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Potential For Sylvatic Mammals And Stray Canines In Transmission Of Leishmaniasis And Trypanosoma Cruzi In Paso Del Norte Border Area

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POTENTIAL FOR SYLVATIC MAMMALS AND STRAY CANINES IN TRANSMISSION OF LEISHMANIASIS AND *TRYPANOSOMA CRUZI* IN PASO DEL NORTE BORDER AREA

By

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Introduction

Epidemiology of Leishmaniasis

The leishmaniases are a group of diseases with a worldwide distribution caused by protozoan parasites that are members of the genus *Leishmania* known to infect mammalian species, including humans by the bite of phlebotomine sand flies[1]. The manifestations of the human leishmaniasis are classified in four clinical forms: visceral, cutaneous, mucocutaneous, and diffuse cutaneous. Cutaneous manifestations can be further subdivided into localized, diffuse, leishmaniasis recidivans, and post-kala-azar dermal leishmaniasis[2]. Leishmaniasis has a wide geographic distribution and a high global incidence of human disease. The World Health Organization (WHO) estimates that 350 million people around the world are at risk for leishmaniasis and develop the disease annually[1]. In addition, approximately 20,000 to 40,000 deaths per year worldwide are attributed to the visceral form of the disease. Leishmaniasis is endemic in 88 countries on five continents[1]. The countries in which leishmaniasis is most endemic include Afghanistan, Brazil, India, and Sudan. With the increase in international travel, immigration, overseas military exercises, and HIV co-infection, leishmaniasis is becoming more prevalent throughout the world[2].

Epidemiology of Leishmaniasis Worldwide

Cutaneous leishmaniasis (CL), also called “oriental sore”, “Aleppo sore”, and “Baghdad boil”, among other names, is more widely distributed than any other form[3]. Cutaneous leishmaniasis is endemic in more than 70 countries with 90% of cases occurring in Afghanistan, Algeria, Brazil, Pakistan, Peru, Syria, and Saudi Arabia[3]. Cutaneous leishmaniasis affects more than 1.4 million persons annually[1]. Surveillance data indicate that the number of cases has increased during the past decade, as documented in Afghanistan[4], Bolivia[5], Brazil[6], Colombia[5, 7], Peru[5], and Syria[8]. Possible explanations for the increase in cases include improved diagnosis and case management[3], as well as inadequate vector or reservoir control and increased detection of cutaneous leishmaniasis associated with opportunistic infections, i.e. HIV/AIDS[9]. However, due to the fact that many CL infections are symptomless or misdiagnosed[3], the global burden is still likely to be an underestimate.

More than 90% of visceral leishmaniasis (VL) cases occur in six countries: Bangladesh, Brazil, India, Sudan, South Sudan, and Ethiopia. The remainders occur in southern Europe, Asia (with the exception of Southeast Asia), the Middle East, and Africa, particularly east and North Africa, and a few countries in Latin America.[1]

Epidemiology of Leishmaniasis in the Americas

Cutaneous leishmaniasis is the most prevalent form of the disease in the Americas, where it occurs from Southern Texas to Northern Argentina[10]. It is considered a neglected tropical disease by the WHO[11]. From 2004-2008, CL was most prevalent in Brazil and Peru[10]. However, with more than 15,000 reported cases in 2009 and 2010, Colombia now ranks second after Brazil (>15,000 per year)[1].

90% of the reported cases of visceral leishmaniasis in the Americas occur in Brazil[10]. Foci also have been reported in Mexico, Central America, and other South American countries (not in Uruguay, Chile, or Canada). Brazil ranks VL control as an important public health priority, reporting is mandatory. In the early 1980s, the first of a series of urban epidemics occurred in the Brazilian city of Teresina, followed by other outbreaks in São Luis, Natal, and Fortaleza[1]. The reported VL incidence in Brazil doubled from a mean of 1,500 cases per year in the 1980s to more than 3,400 per year from 2003 to 2007; the disease now occurs in urban to rural areas as far south as the states of São Paulo and Mato Grosso do Sul, in addition to the traditionally endemic northeast[1]. Colombia ranks in second with the most annual cases (≈ 60) from 2004-2008 and coming in third with about 48 cases per year from 2004-2008[1].

Disfiguring, mutilating, and occasionally life-threatening lesions of mucocutaneous leishmaniasis (ML) have been reported in 25%, 14%, 2%, and 0.3% of persons with *L. braziliensis* infections in Bolivia, Peru, Colombia, and Venezuela, respectively[12]. Mucocutaneous leishmaniasis also occurs in the Central and South Americas, except for Argentina.

Diffuse cutaneous leishmaniasis (DCL) cases, caused by *L. mexicana*

amazonensis, are very rare and have been recorded in Belize, Guatemala, Venezuela, Mexico, and the Amazon region of Brazil[12]. This form develops in as many as 6 out of 20 (30%) patients with *L. m. amazonensis* infection[12].

Overview of the Epidemiology of Leishmaniasis in Texas

Since 1903, 30 autochthonous cases of cutaneous leishmaniasis have been reported in the United States with all located in south central Texas, an area that is now known to be endemic for the disease[13]. In 2008, nine cases of CL were reported in the northern Texas, Dallas- Fort Worth area. In all cases, diagnosis was confirmed by histologic examination. Out of the nine cases, two were confirmed by PCR to be caused by *L. mexicana*. None of these claimed to have traveled outside of their respective local counties[13]. These cases suggest a northern spread in Texas and a shift in the area where this disease is endemic. Further studies must be conducted in order to identify the reasons for the northern spread.

Zoonotic Leishmaniases in the Americas and in Texas

Among the 15 well-recognized Leishmania species known to infect humans, 13 are zoonotic in nature[14]. Cutaneous leishmaniasis, caused by *L. mexicana*, is considered endemic in south-central Texas[15]. In 1990, the first isolations of *Leishmania* in Texas were discovered in sand flies, *Lutzomyia anthophora,* collected from a nest of southern plain wood rats, *Neotoma micropus*[16]. The first rodent host of *L. mexicana* was collected in Texas from a wood rat, *N. micropus*. This finding indicates that the *Neotoma* species is a potential zoonotic reservoir for leishmaniasis[17, 18]. In a study conducted in Texas between 2004 and 2008, eight domestic felines were examined for *L. mexicana* and positively diagnosed through skin biopsies[19]. In addition to the histological examination of skin tissue, positive PCR and sequence results were obtained from biopsy samples from 5 of the 8 cats[19]. All cats were collected from different counties in south east Texas including Burleson (2004), Caldwell (2004), Bell (2006), Hood (2006), Lampasas (2007), Tarrant (2007), Bastrop (2008), and Kaufman (2008) [19]. The results of these studies suggest that cutaneous leishmaniasis is becoming more widespread in Texas; however, the cause of this is unclear. The burrowing wood rat, *N. micropus*, is a mammalian reservoir for *L. mexicana,* and the northern spread of disease within the state may be related to expansion of its range[16, 19].

Leishmania Morphology

Leishmania has two morphological forms, the promastigote and amastigote. In the sand fly vector, *Leishmania* exist as flagellated extracellular promastigotes[2]. The promastigote is spindle shaped measuring 10 - 20μm in length, not including the length of the flagellum. The nucleus and kinetoplast are visible[20]. Promastigotes of *Leishmania* species are motile and elongate, with a flagellum at the anterior end. In human tissue, leishmanial parasites are in the amastigote form and multiply within histiocytes[20]. Amastigotes are ovoid or round and 1.5 μm to 3 μm in diameter[20]. They have a thin cell membrane, a relatively large nucleus, and a rod-shaped kinetoplast that is not always visible in tissue sections because of its orientation within the parasite.

Life cycle of *Leishmania*

Metacyclic promastigotes, the infective form of the parasite, are carried in the salivary glands of female sandflies (*Phlebotomus* species in the Old World; *Lutzomyia* species and *Psychodopygus* species in the New World) [21] and transmitted to a host during a blood meal. The promastigotes are phagocytized by macrophages and other types of mononuclear phagocytic cells, which transform into non-motile, oval amastigotes with no free flagellum[21]. Amastigotes persist and replicate by binary fission within the vacuole. The expanding vacuole then nearly fills the cell, leading to lysis and cell death[21]. Released daughter amastigotes attach to and penetrate other cells. When a sand fly ingests an infected cell, amastigotes transform into promastigotes, which live and develop extracellularly in the fly's alimentary tract and then migrate to the proboscis, the life cycle of *Leishmania,* then begins again[21]. Rarely, transmission can occur via congenital, sexual, occupational, or blood borne through transfusion or IV drug use mode in humans.

Vectors and Criteria for Reservoirs

The leishmaniases are a group of zoonotic diseases that have been known to infect mammalian species, including humans through the bite of phlebotomine sand flies[3, 22]. In the Old World, the most common sub-genera of sand fly is the *Phlebotomus*[22]. In the New World, the sub-generas *Lutzomyia* and *Psychodopygus* most commonly are vectors for *Leishmania*[22]. Phlebotomine sand flies are common inhabitants of a wide range of environments including areas of primary forest, crop plantations, animal shelters, and human houses, from coastal plains to hilly areas[22]. *Lutzomyia* are common inhabitants of caves and only females suck blood of the infected host and transmit the parasite 7-10 after feeding. The infected host must live long enough to sustain the parasite to reproduce and in turn, infect other mammals. The infected mammal is not considered a reservoir if it did not live long enough for it to infect other vectors or provides maintenance of the parasite. Also, the maintenance of parasites in skin lesions and blood must be at densities high enough to infect vectors[23].

Human Leishmaniases (Clinical Forms)

The manifestations of the human leishmaniases are classified in 4 clinical forms, visceral, cutaneous, muco-cutaneous and diffuse[2]. The clinical presentations are extremely diverse and dependent on a variety of host and parasitic factors.

Cutaneous forms of the disease normally produce skin ulcers on the exposed parts of the body such as the face, arms and legs. The disease can produce a large number of lesions, up to 200, causing serious disability and invariably leaving the patient permanently scarred[24].

Visceral leishmaniasis (VL) is usually a disease of young children and adults[25]. Only a small number of infected patients actually display the classical symptoms of this disorder. Visceral leishmaniasis is characterized by irregular bouts of fever, substantial weight loss, swelling of the spleen and liver, and anemia (occasionally serious) [24]. If the patient is untreated, the fatality rate can be as high as 100% within 2 years[25].

In the mucocutaneous form of leishmaniasis, lesions can lead to partial or total destruction of the mucous membranes of the nose, mouth and throat cavities and surrounding tissues[25].

Diffuse cutaneous leishmaniasis is incurable and occurs among individuals who have defective cell-mediated immune responses to leishmanial antigens[26]. This form is extremely disfiguring and is characterized by nonulcerating, non-necrotizing skin lesions that spread over the body. This form is often clinically confused with leprosy because of the nature and distribution of the lesions[26].

Treatments

The treatment of leishmaniasis depends on the clinical manifestation, parasitic species and geographic location[25]. The 2 available medications, sodium stibogluconate (Pentostam), produced in Great Britain, and meglumine antimonate (Glucantime), produced in France, are experimental drugs approved by the Federal Drug Administration, available only through the Centers for Disease Control and Prevention (CDC) [27]. Depending on the species and region, cure rates of 80-100% have generally been reported[25, 27].

Chagas' Disease

Epidemiology of American Trypanosomiasis

American trypanosomiasis, also commonly referred to as Chagas' disease is a potentially life threatening disease caused by the protozoan parasite *Trypanosoma cruzi (T. cruzi).* The disease is endemic in 21 Latin- American countries located in two main ecological zones, the Southern Cone (Brazil, Argentina, Paraguay) and in Northern South America, Central America & Mexico[28]. Chagas' disease was once confined to the Americas, mainly Latin America, but has now spread to other continents[29]. It is widely distributed from southern United States to southern South America. It has been increasingly detected in the United States, Canada, many European and some Western Pacific countries[29]. This is due mainly to the increased migration between Latin Americans and the rest of the world.

Chagas' disease, documented to be dispersed by dozens of species of insect vectors, which are members of the family, Reduviidae. *Triatoma infestans* (Southern Cone) is mainly an intra-domiciliary vector and *R. prolixus* (Northern zone) is both a peri- and intra-domiciliary vector[34]. Humans become infected when the vector invades the household like those common in Latin America. Other modes of transmission include blood transfusions, vertical transmission (from infected mother to child), and organ transplants, laboratory accidents, and food contaminated with the *T. cruzi*[29]. Nearly 7 to 8 million individuals are infected worldwide with *T. cruzi,* mostly in Latin America in Mexico, Central and South America[29]. Chagas' disease has an acute and chronic phase and if left untreated it can be a lifelong infection. The World Health

Organization (WHO) approximates that more than 25 million people are at risk for contracting the disease. In 2008, it was estimated that Chagas' disease killed more than 10,000 people[29].

Epidemiology of Chagas' Disease in the U.S. and Texas

The CDC estimates that more than 300,000 persons with *T. cruzi* infection live in the United States[30]. Most people with Chagas' disease in the United States acquired their infections in endemic countries. Although there are triatomine bugs in the U.S., only rare vector-borne cases of Chagas' disease have been documented[30]. The only three known cases of direct transmission of *T. cruzi* from vector to human have been documented in the United States. Two of the cases occurred from infant twins in 1955 from Texas and the third case was a woman from California who became ill with Chagas' disease in 1982[31]. The rarity of human cases probably is due to better housing conditions in the United States, which don't provide a suitable dwelling for the triatomine vector to thrive unlike many impoverished homes in Latin America.

In contrast to the three documented human cases of *T. cruzi,* more infections have been recorded among triatomine arthropods and wild and domestic mammals in the southern half of the United States. For example, a study in southeast Georgia trapped raccoons and opossums over a span of two years. Of the 39 opossums, 6 (15.4%) and 12/54 raccoons captured (22.%) tested positive for *T. cruzi* in blood cultures[32].

T. Cruzi Morphology

T. cruzi exists in three morphological forms: trypomastigote, epimastigote, and amastigote[33]. Trypomastigotes are the non-dividing form located in the feces of the vector and in the bloodstream of infected vertebrates. Trypomastigotes are approximately 20mm long and have a slender, irregular shaped membrane[33]. They have a centrally positioned nucleus, and a kinetoplast located towards the posterior. A flagellum stems from the kinetoplast and runs through the remainder of the parasite and extends beyond it. The epimastigote is found in the intestinal tract of the vector and the kinetoplast is found anterior and adjacent to the nucleus[33]. The flagellum of epimastigotes emerges from the middle of the membrane. The amastigote is the dividing form, found in the cytoplasm of vertebrate cells. Unlike the trypomastigote and epimastigote, the amastigote has no protruding flagellum and are round/oval shaped[33].

Life cycle of *T. Cruzi*

Trypomastigotes are carried in the feces of the triatomine vector and are transmitted to a host during a blood meal when they are released near the site of the bite wound[33]. Once the trypomastigotes penetrate various cells located near the bite wound in the host, they differentiate into intracellular amastigotes[33]. The amastigotes replicate through binary fission in cells of the infected tissue and then differentiate into trypomastigotes and burst out of the cell into the bloodstream. Trypomastigotes cannot replicate in the bloodstream unless they are either ingested by another vector or enter other cells. *R. prolixus* or *T. infestans,* is infected by taking a blood meal from an infected animal or human with circulating trypomastigotes in their bloodstream[33]. The trypomastigotes differentiate into epimastigotes in the mid-gut of the vector and multiply. Typomastigotes also differentiate into infective metacyclic trypomastigotes in the hindgut of the vector where they are carried until the arthropod releases them near the site of the bite wound of a reservoir[33]. *Trypanosoma cruzi* can also be transmitted through blood transfusions, organ transplantation, transplacentally, and through laboratory accidents[29].

Chagas' Disease Vectors

Chagas' disease, documented to be dispersed by dozens of species of insect vectors, which are members of the family, Reduviidae. *Triatoma infestans* (Southern Cone) is mainly an intra-domiciliary vector and *R. prolixus* (Northern zone) is both a peri- and intra-domiciliary vector[34]. Both vectors transmit Chagas' disease. They thrive under poor housing conditions (for example, mud walls and thatched roofs), thus, in endemic countries, poor people living in rural areas are at greatest risk for acquiring infection[34]. Public health interventions aimed at preventing transmission have decreased the number of newly infected people and completely halted vector borne transmission in some areas. In 1991, the Southern Cone initiative was launched by the collective input from each of the countries governments. It focused on the interruption of *T. cruzi* transmission by eliminating domestic vectors (particularly *T. infestans*), together with extended screening of blood donors to reduce the risk of transfusional transmission, and the promotion of maternal screening for infection followed by specific treatment of infected newborns[34]. With the success of the initiative and similar others launched in 1997 for Central America, prevalence estimates steadily declined[34].

Chagas' Disease Reservoirs

Trypanosoma cruzi has been found to infect more than 100 mammalian species in eight different orders[35]. In the United States, the disease exists almost exclusively as a zoonosis; only five autochthonous insect-borne cases have been reported in humans[36]. *Trypanosoma cruzi* has been identified in a variety wild animals such as raccoons, opossums, wood rats, raccoons, armadillos, and coyotes for the past 70 years [32, 37, and 38]. Although the potential for dogs to serve as reservoirs for Chagas' disease has not been well studied, there is some evidence suggesting their potential involvement in domestic transmission in the U.S. as well. More recently, a study identified *T. cruzi* in forty-eight different dog breeds in 48 of 254 Texas counties[38]. In the histopathologically confirmed canine cases, acute death occurred in 42%, approximately half of which were under one year of age. Nearly all cases with histopathology data reported myocarditis (97.9%) and eighty two percent observation of *Trypanosoma cruzi* organisms. The study results provide strong evidence that an active canine Chagas disease transmission cycle is present throughout Texas, affecting a broad range of dog breeds and age groups[38].

Clinical Aspects of Chagas' Disease and Treatment

Chagas' disease has an acute and a chronic phase, both of which are mostly asymptomatic and if untreated could be life threatening. Immediately after infection, the acute phase of Chagas' disease occurs and may last up to a few weeks or months. During the acute phase, trypomastigotes are circulating in the blood and infection may be mild or asymptomatic. Symptoms of the acute phase are not always present but can include fever, swelling around the site of the bite wound, and in very rare cases, severe inflammation of the heart muscle or the brain and lining around the brain[39]. Chronic Chagas' disease occurs subsequent to acute infection. A prolonged asymptomatic form called, "chronic indeterminate" can occur. During this phase, very few or no trypomastigotes are circulating in the blood and some individuals may never experience Chagas' related symptoms during the course of their lives. The CDC estimates that 20-30% of infected individuals will experience incapacitating or life threatening symptoms[39]. Symptoms of chronic Chagas' disease include heart rhythm abnormalities, a dilated heart that doesn't pump blood well, and a dilated esophagus or colon that can lead to difficulties with eating and passing stool. All of the symptoms can lead to sudden death[39]. In people who have suppressed immune systems (for example, due to AIDS or chemotherapy), Chagas' disease can reactivate and parasites can be found in the circulating blood. This occurrence can potentially cause severe disease[39].

In the U.S., medications to treat Chagas' disease are only available through the Centers for Disease Control and Prevention. Common treatments for acute Chagas' disease in other endemic regions most affected include the prescription medications Benznidazole and Nifurtimox[40]. Treatments for chronic Chagas' disease cannot cure the

disease but can be used to alleviate symptoms. To treat heart related Chagas' complications, infected individuals may receive a pacemaker or other devices to regulate their heart rhythm or in extreme cases, a heart transplant. Treatment for digestive-related complications may include a change in diet, some medications, corticosteroids, or in severe cases, surgery[40].

Study Rationale

It is well recognized that *T. Cruzi* is endemic in the United States, as evidenced by infections reported in dogs and a wide range of mammalian wildlife species. The Southwest region of Texas has a wide variety of wild mammals and domestic dogs, which have been described as hosts of *Leishmania* and *T. Cruzi* and some of them are considered to play an important role as reservoirs. Additionally, environmental changes, such as climate change, may expand the range of vector and reservoirs northward affecting peri-urban and urban centers. In North America (Mexico, United States, and Canada), leishmaniasis is a vector-borne disease that is autochthonous in Mexico and Texas and has begun to expand its range northward[41]. From a public health viewpoint it is important to evaluate the presence of *Leishmania* and *T. cruzi* in sylvatic mammals and canines and determine whether or not they are acting as disease reservoirs.

The present study sought to identify whether *T. Cruzi* and *Leishmania mexicana* are present in sylvatic mammals, which include rodents, raccoons, and stray dogs in the El Paso County, Texas and surrounding areas (Texas state regions 9 and 10). DNA extraction and amplification of *Trypanosoma cruzi* and *Leishmania* from sylvatic mammal and stray canine heart, spleen, and skin tissue will be conducted for the identification of *Leishmania mexicana* and *T. cruzi* by PCR.

Specific Aim

The major aim of this study is to investigate whether *Leishmania mexicana* and *T. Cruzi* are present in sylvatic mammals or stray canines and to determine if these are potential reservoirs of these parasites in the El Paso County, Texas and surrounding areas (Texas state regions 9 and 10).

Hypothesis

It is hypothesized that *T. cruzi* and *Leishmania mexicana* are mainly affecting wild mammals in the southern portion of Texas and live in close proximity with traditional vectors of the parasites. The identification of *T. cruzi* and *Leishmania mexicana* in tissue samples collected from sylvatic mammals and stray canines will show that they are possible reservoirs of these parasites.

Methods and Materials

Sample Collection

Tissue samples of sylvatic mammals and stray canines were collected for identification of *T. cruzi* and *Leishmania mexicana.* Heart and skin tissue samples were collected from stray canines provided by the City of El Paso Animal Services. UTEP approval from the Institutional Animal Care and Use Committee (IACUC) was not required as the canines were not captured nor euthanized specifically for this study. Dr. Ken Waldrup, the regional veterinarian for the Texas State Department of Health Services collected the tissue samples once a week for 10 weeks from stray canines during October 2011 and July 2012. He also collected heart, lung, and skin tissue samples from sylvatic animal species such as raccoon's (*Procyon lotor)*, White-throated wood rats (*Neotoma leucodon)*, Hispid cotton rats (*Sigmodon hispidus),* White-ankled mice (*Peromyscus pectoralis)*, and White-footed mice (*Peromyscus leucopus*.) Sampling and tissue collection provided by Dr. Waldrup was obtained through live traps, which captured animals in the wild in the Mason Mountains.

DNA Extraction

Genomic DNA extraction from sylvatic mammal and stray canine heart, spleen, and skin tissue was conducted to prepare the samples for PCR identification of *Leishmania mexicana* and *T. cruzi*[42]. Two milliliters of SNET (solution to denature proteins) and 100 microliters of proteinase K was added to each tissue sample in a conical tube. The tube was then incubated overnight at 55 degrees Celsius with agitation in a Gyromax Benchtop Incubator Shaker[43] at about 300 rpm. Once incubation was complete, the sample was placed on the vortex machine for 10 seconds until the tissue dissolved. Next, one milliliter of phenol: chloroform: isoamyl alcohol was added to the tube and sealed. The sample was then vortexed for 10 seconds and placed on a rocking platform for 30 minutes at room temperature. After the 30-minute incubation, the sample was centrifuged for 5 minutes at maximum speed at room temperature. After centrifugation, 750µl of the aqueous phase was placed in a 1.5-milliliter microfuge tube with an equal volume of ice-cold isopropanol to precipitate the DNA. Then the sample was mixed by vortexing the tube for 5 seconds and then centrifuged for 15 minutes at max speed at 4 degrees Celsius. Next, the isopropanol was removed and the pellet was rinsed with 100 microliters of 70% ethanol. If the pellets were loose, the tube was centrifuged again for 5 minutes to secure the pellet to the bottom of the tube. Next, the 70% ethanol was removed and the pellet was allowed to air dry for 10-15 minutes at room temperature. Finally, the pellet was suspended in 100 microliters of nuclease free water and vortexed. To measure the concentration of DNA the NanoDrop was used and the samples were stored at -20 degrees Celsius.

Primers and PCR

The PCR primer targeting *Leishmania genus* is a 720 base pair sequence- LINR4/ LIN19[44]. The primer targeting *Leishmania mexicana* is a 790 base pair sequence of oligonucleotides- IR1/LM17[44]. The primer targeting *T. cruzi* is a kDNA mini-circle- P35/P36[44]. The positive control for all samples is a 227 base pair sequence known as the IRBP gene[44]. The small length of target gene sequences requires a 1.8% agarose gel stained with ethidium bromide. The gels were run at 100 volts for 45 minutes[45]. Positive controls for each target gene and a negative control (primers) were run as well. All primers were obtained from Invitrogen[46].

Data Analysis

Age, gender, and GPS location of each stray canine and wild mammal was collected and inputted into an excel spreadsheet.

Mr. Jaime Javier, GIS specialist from the City of El Paso's Department of Information Technology Services, mapped the GPS locations of the canines and sylvatic mammals. The maps depict the geographic distribution of *Leishmania* and *T. cruzi* infections.

Results

A total of 10 (10%) PCR confirmed cases of *T. Cruzi* and no cases of *L. mexicana* were identified among 96 stray canine samples collected in the El Paso region and surrounding areas (Table 1 and 2). PCR's of a *T. cruzi* canine positive and negative sample are shown in Figure 1 and 2. In Figure 1, the *T. cruzi* sample matches the profile to that of the positive control unlike the sample in Figure 2, which does not show any DNA matching the size of the positive control. Seven of the ten *T. cruzi* cases were all from the same animal group collected in October 2011. Nine of the ten cases were samples collected during the fall months i.e. September through December. Only one *T. cruzi* positive canine case had visible lesions on its back. Among the 96 canine samples, 2 (2%) were PCR confirmed to be of *Leishmania* genus but not *L. mexicana* (Table 3 and Figure 3). In Figure 3, the *Leishmania* genus sample matched the profile to that of the positive control. The canine samples were from 41 counties within El Paso and Regions 9 and 10. El Paso was the only county out of the 41 to contain *T. cruzi* positive canine cases. Figures 4 and 5 show the distribution of T. cruzi cases and both *Leishmania* genus cases in the Texas Regions 9 and 10, respectively.

A total of 13 (65%) PCR confirmed cases of *T. cruzi* (Table 4) and 1 (1%) case of *L. mexicana* (Table 5) was found among the 20 sylvatic mammals collected in the Mason Mountains. Figure 6 depicts the distribution of *T. cruzi* sylvatic cases in the Mason Mountains. Among the 20 sylvatic samples, 12 (60%) were PCR confirmed to be of *Leishmania* genus but not *L. mexicana* (Table 6). PCR of a sylvatic Leishmania genus and *T. cruzi* positive and negative sample are shown in Figure 7. The heart and skin tissue from the sample shown in Figure 7 was positive for Leishmania genus but

not for L. mexicana and only the heart sample tested positive for *T. cruzi.* The distribution of sylvatic cases is demonstrated in Figure 8. Five species were represented among the *T. cruzi* positive sylvatic mammals with raccoons being the top recorded species (Table 1).

The age of *T. cruzi* canine cases when available ranged from 6 months to adulthood (~6 years), 90% of the cases were adults. Approximately 60% of the cases were male. The age of *T. cruzi* sylvatic cases ranged from 6 months to adulthood, 92% of the cases were adults. Approximately 62% of the cases were male. Among the *T. cruzi* canine cases, 7 dog breeds were represented, with the top two breeds reported as Pit Bull and Chihuahua (Table 1). Five species were represented among the *T. cruzi* positive sylvatic mammals with raccoons being the top recorded species (Table 4).

Table 1 Stray Canine Chagas' Disease

	Chagas' Disease	
Demographic Characteristics	Positive Cases	Negative Cases
Age	10 (10%)cases	86 (89.5%)cases
Mean	6 years	6 years
Range	6 mths - 6 yrs	6 mths - 6 yrs
>1 year	90%	90%
Sex		
Male	5(50%)	39(45%)
Dog Breed		
Blue Heeler X	1(10%)	2(2.3%)
Black Lab X	1(10%)	4(4.6%)
Pit Bull X	3(30%)	17(19.8%)
Chihuahua	2(20%)	8(9.3%)
Sharpei X	1(10%)	0
Poodle X	1(10%)	4(4.6%)
German Shephard	1(10%)	26(30.2%)
Chow	0	2(2.3%)
Rottweiler	0	2(2.3%)
Cairn Terrier	0	6(6.9%)
Collie	0	1(1.2%)
Boxer	0	4(4.6%)
Lhasa apso	0	1(1.2%)
Cocker Spaniel	0	2(2.3%)
Corgi	0	1(1.2%)
Labrador Retriever	0	4(4.6%)
Fox Terrier	0	1(1.2%)

Table 2. Stray Canine *L. mexicana* Cases

	L. Mexicana	
Demographic Characteristics	Positive Cases	Negative Cases
Age	0	96(100%)
Mean		6 years
Range		6 mths - 6 yrs
>1 year		90%
Sex		
Male		44(46%)
Dog Breed		
Blue Heeler X		3(3.1%)
Black Lab X		5(5.2%)
Pit Bull X		20(20.8%)
Chihuahua		10(10.4%)
Sharpei X		1(1%)
Poodle X		5(5.2%)
German Shephard		27(28.1%)
Chow		2(2%)
Rottweiler		2(2%)
Cairn Terrier		6(6.2%)
Collie		1(1%)
Boxer		4(4.2%)
Lhasa apso		1(1%)
Australian Cattle Dog		1(1%)
Cocker Spaniel		2(2%)
Corgi		1(1%)
Labrador Retriever		4(4.2%)
Fox Terrier		1(1%)

Table 3. Stray Canine *Leishmania* genus Cases

	L. genus	
Demographic Characteristics	Positive Cases	Negative Cases
Age	2 (2%)	94(97.9%)
Mean	6 years	6 years
Range	6 years	6 mths - 6 yrs
>1 year	100%	90%
Sex		
Male	0	44(46.8%)
Dog Breed		
Blue Heeler X	0	3(3.2%)
Black Lab X	0	5(5.3%)
Pit Bull X	1(50%)	19(20%)
Chihuahua	1(50%	9(9.6%)
Sharpei X	0	1(1.1%)
Poodle X	0	5(5.3%)
German Shephard	0	27(28.7%)
Chow	0	2(2.1%)
Rottweiler	0	2(2.1%)
Cairn Terrier	0	6(6.3%)
Collie	0	1(1.1%)
Boxer	0	4(4.2%)
Lhasa apso	0	1(1.1%)
Australian Cattle Dog	0	1(1.1%)
Cocker Spaniel	0	2(2.1%)
Corgi	0	1(1.1%)
Labrador Retriever	0	4(4.2%)
Fox Terrier	0	1(1.1%)

Figure 1 T. cruzi positive canine sample collected on October 6, 2011

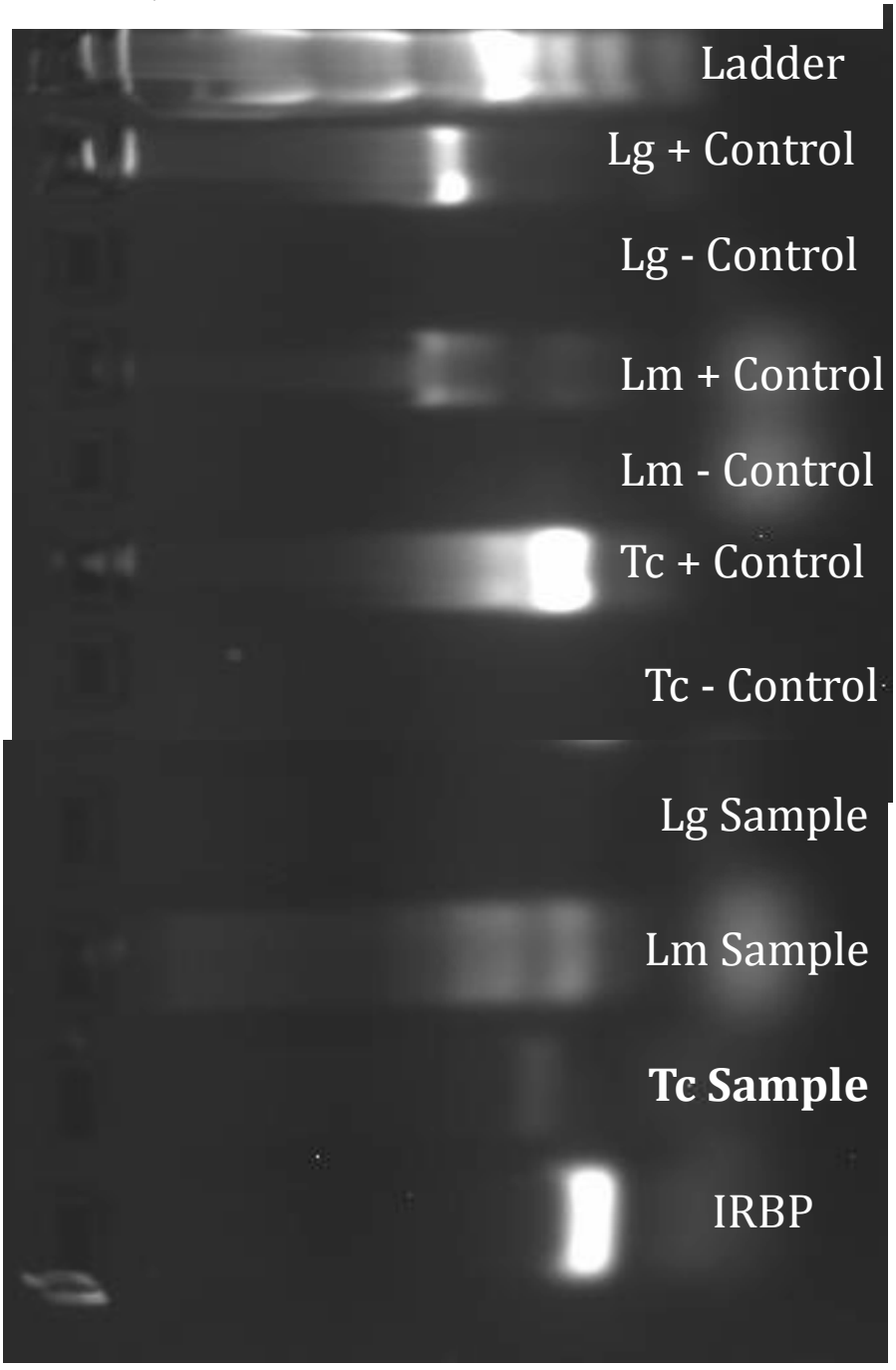


Figure 2. *T. cruzi* negative canine case collected on March 17, 2012

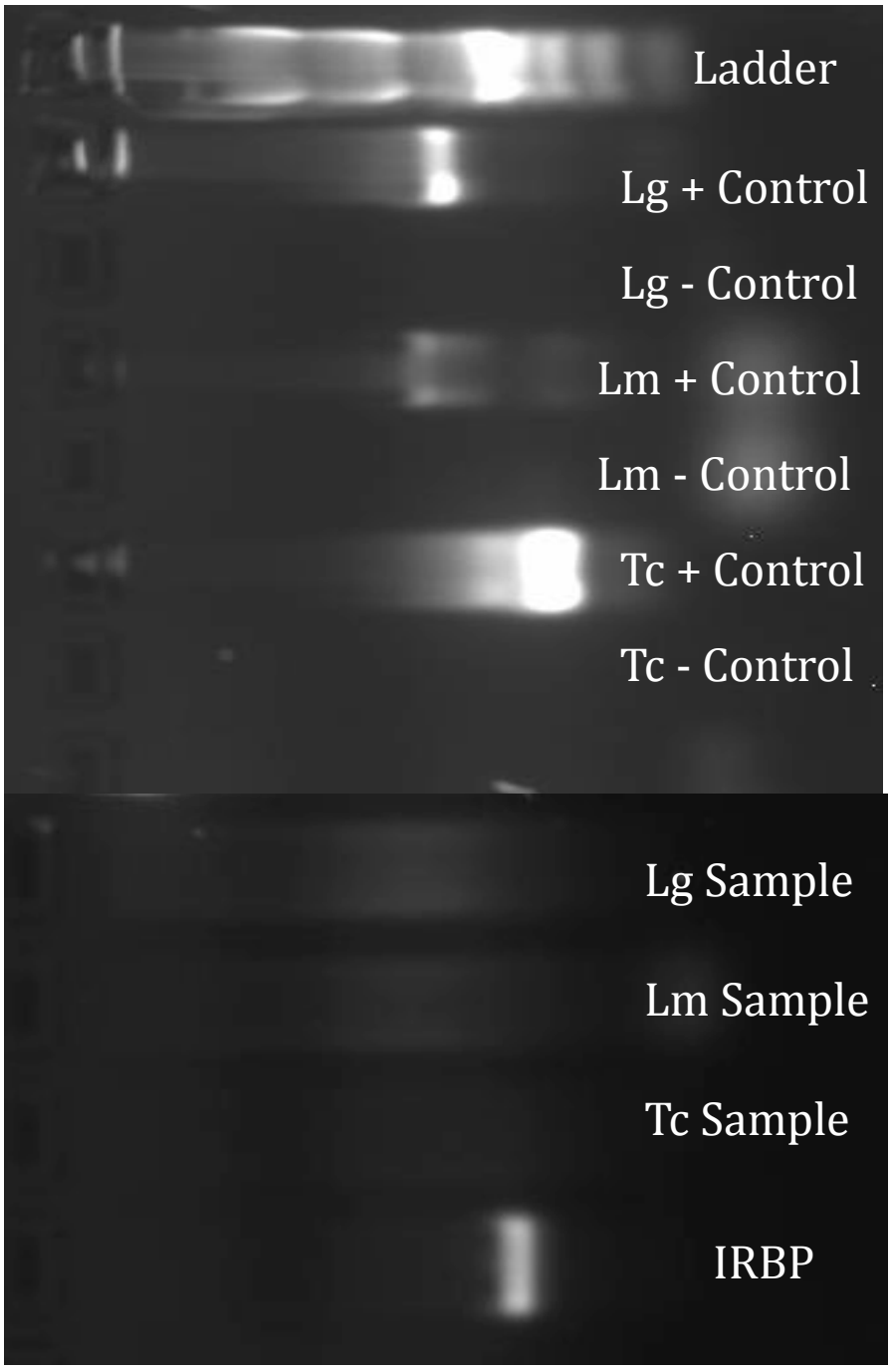


Figure 3. Positive canine *Leishmania* genus skin sample collected in March 2012

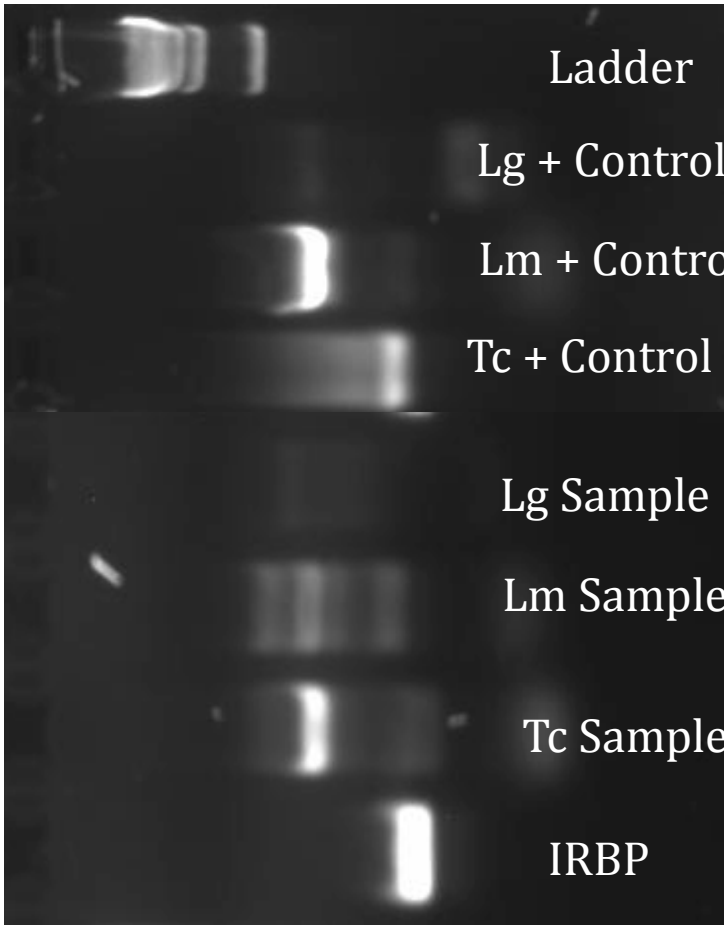


Figure 4. Locations of *T. cruzi* cases among the 96 total canines collected for sampling within El Paso and Regions 9 and 10

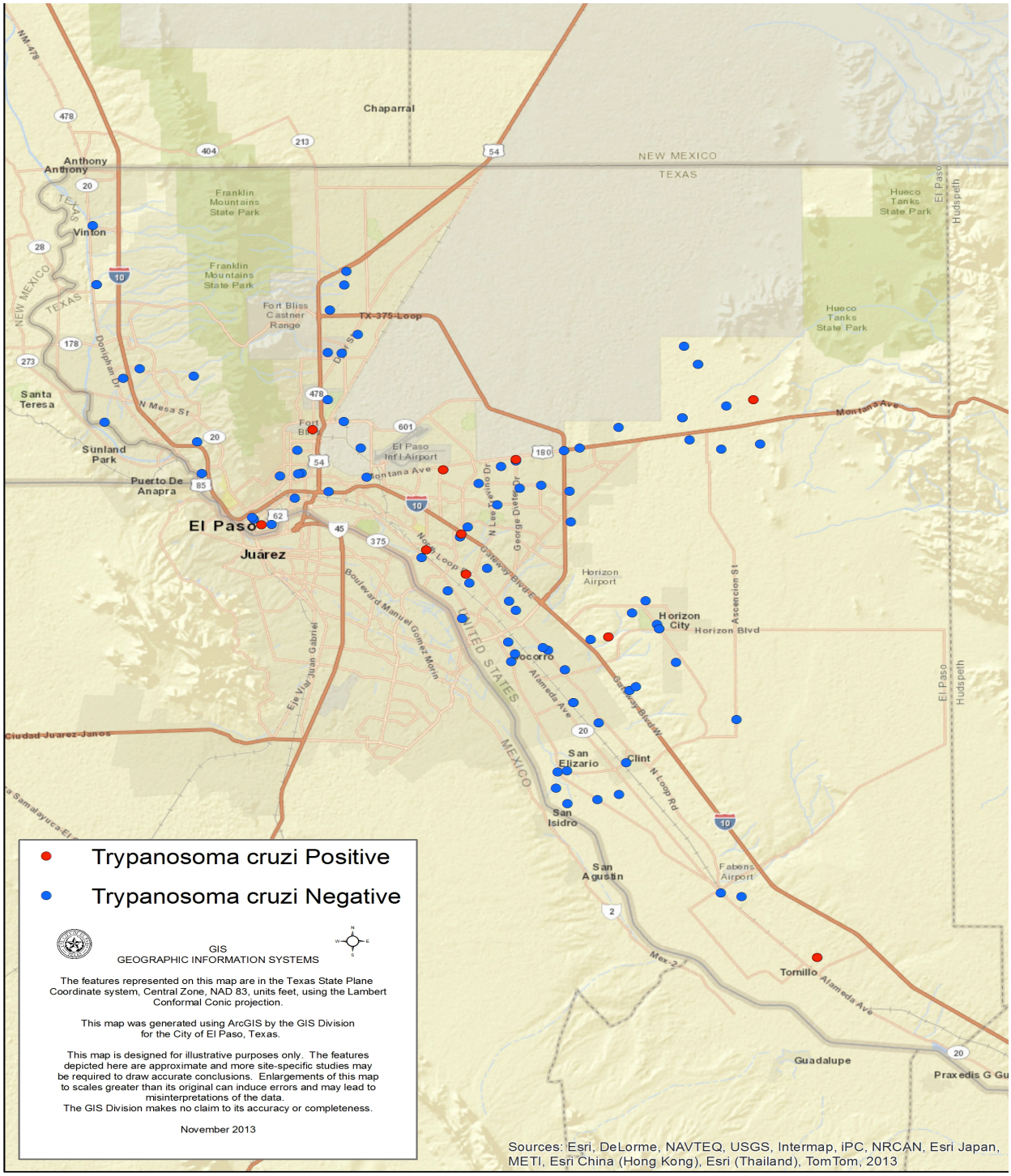


Figure 5. Locations of the 2 (2%) *Leishmania* genus within El Paso and Regions 9 and 10

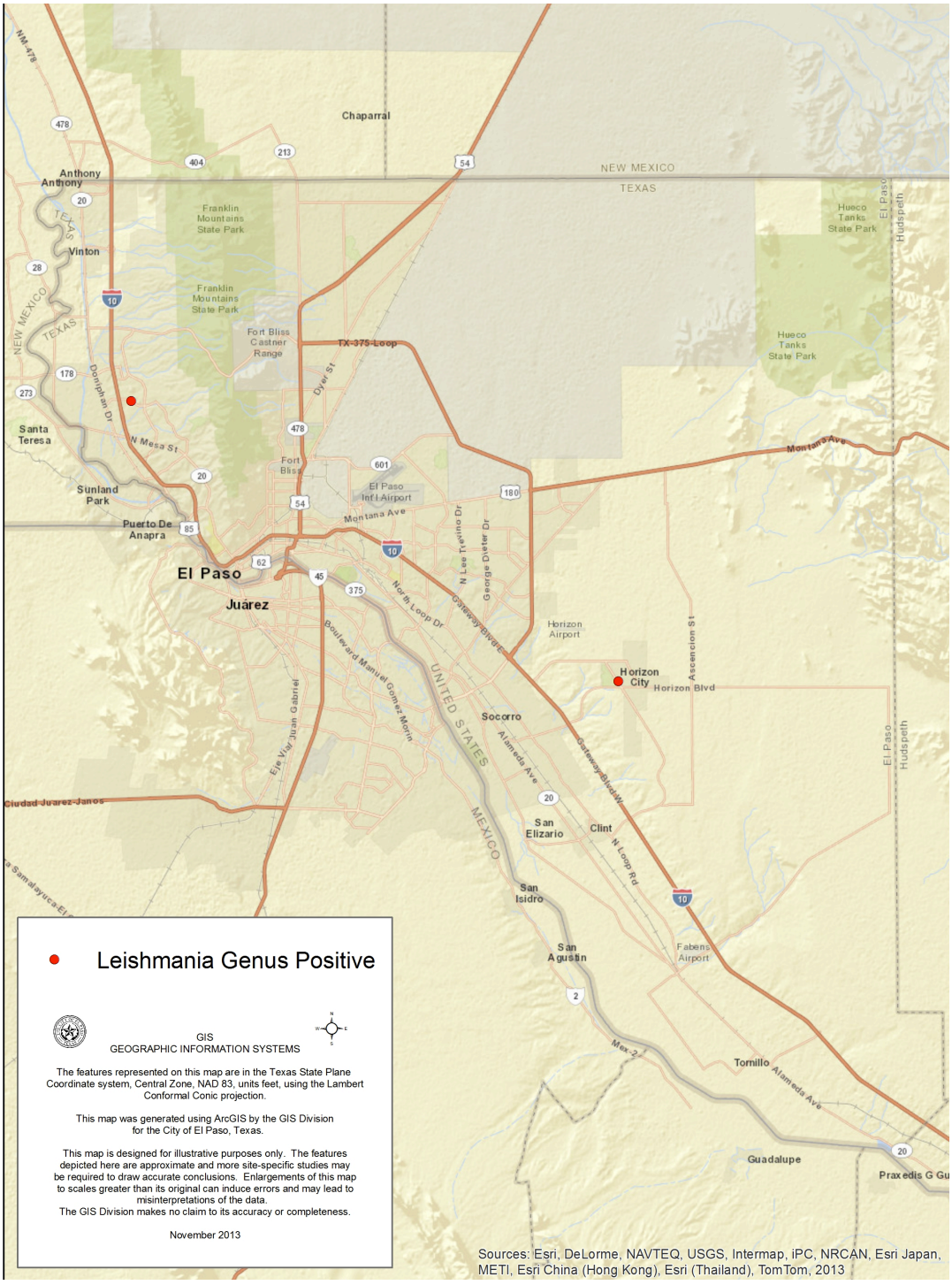


Table 4. Sylvatic mammal Chagas' disease cases

	Chagas' Disease	
Demographic Characteristics	Positive Cases	Negative Cases
Age	13 (65%)cases	7 (35%)cases
Mean	6 years	6 years
Range	6 mths - 6 yrs	6 mths - 6 yrs
>1 year	92%	90%
Sex		
Male	8(62%)	4(28%)
Species (Common Name)		
Procyon lotor (Raccoon)	5(38%)	1(14%)
Sigmodon hispidus (Hispid Cotton Rat)	1(8%)	2(28.5%)
Peromyscus pectoralis (White ankled mouse)	3(23%)	1(14%)
Peromyscus leucopus (White footed mouse)	3(23%)	0
Peromyscus truei (Pinon mouse)	1(8%)	0
Peromyscus attwateri (Texas mouse)	0	2(28.5%)
Neotoma leucodon (White-throated wood rat)	0	1(14%)

Table 5. Sylvatic mammal *L. mexicana* cases

	L. Mexicana	
Demographic Characteristics	Positive Cases	Negative Cases
Age	1	19
Mean	n/a	6 years
Range	n/a	n/a
>1 year	100%	90%
Sex		
Male	1(100%)	11(57.8%)
Species (Common Name)		
Procyon lotor (Raccoon)	0	6(31.5%)
Sigmodon hispidus (Hispid Cotton Rat)	0	3(15.8%)
Peromyscus pectoralis (White ankled mouse)	0	4(21%)
Peromyscus leucopus (White footed mouse)	0	3(15.8%)
Peromyscus truei (Pinon mouse)	1(100%)	0
Peromyscus attwateri (Texas mouse)	0	2(10.5%)
Neotoma leucodon (White-throated wood rat)	0	1(5.2%)

Table 6. Sylvatic mammal *Leishmania* genus cases

	Leishmania genus	
Demographic Characteristics	Positive Cases	Negative Cases
Age	11	9
Mean	6 years	6 years
Range	6 mths - 6 yrs	6 mths - 6 yrs
>1 year	92%	90%
Sex		
Male	8(72.7%)	4(44.4%)
Species (Common Name)		
Procyon lotor (Raccoon)	3(27.2%)	3(33.3%)
Sigmodon hispidus (Hispid Cotton Rat)	2(18.2%)	1(11.1%)
Peromyscus pectoralis (White ankled mouse)	1(9%)	3(33.3%)
Peromyscus leucopus (White footed mouse)	3(27.2%)	0
Peromyscus truei (Pinon mouse)	0	1(11.1%)
Peromyscus attwateri (Texas mouse)	2(18.2%)	0
Neotoma leucodon (White-throated wood rat)	0	1(11.1%)

Figure 6. Distribution of *T. cruzi* sylvatic cases in the Mason Mountains

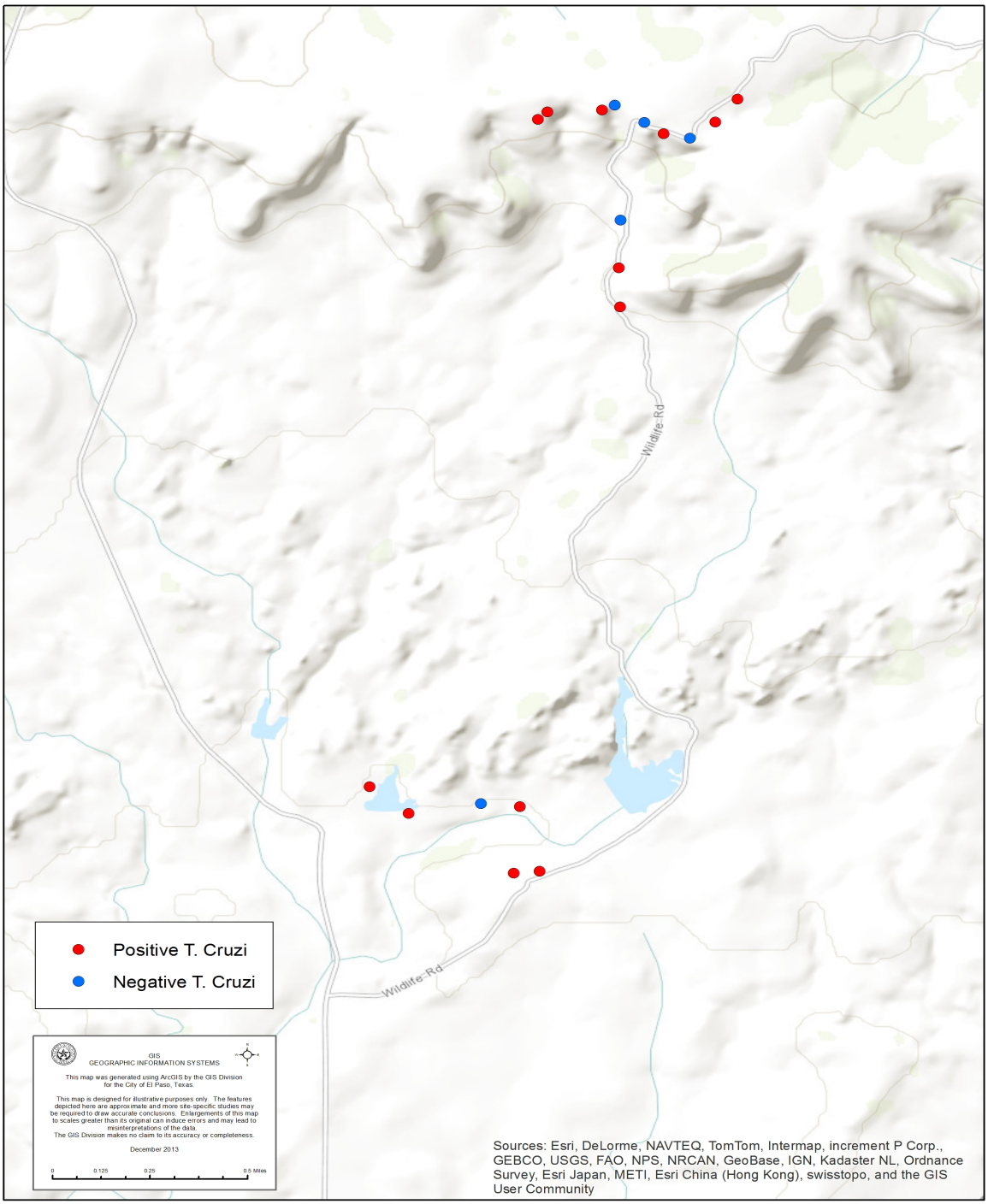


Figure 7. PCR of a sylvatic *Leishmania* genus and *T. cruzi* positive and negative sample

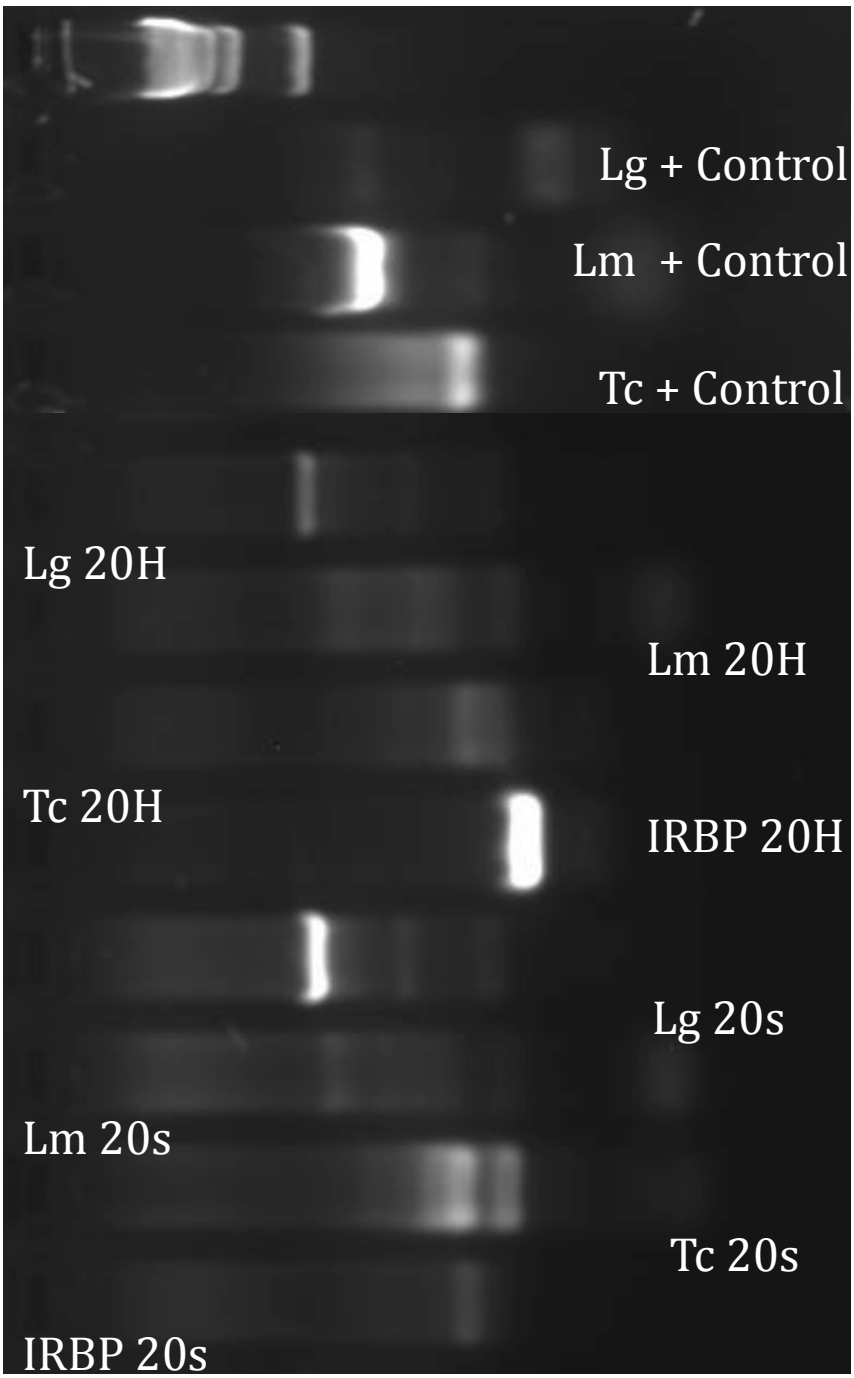
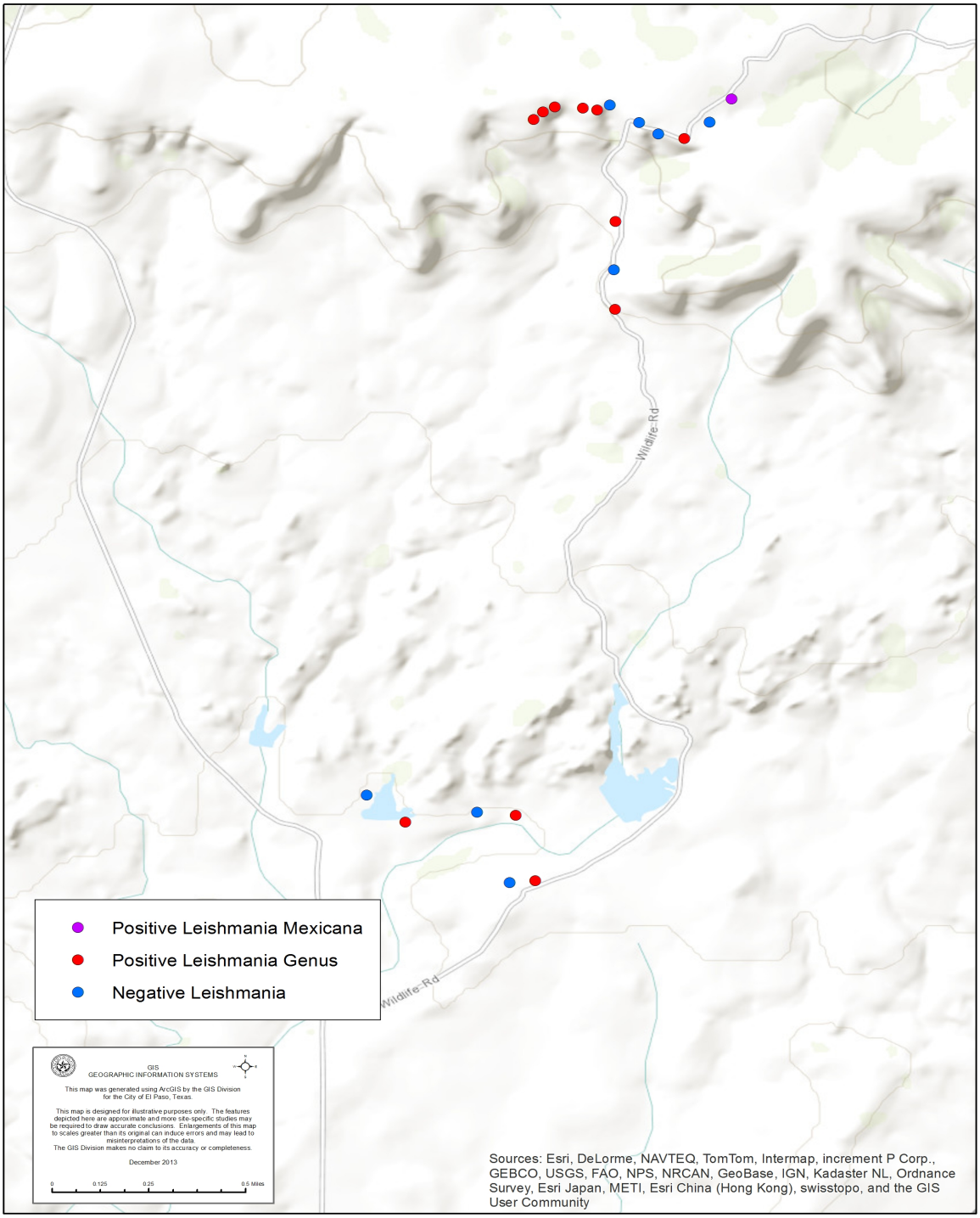


Figure 8. Distribution of *L. mexicana*, *Leishmania* genus, and *T. cruzi* sylvatic cases in the Mason Mountains



Discussion

This represents the first study of Chagas' disease and leishmaniasis in stray canines and sylvatic mammals to be conducted in El Paso and surrounding areas. The presence of positive cases during the study period indicates that Chagas' disease and leishmaniasis among dogs and sylvatic mammals in Texas is not a rare occurrence nor is it geographically constrained to a particular region within the state.

Nine out of the ten positive *T. cruzi* stray canine cases were collected during fall months (October and November). With more than half of the cases identified during those months, it could possibly indicate transmission during late summer months. To accurately identify whether that is valid, further testing of stray canines during longer periods of time must be conducted. The prevalence of disease in the canines was more likely due to lifestyle factors (e.g., occasionally insectivorous and exposure to wild vertebrate hosts) rather than breed predilection for the pathogen. All cases were spatially distributed, which did not indicate any patterns of infection within an area.

Twenty-nine out of the 96 canine samples had visible lesions on the back, legs, or face, and tissue samples were collected from those lesions .Out of the twenty-nine, only one sample tested positive for *T. cruzi*. Among the 96 canine samples, 2 (2%) were PCR confirmed to be of *Leishmania* genus but not *L. mexicana*. This suggests that another species of Leishmania exists in El Paso and surrounding areas besides *L. mexicana*. Out of those two cases, one of the samples was collected from a lesion on a stray canine.

Among the sylvatic mammals temporal *T. cruzi* infection could also be a factor,

however, samples were only collected in October, therefore further research must be conducted to make a valid conclusion. Five species were represented among the *T. cruzi* positive sylvatic mammals with raccoons being the top recorded species. The first cases of *T. cruzi* in raccoons were identified in Georgia, USA[32], therefore, the identification of *T. cruzi* in raccoons in Texas indicates that it is not a rare occurrence nor is it geographically constrained to a particular state.

The resulting case count of this study underestimates the true prevalence of infection in dogs and sylvatic mammals in El Paso and surrounding areas.

Conclusions

These data should serve to increase awareness regarding the prevalence of the diseases in sylvatic and stray mammals among veterinarians and public health practitioners, leading to a greater emphasis placed on prevention methods that can be used to break the transmission cycle. This presence of the parasites warrants further evaluation of U.S. canines and sylvatic mammals as Chagas' disease and leishmaniasis reservoirs, including duration of parasites in the blood and infectivity and pathogenicity of parasite strains to humans.

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Jacqueline Mariscal is the eldest daughter of Anna and Richard Mariscal. She is the first to graduate from a University having completed her bachelors in Biology at UTEP in 2005 and acquired her Masters of Public Health (MPH) in 2013. Her post undergraduate accomplishments include being the former President for the program's student organization known as Students for Public Health (SPH). From 2009 to present, Jacqueline has been a collaborator in various research projects on infectious diseases and public health issues, researching influenza as an undergraduate and Leishmaniasis, Chagas disease and Rabies as a graduate student. Jacqueline is also a current recipient of the Sandy Tyler Endowed Fellowship. She has participated in international research in Vallenar, Chile with the Minority Health International Research Training program conducting a needs assessment, implementing an intervention, and evaluating a program to improve the lifestyle behaviors for health care professionals at the Providence Hospital in Huasco, Chile. Jacqueline was the only UTEP graduate student to be chosen by the American Public Health Association to speak at their national conference in San Francisco on her thesis research. Jacqueline's future goals include becoming a physician and working for the Centers for Disease Control as an infectious specialist.