Figure 18.

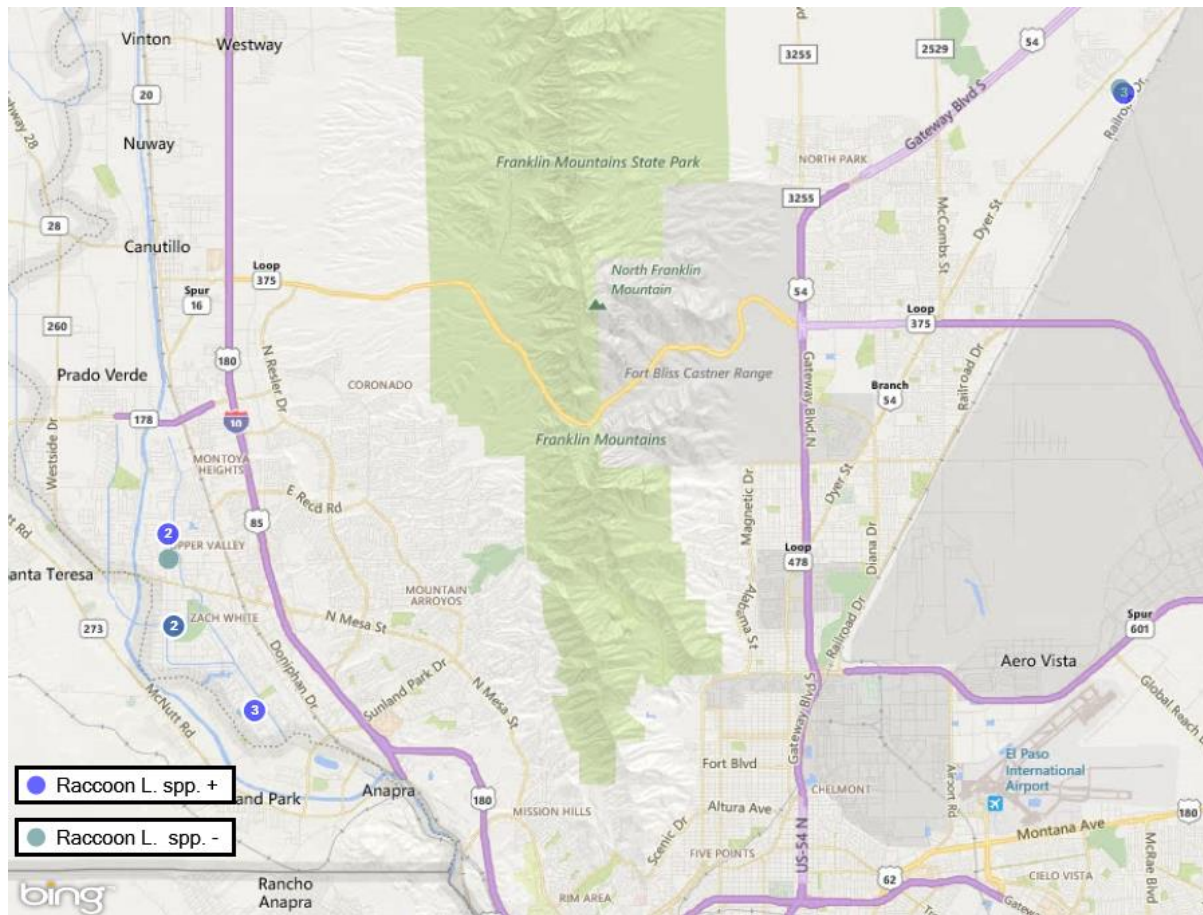


Figure 17: Geographic distribution of *Leishmania* spp. tested raccoons



Figure 18: Raccoon skin lesion.

Coyotes were mostly found in the outskirts of the city and four out of the seven coyotes tested for *Leishmania spp.*, four tested positive via PCR (Figure 19). Coyotes were collected between October 2014 and January 2016; out of the seven, only one was male and they were all adults. There were no records of lesions for all of the coyotes; however, the four that had information collected were severely afflicted with sarcoptic mange.

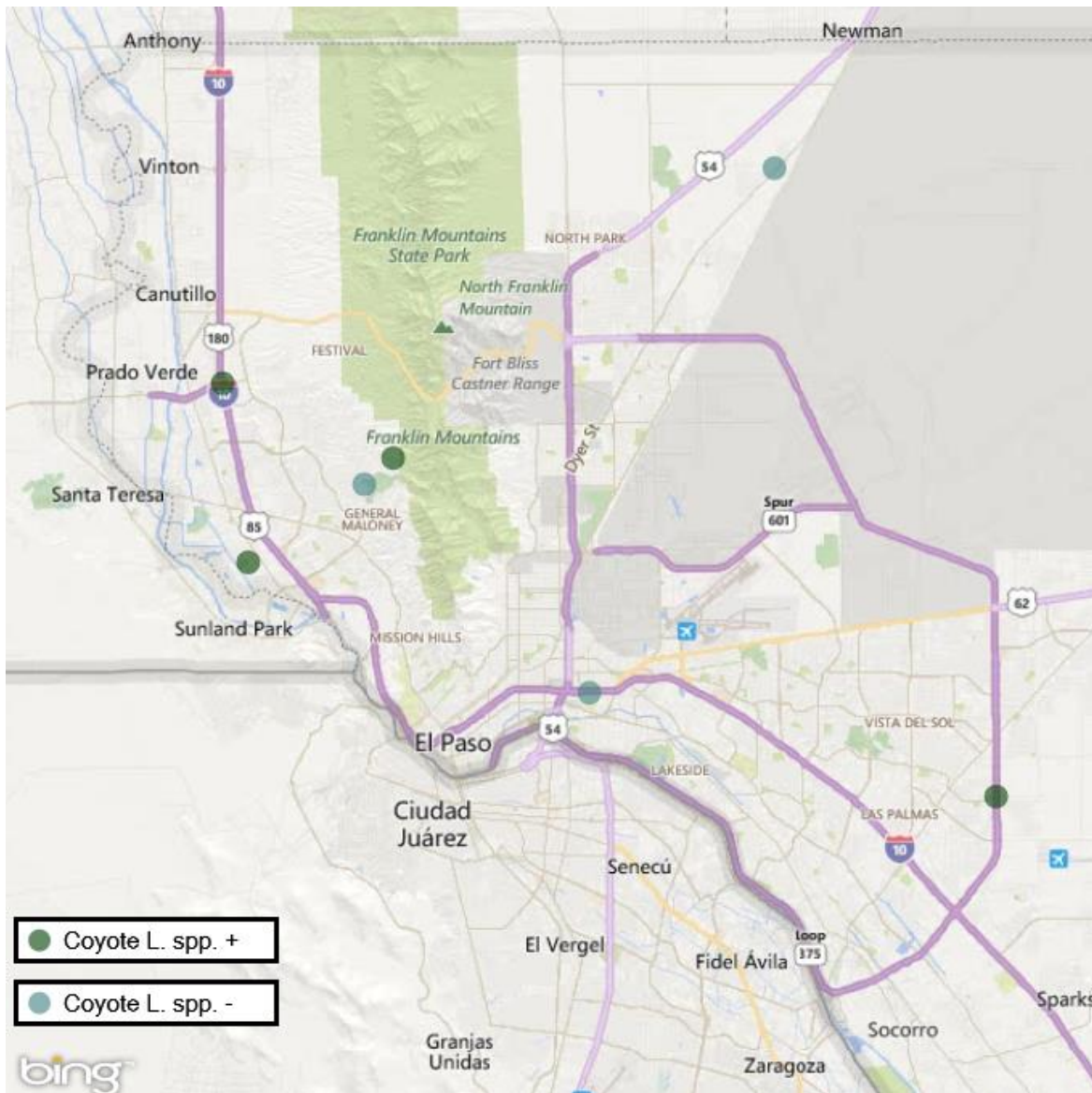


Figure 19: Geographical distribution of coyotes tested for *Leishmania spp.*

6.3 DNA sequencing

Ten samples were chosen from the twenty-nine samples PCR positive for *T. cruzi*, and the twenty-one positive samples for *Leishmania spp.*. Given that there were twelve samples that were positive for both diseases, another five samples were chosen from those, so the PCR samples of the same animal were tested for both *T. cruzi* and *Leishmania spp.*. All of the samples

sequenced for *Leishmania spp.* tested positive for *Leishmania mexicana*. The raccoon sample that was positive for *Leishmania spp.* in the skin, although it was to be considered as negative due to negative results in IRBP, was also sequenced and was also positive for *Leishmania mexicana* (Table 8)

Table 8: *Leishmania spp.* CLUSTAL multiple sequence alignment

```

PL20SK -----
CL1H  -----TCATCCATCGCGACACGTTATGTGAGCCGTTATCCACACACGCACCCCCCSG
CL4SP  -----TCCATCGCGACACGTTATGTGAGCCGTTATCCACACACGCACCCCCCSG
2H     TCCGAAGTCATCCATCGCGACACGTTATGTGAGCCGTTATCCACACACGCACCCCCCSG
3SK    --CCAGATCATCCAACGCGACACGTTATGTGAGCCGTTATCCACACACGCACCCCCCSG
PL22SK GATCCAGWCATCCATCGCGACACGTTATGTGAGCCGTTATCCACACACGCACCCCCCSG
R2H    ---CCAGTCATCCATCGCGACACGTTATGTGAGCCGTTATCCACACACGCACCCCCCSG
CL5SK  -----CATCCATCGCGACACGTTATGTGAGCCGTTATCCACACACGCACCCCCCSG
R8SP   --TGCAGTCATCCATCGCGACACGTTATGTGAGCCGTTATCCACACACGCACCCCCCSG
LMC    -----

PL20SK -----CGCGCTTGGGGAGGCCTATTCT-----TTCGATAGGCGC
1H     CCCAAAAMCGGAAAACGCSGWWTGAAACGGGCATTTTCTCGGCTTTTGTATTTACGC
4SP    CCCMAAAAMSGRAAAASGCSGWWTGAAACGGGMATTTTCCCGGCTTTTGTATTTACGC
2H     CCCMAAAAMSGRAAAASGCGG-WWTGGAACGGGSMTTTTTCCCGGCTTTGGWATATCCC
3SK    CCCMAAAAMGGRAAAMGSCGK-WWTGGAACGGGSMTTTTTCCCGGCTTTGGWTATACCC
22SK   CCCMAAAAMGGRAAAMGCCGK-WWTGGAACGGGSMTTTTTCCCGGCTTTGGWTATACCC
R2H    CCCMAAAAMGGRAAAMGGCGK-WWTGGAACGGGSMTTTTTCCCGGCTTTGGWTATACCC
5SK    CCCMAAAAMGGRAAACGCCGK-WTTGAAACGGGSATTTTCCCGCTTTTGGATATACGC
R8SP   CCCMAAAAMGGRAAACGCCGG-WTTGAAACGGGSATTTTCCCGGCTTTTGGATATACCC
LC     -----ACTCTCGGGAGGCCTATTCT-----TTCGATAGGCGC
          *  *  * *  *  *  *  *

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T. cruzi PCR samples that showed positive results in the agarose gels, were also positive in the sequencing results for *T. cruzi* (Table 9).

Table 9: *T. cruzi* CLUSTAL multiple sequence alignment

C13Sp	--GCATCACACGTTGTGGTCCAAATTTTGTTCGATTGTGAATGGTGAGTCAGAGR
M12Sk	--GCAT-CAACGTTGTGGTCMAAAATTTTGTTCATTGTGAATGGTGGAGTCAGAGR
C14H	----CTCACACGTTGTGGTCCAMATTTTGTTCGATTGTWCATGRTGGCAGTCCCAGA
UC9H	----ATCACACGTTGTGGTCCATT-TTTGTTCGATTATGG-TGSTGGGACTCCCACC
C14Sk	--GCAT-CCACGTTGTGGTCCAT-TTTGTTCGATTGTGAATGGTGGAGTCAGAGS
Tc	CTGCATCACACGTTGTGGTCCAAATTTTGTTCGATTGTGAATGGTGGAGTCAGAGR
	. * . * * * * * * * * : * * * * * * * * * * * * * * * * *
C13Sp	CACTCTCTGWCATATCTGTTTGMGTGTTACACACTGGACACCAAACAACCCTGAACTA
M12Sk	CACTCTCTGTCAATATCTGTTTGCCTGTTACACACTGGACACCAAACAACCCTGAACTA
C14H	CACTCYCTGAAAMTATCTGTTTGCCTGTTACACACTGGACACCAAACAACCCTGAACTA
UC9H	CTCCKCCSGTGGATATCTGTTTGCCTGTTAC-CACTGGACACCAAACAACCCYTGAACTA
C14Sk	CACTCTCTGTCAATATCTGTTTGCCTGTTACACACTGGACACCAAACAACCCTGAACTA
Tc	CACTCTCTGTCAATATCTGTTTGCCTGTTACACACTGGACACCAAACAACCCTGAACTA
	* : *
C13Sp	TCCGCTGCTTGGAGGA-----
M12Sk	TCCGCTGCTTGGAGG-----
C14H	TCCGCTGCTTGGAGGACC---
UC9H	TCCGCTGCTTGGAGGARCCCC
C14Sk	TCCGCTGCTTGGAGGARCCC-
Tc	TCCGCTGCTTGGAGGA-----
	* *

6.4 Data analysis

Results were analyzed using chi-squared test in order to compare the significance of difference between each sylvatic animal species for each of the diseases. The animal species are ranked in order of significance. The degree of freedom was determined to be 1, meaning the chi-square result will have to be higher than 3.84 in order for it to be statistically significant ($p \leq 0.05$). Species are in order of highest to lowest prevalence for each disease (Ott, 1984).

Based on the prevalence and χ^2 test, *Leishmania spp.* had no significant difference between the two highest prevalence rates Raccoon (71.43) and Coyote (57.17), $p > 0.05$. There was also no difference between the Fox (10.81) and the Stripped skunk (0.00), $p > 0.05$. However, the difference between the coyote and fox was statistically significant $8.49 \leq 0.05$ (Figure 20).

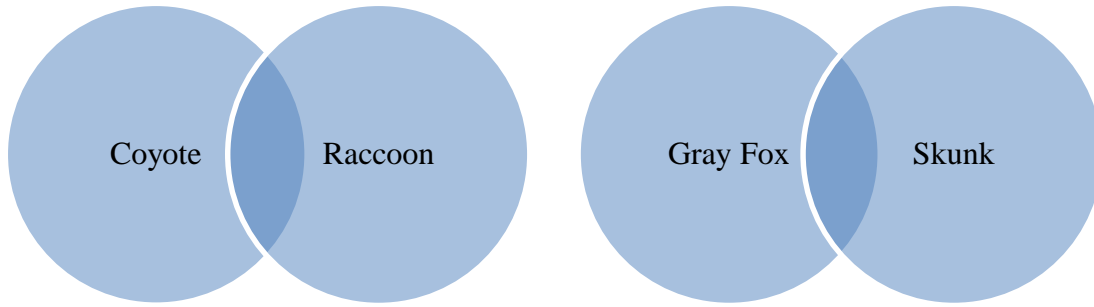


Figure 20: Difference between species tested positive for *Leishmania spp.* based on χ^2 test

The results of the *T. cruzi* samples showed statistical significant difference between the coyote (100) and the gray fox (48.65), $6.33 \leq 0.05$. There was no statistical difference between the gray fox (48.65) and the raccoon (35.71), $\chi^2 = 0.68 > 0.05$; or between the raccoon (35.71) and the skunk (12.50), $\chi^2 = 2.87 > 0.05$. However, there was a statistical difference between the gray fox and the skunk, $\chi^2 = 8.43 \leq 0.05$ (Figure 21).

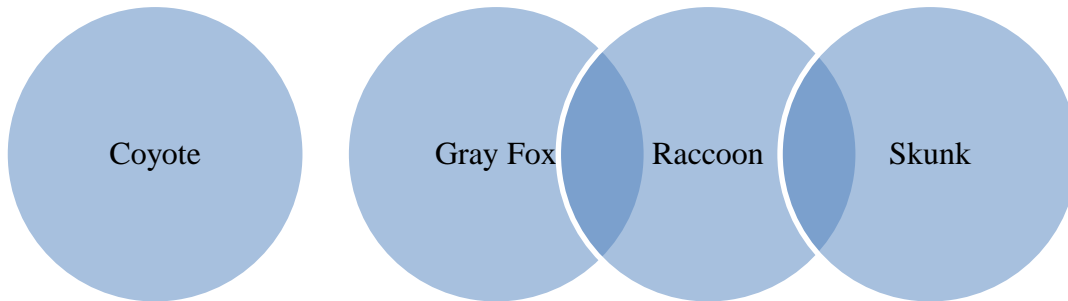


Figure 21: Difference between species tested positive for *T. cruzi* based on χ^2 test

7. Discussion

Based on the PCR results, the expected results were different from the final results. The percentage of positive results of *T. cruzi* was 40.24% compared to the expected results of 65%. *Leishmania spp.* results were also different and much higher than expected with 21.95% of the samples being positive, compared to the 5% that was expected. It is important to note that although these are wild animals that are usually in low populated areas, they are also found throughout the city, which can lead to more easily passing these diseases to other hosts including humans.

The gray fox, *Urocyon cinereoargenteus*, was found in all areas of the city, including the Downtown area, compared to the rest of the animals that were found mostly in low populated areas close to the Rio Grande, and the Franklin Mountains. The gray fox samples positive for *T. cruzi* are relatively higher than reported in other states, with some studies reporting zero cases from the animals tested (Brown et al., 2010). A study in North Carolina and Virginia, with a total of 54 gray fox, only had six foxes testing positive for *T. cruzi* (Rosypal et al., 2010). A study in central Texas, found eight foxes, out of 58 tested to be positive for *T. cruzi* (Curtis-Robles, Lewis, & Hamer, 2016). *Leishmania spp.* was also reported in one fox of the North Carolina and Virginia study (Rosypal et al., 2010).

It is also important to take into account the home range of these animals, given that although some of them were picked up at one location, some of them can travel large distances. For the coyotes, *Canis latrans*, males can travel between 30 and 26 km a day and females travel between 28 and 17 km a day (Lkaundre & Keller, 1981). Having said this, it is not clear where the coyotes could have picked up either of these diseases. Raccoons, *Procyon lotor*, are known to

live near bodies of water, which may be the reason why they were mostly found near the Rio Grande. The home range of the raccoons are anywhere between 0.21 km² and 1.82 km² (Prange, Gehrt, & Wiggers, 2004). Raccoons were also found to be positive in ten out of the twelve tested samples for *Leishmania spp.*, which can be due to *Lutzomia spp.* also benefiting from the ecosystem near the Rio Bravo.

All of the collected species that were positive for either Leishmaniasis or Chagas' disease were found in the West side of El Paso. This is probably due to the characteristics of the area given that it is an area where agriculture and farming is practiced. The results retrieved from the striped skunk are very similar to other studies where the *Mephitis mephitis* has shown to be seropositive for *T. cruzi* in Arizona (9%, N=34) and Georgia (3%, N=3), and the same study found negative results in California (0%, N=6) (Brown et al., 2010). The home range of the skunk is of about 1.1 km for females and 1.3 for males meaning that they got infected for both of these diseases somewhere close to where they were collected (Weissinger, Theimer, Bergman, & Deliberto, 2009).

Based on the results of the χ^2 , the sylvatic animals infected with leishmaniasis were divided in two groups: the skunk and the fox with a high prevalence, and the raccoon and coyote with a lower prevalence. It was statistically significant the difference between these two groups (Figure 20: Difference between species tested positive for *Leishmania spp.* based on χ^2 test).

With the results of χ^2 on the *T. cruzi* results, in Figure 21 it can be seen that the sylvatic animals were divided into three categories: the coyote with the highest prevalence; in the second category the gray fox with significant difference with the coyote and the skunk, however, no difference with the raccoon, and in the third group are the raccoon and the skunk with no significant difference between each other. The difference between these groups could have been

due to factors such as the diet and other *Leishmania spp.* and *T. cruzi* hosts and vectors present in this area transmitting the disease.

Studies have shown that consuming food with the parasite can cause infections (Roellig, Ellis, & Yabsley, 2009). From the four species collected, only the coyotes are primary carnivores and the consumption of infected prey could be the reason why their prevalence in *T. cruzi* was so high. Skunks feed on vegetation but are mainly scavengers, raccoon and gray fox are omnivorous feeding on other small animals but also fruits and crops (Hall, 2005).

Although a study from central Texas found raccoons to have a prevalence of 70, this study only showed a prevalence of 35.71 (Curtis-Robles et al., 2016). The coyotes (14.3 prevalence) and foxes (13.8) from the same study in Texas had a much lower prevalence compared to this study with a prevalence of a 100 and 48.65 respectively. As mentioned before, the climate in El Paso, Texas, is much different from that in central Texas and the *T. cruzi* vectors found in this area are different from other parts of Texas. *T. rubida* has only been found to be positive for *T. cruzi* in two counties of Texas, both in the Western part of Texas including El Paso County. *T. indictiva* and *T. protracta* have also been found in the region although they have not shown any positive results for *T. cruzi*. The difference of vectors in this region could be a reason for the difference in prevalence of the same species tested in both studies.

8. Conclusion

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 that have cardiomyopathy or lymphadenopathy also as a symptom. Recognizing this diseases as a possibility will allow the health care provider a better case management and for the patient to fully recover when they are diagnosed on time. It is also important to teach the population about the diseases for them to know what to do in case they notice any of the symptoms. However, prevention is a key factor to avoid these diseases which should be the main focus of any education efforts made to keep the El Paso community healthy.

Identifying the local hosts of *Leishmania spp.* and *T. cruzi* will allow to create targeted intervention programs tailored to the El Paso county area which will help in the efficiency of raising awareness. Although the species in this study are wild animals and rarely come in close contact with people, they do help on maintaining the disease in the area.

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9. Vitae

Mariel Matamoros has a B.S. in Microbiology with a minor in Chemistry from The University of Texas at El Paso. She began her Masters' in Public Health in 2013 where she also began her research in Leishmania and Chagas' disease. In the summer of 2014 she visited Ecuador where she attended Symposium of Tropical Diseases and a workshop on Leishmaniasis. Being that this county is endemic for the disease she was able to see the physical and psychological effects of the disease on people.

Besides doing laboratory research, she is also doing an internship with the City of El Paso Department of Public Health in the Public Health Preparedness Program. She is the Coordinator for the Radiation Response Volunteer Program, a program funded by the Conference of Radiation Control Program Directors (CRCPD). Since its inception in 2014, Mariel has assisted in the implementation of the project's objectives, including conducting community outreach, recruiting radiation professionals, coordinating recruitment and volunteer training with the West Texas Medical Reserve Corp, and exercising the newly developed Community Reception Center plan. For her efforts in the community along with the work of her coworkers, she was awarded with the honor of being part of the 2016 Pillars of Public Health by El Paso Department of Public Health.