TRANSMISSION DYNAMICS OF TRYPANOSOMA CRUZI

By

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A Thesis Submitted to The Honors College
In Partial Fulfillment of the Bachelors degree
With Honors in
Ecology and Evolutionary Biology
THE UNIVERSITY OF ARIZONA
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Approved by:

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Last updated: Nov 15, 2009
Statement of Purpose:

This thesis will examine three aspects of Trypanosoma cruzi transmission dynamics. The first project examines the prevalence of T. cruzi and the non-pathogenic trypanosome T. rangeli in pack rats in peri-urban areas of Tucson, Arizona. As a continuation of this study, we conducted a similar survey in Mariano Melgar, a district in the endemic region of Arequipa, Peru. These projects focus on the importance of the rodent reservoir both in endemic and non-endemic areas for human Chagas disease, and hypothesize that wild rodent populations may be contributing to continued disease transmission. The final study examines the potential for oral transmission of T. cruzi and focuses on the guinea pig as a reservoir host. Combined, these studies provide a holistic understanding of the rodent reservoir host.

Understanding the transmission dynamics in both wild rodent and guinea pig populations can help us to better determine the role they play in human Chagas Disease transmission. Such research will provide insight for future intervention studies. The infectivity study in guinea pigs will also provide valuable information for potential modes of transmission in reservoir species. These studies will also provide a comparative analysis of different transmission cycles. By examining these cycles we can better understand the ecology of T. cruzi and its reservoir hosts.

Statement of Relevance:

Chagas disease is an infectious disease caused by the flagellate parasite Trypanosoma cruzi and is endemic to South America, Central America, and Mexico1,5. Chagas spreads by direct infection from the vector, through blood or organ transplants, or congenitally. With its multiple modes of transmission and innate sustainability by a wide variety of reservoir hosts, Chagas has affected an estimated 10 -20 million people9. The majority of affected people live in rural and peri-urban regions where the type of housing increases contact between vectors and reservoirs8. The parasite T. cruzi is transmitted by the fecal matter of kissing bugs of the family Triatomidae. The parasite in the contaminated feces enters through a wound or mucosal surfaces and multiplies intracellularly2.

Chagas disease has both an acute and chronic phase. The acute phase of the disease can be asymptomatic for several years or present with non-specific symptoms such as inflammation at the initial infection site, fever, and malaise, and is thus often misdiagnosed or neglected. Nearly 40% of those initially infected contract the chronic disease9. The chronic phase manifests in severe gastrointestinal and heart problems that often lead to heart failure. In endemic areas, chronic stage Chagas disease is the number one cause of cardiovascular death9. While several public health initiatives have successfully slowed disease transmission in some areas, it remains a persistent heath problem particularly for those of lower socioeconomic status in rural and peri-urban areas1.

The prevalence of Chagas disease in the United States remains low, however the immigration from endemic areas has increased this prevalence6. Blood and organ donations and congenital transmission are the main modes of transmission of the disease in the United States, rather than the more common mode of transmission involving contaminated feces from the vector in endemic regions6. Although transmission from several vector species of triatomids including: T. rubida, T. protracta, and T. rucurva remain rare in the United States, the parasite remains endemic within the population of small animal hosts native to the southern and southwestern regions of the United States, where they act as reservoir hosts for the parasite6.

It has been estimated that over 150 mammal hosts are capable of harboring the parasite1.
The main reservoir hosts of particular importance to Peru are dogs, guinea pigs, and several species of wild rodents, namely *Rattus rattus*, *Ratus norvegicus*, and *mus musculus*. These reservoirs continue the cycle of infection, and are likely to increase disease transmission to humans. While there have been several studies that demonstrate infection in dogs and guinea pigs in peri-urban areas, to the author's knowledge there has been no study conducted that has demonstrated the prevalence of infection by *T. cruzi* in wild rodent populations in Peru. While similar field studies have been conducted in Ecuador and Chile, it remains unknown in Peru if wild rodents play a significant role in continued transmission of disease.

Infection of *T. cruzi* is well described in reservoir hosts such as guinea pigs and dogs in Peru, though the exact mode of infection from the vector remains largely unknown. Many assume the classical mode of transmission in which the parasite gains access to the host through mucosal surfaces or open wounds or abrasions as the predominant mode of transmission. However, recent studies suggest that horizontal transmission via oral ingestion of infected triatomids may be an alternative mode of transmission. Roellig and colleagues found that raccoons fed nymphal stages of infected triatomids were later developed *T. cruzi* infection, demonstrating mammalian oral transmission of *T. cruzi*. By conducting a similar study using the guinea pig, we will better be able to understand the mode of transmission that may be occurring in peri-urban areas in Peru.

The combination of the studies in both Peru and Arizona will provide a better understanding of the transmission dynamics of *T. cruzi*. The infection study in combination with analysis of disease prevalence in wild rodents will better demonstrate the continued transmission of Chagas Disease in the peri-urban environment in Peru, and potentially inform more effective Chagas Disease interventions.

**Thesis Abstract:**

*Trypanosoma cruzi* is the causative agent of Chagas disease, a parasitic disease that affects 10-20 million people in Central and South America. While there have been studies that indicate several common reservoir hosts, little has been done to demonstrate infection in the wild rodent population. There has also been little research to ascertain the exact types of transmission that may be occurring in peri-urban areas. The guinea pig is well documented as a reservoir host, yet exactly how they become infected with the parasite remains unknown. In this study, we want to understand the prevalence of disease in the wild rodent reservoirs and demonstrate possible means of transmission by examining if guinea pigs can become infected by ingestion of infected triatomid hosts. Understanding the modes of infection in the guinea pig as well as understanding the prevalence of disease in wild rodents will enable a broader grasp of the transmission dynamics and the infectivity of Chagas Disease.

**Projects: Transmission Dynamics of Trypanosoma cruzi in Rodent Populations**


2) A Survey of *Trypanosoma cruzi* in Rodent Reservoir Populations in Peri-Urban Areas in Arequipa Peru.


Works Cited


**Title Project One:**

Abstract:

*Trypanosoma cruzi* is the causative agent of Chagas disease. Chagas disease affects 20 million people per year. Due to immigration from endemic areas, the disease poses an emerging health concern to the Southwestern United States. Another trypanosome, *Trypanosoma rangeli*, while non pathogenic, has been known to infect the same vectors and reservoirs as *T. cruzi*, although no study has surveyed for *T. rangeli* in the Tucson, Arizona vicinity. The majority of the diagnostic tests for *T. cruzi* cross react with *T. rangeli*. This cross reactivity could result in false positives and overestimates of disease prevalence. In this study, we seek to understand if *T. cruzi* remains endemic within the vector and reservoir populations of Tucson, Arizona, and if *T. rangeli* exists within the same populations. Collection of both the main reservoirs and vectors continues, but of the 45 pack rat reservoirs sampled thus far, 12 have been identified as microscopically positive for trypanosomes. Possible candidates of *T. rangeli* infection are identified based on morphological characteristics of the parasite such as distance from the nucleus to the kinetoplast and relative size of the organism. More specific PCR diagnostic techniques will be employed to determine the presence of either *T. cruzi* or *T. rangeli* in the samples. In order to understand the potential risk that Chagas disease poses to the Southwestern United States, it is important to understand which parasite exists within the reservoir population. By using specific tests to determine which parasite is present we are able to limit false positives for *T. cruzi* and are better able to determine the potential risk that Chagas disease poses to Tucson, Arizona.

Specific Aims:

- Determine local ecology of pack rat populations in peri-urban locations in Tucson, AZ.
- Determine the prevalence of *T. cruzi* in the local pack rat populations of Tucson, AZ.
- Determine the prevalence of *T. rangeli* in the local pack rat populations in Tucson, AZ.

Introduction:

*Trypanosoma cruzi* is the causative agent of Chagas disease. Chagas disease affects an estimated 14-20 million people in Latin America and poses an emerging public health concern to the Southwestern United States (Scholfield 2007). The prevalence of Chagas disease has increased due to immigration from endemic areas (Klotz 2008). Blood and organ donations and congenital transmission remain the main modes of transmission of the disease, rather than the more common mode of transmission involving contaminated feces from the vector (Klotz 2008). While transmission from several vector species of Triatomids including: *T. rubida*, *T. protracta*, and *T. rucurva* remain rare in the United States, the parasite remains endemic within the population of small animal hosts native to the South and Southwest (Wood 1938).

Another species, *Trypanosoma rangeli*, while non pathogenic, also represents an epidemiological concern to the American Southwest. Both *T. rangeli* and *T. cruzi* infect the same vector and reservoir hosts, and have similar morphologies and immunological cross-reactivity which confound many diagnostic tests (Vallejo 1999). This cross-reactivity results in inaccurate estimates of the parasite in both the vector and reservoir populations (Vargas 2000). In Latin
America, *T. rangeli*’s and *T. cruzi*’s geographical distributions overlap, often resulting in co-infection (Vargas 2000). While *T. cruzi* infects both Triatomids and pack rats in the Southwestern United States, no previous study has surveyed for *T. rangeli*, and it remains unknown if the parasite confounds any of the past or current studies.

The majority of epidemiological studies do not consider the possibility of *T. rangeli* confounding the prevalence of *T. cruzi* within a population study. The majority of PCR (polymerase chain reaction) primers used to detect the presence of *T. cruzi* cross react with *T. rangeli*, resulting in false positives (Vargas 2000). The 121/122 primer set is the standard PCR primer set for detection of *T. cruzi* throughout Latin America, however it cross reacts with many other parasites. While the 121/122 primer does differentiate between *T. rangeli* and *T. cruzi*, it is not a specific primer set for *T. rangeli*. We therefore utilized the R1/R2 primer set for the detection of *T. rangeli* (cite). The R1/R2 primer set is specific for the p542 region found only in *T. rangeli*. The C6 primer set was also employed to detect for *T. cruzi*. The C6 primer sequence utilizes a repeated sequence that does not appear in other species of Typanosome(cite).

*T. cruzi* was first described in Latin America in 1909 and in Arizona in 1939 (Schuck 1945). Since then, several vector studies in the Southwestern United States, including in Arizona, Texas, and California, have shown incidence rates ranging from 4.9% to 37.5% (Schuck 1945, Eads 1963, Burkholder 1980). Several studies have involved pack rats (*Neotoma albigula*) and small rodents. In Arizona, the last study looking at pack rats occurred in 1938 by Sherwin Wood. More recently, a similar study in Texas confirmed the pack rat as a reservoir host (Burkholder 1980). Both studies used microscopy for detection of *T. cruzi* but did not survey for *T. rangeli*.

In this study, we want to understand if *T. cruzi* remains endemic within the vector and reservoir populations of Tucson, Arizona, and if *T. rangeli* exists within those same vector and reservoir populations. We surveyed the pack rat populations of Tucson for the presence of both *T. cruzi* and *T. rangeli* using specific PCR diagnostic techniques. Pack rats were captured in peri-urban areas of the city, where the insects and pack rats potentially pose the greatest health risk to humans. Their blood was then examined using microscopy and specific PCR.

**Methods:**

**Capture of Rats:**
Pack Rats were captured using HavaHart traps in South Western and Northern areas of Tucson and Oro Valley. The traps were placed in peri-urban areas near active middens in areas that were deemed to represent the greatest public health risk. Active middens were determined by amount of newly foraged material and presence of feces. The traps were placed near the middens at dusk and were collected the following morning. Peanut butter was used as bait. The geographical coordinates were recorded again using the Venture etreck Global Positioning Device.

**Anesthesia and Euthanasia of Pack Rats:**
Isoflourine was used as anesthesia. Pack rats were placed within the anesthesia chamber until the rat became unresponsive. A cardiac stick was preformed while the animal remained under the influence of anesthesia. Blood collected was used for microscopy and molecular tests. After the cardiac stick was performed the pack rats were placed back in the anesthesia chamber and were euthanized with an overdose of Isoflourine.
Microscopy and DNA extraction of Pack Rat Blood Samples:
Blood collected from the cardiac stick was used for thin blood smears. These smears were fixed by placing the slides in methanol for five minutes and were then allowed to dry completely. The slides were then stained overnight with Geimsa stain and were examined microscopically for the presence of trypanosomes the next day. The remaining blood from the cardiac stick was collected in Heparin and Serum Separating Tubes. These were centrifuged for ten minutes and the serum and serum collected. The DNA was extracted from the remaining blood in the Heparin Tubes using the QIAamp DNA Blood Mini Kit (QUIAGEN).

PCR Analysis:
As described by Gilman et al, PCR amplifications for both *T. cruzi* and *T. rangeli* were performed using the primer set 121/122 (5'-AAATAATGTACGGGKG-AGATGCATGA- 3' and 5'-GGTTCGATTGGGTGTTGTAAT ATA-3'). A total volume of 25µL contained a combination of the following concentrations: 2.5µmol/L MgCl₂, 200µmol/L dNTP's, 0.2 bovine serum albumin (BSA), 1XPCR buffer, and 2µL of DNA. The reactions were heated to 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds, and a final extension at 72°C for 7 minutes.

PCR amplification using the C6 specific primer for *T. rangeli* were performed using the C6 primer set (5'-GATGCGCATTTGTTACGA-3' and 5'-CTGGCTGGCCCTTGTATCC-3'). A total volume of 25µL contained a combination of the following concentrations: 2.5 units of taq DNA polymerase in a buffer of 10mM Tris pH 8.3 50mM KCl, 5.0mM MgCl₂, .25mM of each of 4 nucleotide triphosphates, and .4mM of each primer. The Samples were denatured for 5 mins at 95°C and amplified for 1min at 95°C, 1 min at 60°C, and 1 min at 72°C (repeated for 35 cycles).

As described by Vargas et al, PCR amplification for T. rangeli were performed using the R1/R2 primer set (5'-CGCGGCTCGCACTGCACCTC-3') and R2 (5'-GGCGCATCACCGAGCACTG-3') for amplification of the P542 element. A total volume of 50µL using 1.25 units of Taq DNA polymerase in a buffer composed of 10mM Tris-HCL, pH 8.3, 50mM KCl, 4mM MgCl₂, 0.4 mM each of the four nucleoside triphosphates, and 0.5µM of each of the primers. Samples were denatured for 4 min at 94°C, then amplified through 30 cycles: 1 min at 94°C, 1 min at 65°C, and 1 min elongation at 72°C.

Results:
In total 53 pack rats were captured in peri-urban areas in Tucson and Oro Valley. The distribution between male and female pack rats were nearly even. Smaller pack rats seemed to present with higher levels of parasitemia than the larger rats. Of the 53 pack rats surveyed 14, 26.4%, were microscopically positive for trypanosomes. Of the 14 positive samples 3 appeared to have heavy infections based on the number of organisms appearing in the viewing plane. Based on microscopic analysis, *T. rangeli* infection is suspected. The morphology of the parasites were more indicative of *T. rangeli*, with overall larger size and greater distance between the nucleus and kinetoplast (Figure 1).
PCR analysis of the DNA extracted from the clotted blood of microscopically positive samples were mostly negative for amplification of trypanosome DNA. Of the total microscopically positive samples only six have demonstrated potential positive results for *T. cruzi* via PCR analysis, indicating problems with the DNA extraction. None of the PCR samples indicated positive *T. rangeli* infection despite morphological indications.

**Discussion:**

Based on microscopy, it appears that trypanosomes are present within the population of pack rats in Tucson, Arizona, confirming our hypothesis that trypanosomes remain endemic within the pack rat population. We have not been able to amplify *T. rangeli* from the DNA samples. We were able to amplify *T. cruzi* DNA from DNA extracted from the plasma from the serum of sample NA034, and five other blood samples. The samples were positive for *T. cruzi* despite morphological characteristics more indicative of *T. rangeli*.

We have been able to confirm positive trypanosome samples from various areas in Tucson, and it appears that trypanosome infection remains widespread in the pack rat population in the Tucson area. There does not appear to be an association between infection and sex of the animal, however there does appear to be a loose association between the age of the animal and infection. Infection also appears more common in pack rats that were collected during the summer months of June and July than those collected in August and September. We do not currently have a large enough sample size to correlate significance, however based on current trends we believe that seasonality could be a contributing factor. The common vector of Chagas Disease, the kissing bug or *Triatoma rubidea*, actively mate during the summer months, possibly allowing for greater potential to spread disease.

Future directions will include more specific testing for both *T. cruzi* and *T. rangeli* infection, including using a DNA extraction technique using the blood clot for specificity. We also want to conduct serological testing to confirm infection. While there has been only two cases of Chagas Disease in the United States that was contracted directly from the classical mode of infection (Schiffler), the disease appears to remain endemic within small animal hosts. This data is supported by not only our current research, but also continuing research in other regions of the world. By understanding the transmission dynamics that are occurring within the rodent population, we will build a better understanding of how rodent reservoirs continue the cycle of infection of trypanosomes. It will also allow us to ascertain which parasite, between *T. cruzi* and *T. rangeli*, exists within the pack rat population, and will demonstrate if prevalence rates for *T. cruzi* have been overestimated. By both understanding the prevalence of
trypanosomes in the rodent population as well as identifying the predominate trypanosome, we will be able to more effectively determine the risk that Chagas Disease poses to the Southwestern United States.
Works Cited


Title Project Two:

A Survey of *Trypanosoma cruzi* in Rodent Reservoir Populations in Peri-Urban Areas in Arequipa, Peru.

Abstract:

*Trypanosoma cruzi* is the causative agent of Chagas disease, a parasitic disease that affects 10-20 million people in Central and South America. While there have been studies that indicate several common reservoir hosts, little has been done to demonstrate infection in the wild rodent population. In this study, we want to understand the prevalence of disease in the wild rodent reservoirs. Out of 34 rats surveyed 22 (64.7%) were positive for Trypanosomes. Of this sample 6 were positive by microscopy, 11 were positive by PCR (121/122 primer set), and 13 were positive by ELISA. In total, 16 out of 34 (47.0%) rats sampled were positive for *T. cruzi* by either ELISA or PCR (121/122 primer set). Understanding the prevalence of disease in wild rodents will enable a broader grasp of the transmission dynamics and the infectivity of Chagas disease and could provide a potential target for future Chagas disease interventions.

Specific Aims:

- Determine the local ecology of rat populations across an economic gradient in the peri-urban areas in the region of Mariano Melgar in Arequipa, Peru.
- Determine the prevalence of *Trypanosoma cruzi* in local rat populations.

Introduction:

*Trypanosoma cruzi* is the causative agent of Chagas disease, a parasitic disease estimated to affect 10-20 million people in Central and South America. The parasite is also estimated to be able to infect over 150 reservoir hosts, perpetuating infection. The main reservoir hosts of particular importance to Peru include dogs, guinea pigs, and several species of wild rodents namely *Rattus rattus*, *Ratus norvegicus*, and *mus musculus*. These rats are generally considered commensal and are typically found in urban and peri-urban environments. Wild rodent reservoirs have the potential to continue the cycle of Chagas infection, and could perpetuate disease transmission to humans. While there have been several studies that demonstrate infection in dogs and guinea pigs in peri-urban areas in Peru, no study has demonstrated the prevalence of infection by *T. cruzi* in wild rodent populations. Field surveys for *T. cruzi* have been conducted in Ecuador, Chile, and Panama but it remains unknown in Peru if wild rodents play a significant role in continued transmission of disease.

Rodents act as reservoirs for many diseases including Toxoplasmosis, hantavirus, salmonella, Leptospirosis, and tricanosis. While some studies have surveyed for Chagas disease in rats, it remains largely unknowns if rats play a significant role in continued disease transmission. All previously reported studies also focus on the rat reservoirs outside households. Virtually no studies have examined the rat reservoir within home.

In this study, we plan to examine the transmission dynamics of *Trypanosoma cruzi* by conducting a survey for *T. cruzi* in wild rodent populations to ascertain if transmission occurs in the wild rodent population. By understanding the transmission dynamics that occur in wild rodents the peri-urban setting we will better be able understand the importance of rats in
continuing disease transmission. With this knowledge we can better understand the importance of the rodent reservoir, and potentially integrate this knowledge into future Chagas Disease interventions and public health initiatives.

Site Justification:

Chagas disease has been well described in Arequipa, Peru. The ecology in the outer regions of the city include several new *pueblos jóvenes* whose unique urban and peri-urban zones facilitate differing modes of Chagas disease transmission. These areas represent a unique opportunity to study transmission dynamics. The region of Mariano Melgar encompasses a mix of both urban, well-established houses and the poorer constructions in *pueblo jóvenes* regions. For this survey we selected a transect that began in a well established area of middle income households and continued up the hillside to the poorer *pueblo jóvenes* regions.

Methods:

**House Selection:**
Homeowners within the transect region were asked by a member of the research team if they had problems with rats in their houses. If it was reported that a rat problem existed, permission was sought to place traps within the house. The recruitment process involved an explanation of the purpose and importance of the experiment to the homeowner.

**Capture of Wild Rodents:**
Wild rodents were captured using Tomahawk traps. A total of three traps were placed and left overnight within the houses in areas indicated by the owners. A mixture of tuna and ketchup was used as bait. The traps were collected the next day at the owner's convenience. If the trapping was unsuccessful, the research team would return to the house for a total of three additional attempts. The geographical coordinates of trap locations were recorded using the [Garmin] global positioning device, and inputted as map points using the ArcGIS software. The corrals and location of the traps were also recorded in a field notebook.

**Anesthesia and Euthanasia of Rodents:**
Isoflurane and Sevoflorane were used as anesthesia. The rats, while still in the Tomohawk traps were placed individually, within an anesthesia chamber until unresponsive. A cardiac stick was then performed while the animal remained under the influence of anesthesia. After the cardiac stick was executed, the rodent was euthanized with an overdose of Isoflurane or Sevoflourane.

**Microscopy and DNA extraction of Wild Rodent Blood Samples:**
Blood collected from the cardiac stick was used for thin blood smears. Smears were then fixed in methanol and stained with Geimsa stain and examined microscopically for the presence of *typanosomes*. The remaining blood from the cardiac stick was collected and centrifuged in eppendorf tubes and the serum collected.

**DNA Extraction:**
The blood clot was used for the DNA extraction, rather than whole blood or serum, for greater sensitivity. In an eppendorf tube, 500 µL of the blood clot was washed with 100µL of the wash buffer (10mM Tris HCL ph 7.6, 5 mM MgCl₂, and 10mM NaCl). This mixture was centrifuged at 13000rpm for 2 minutes, and the resulting supernatant discarded. Enough wash buffer then
was added to bring the solution to 1.0 mL, and was resuspended by vortexing and then centrifuging at 13000rpm for two minutes. This procedure was repeated three times and then resuspended in 500µL of buffer wash. After adding the buffer wash, 0.5% SDS (25µL) from stock, 10% SDA, and 0.5 mg/mL (12.5µL) proteinase K from stock 20mg/mL was added. This mixture was then incubated for two hours at 56 degrees Celsius, and vortexed every half hour. The DNA was extracted from the remaining blood solution using the QIAamp DNA Blood Mini Kit (QUIAGEN) standard operating procedure for blood and body fluid beginning with incorporation of ethanol.

**PCR and ELISA tests for Identification of Trypanosoma cruzi:**
As described by Fitzwater et al, PCR amplifications were performed using the primer set 121/122 (5'-AAATAATGTACGGGKG-AGATGCATGA- 3' and 5'-GGTTCGATTGGGGTTGGTGTAAT ATA-3'). A total volume of 25µ containing a combination of the following concentrations: 2.5µmol/L MgCl₂, 200µmol/L dNTP’s, 0.2 bovine serum albumin (BSA), 1XPCR buffer, and 2µL of DNA. The reactions were heated to 94°C for 3 minutes, followed by 35 cycles at 94°C for 30 seconds, and a final extension at 72°C for 7 minutes.

**Results:**
Traps were placed throughout the study area in houses that have had current or previous problems with rats. A total of 34 rats were captured. In total, 41 houses were surveyed and rats were captured at 11 of these houses. Of the 34 rats captured, one belonged to the species *Rattus rattus*, known colloquially as the black rat, and the rest belonged to the species *Rattus norvegicus*, known as the brown rat. The 33 brown rats were all captured in houses that raised other animals such as guinea pigs, chickens, or rabbits, and were captured in the middle and upper sections of the surveyed area. The black rat was captured in a more urban setting, toward the bottom of the surveyed area.

The majority of the rats were captured in the center of the surveyed area, especially in houses where other domestic animals were present. In total, 21 rats out of 34 were captured towards the center of the transect. The bottom of the surveyed area consisted of more established middle class households, with less domestic animals. Only one rat was captured in this region. The top of the surveyed area consisted of *pueblos jovenes*, or newly established settlements. These houses tended not to have as many domestic animals as those surveyed in the middle region. A total of 12 rats were captured at the top of the studied area. All rats were captured in kitchens, bathrooms, storage areas, and animal pens. The largest number of rats were captured in animal pens, storage areas and kitchens (Table 1). These areas typically had food scraps or other stored foods readily available. These areas also typically housed domestic animals or animals were free to enter the spaces.

In total, six rats were microscopically positive for trypanosomes. These rats were captured toward the upper area of the study region. Out of the six positive rats four had heavy infections. Based on morphological characteristics, *T. cruzi* or *T. lewisi* infection was suspected.

Out of the 34 rats surveyed 22 (64.7%) were positive for Trypanosomes. Of this sample 6 were positive by microscopy, 11 were positive by PCR (121/122 primer set), and 13 were positive by ELISA. In total, 16 out of 34 (47.0%) rats sampled were positive for *T. cruzi* by either ELISA or PCR (121/122 primer set) and 7 samples (20.6%) were positive by both PCR and ELISA. None of the microscopically positive samples were positive by either ELISA or PCR. The positive samples were from nearly all areas of the sampled houses including the
highest areas of the study. The single sample from the lowest area of the study region was not positive for *T. cruzi*. In a separate, unpublished study by Dr. Micheal Levy, which surveyed for Chagas disease in triatomids, guinea pigs, rabbits, and dogs found 18 houses with positive triatomids. Of these 18 houses only one rat from these houses was found positive. The other positive rats were from houses identified by this separate study as having negative animals and triatomids.

Table One:

* N/A – did not place traps within these areas

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Figure One:

PCR results rats 15 - 34

Figure Two:
Discussion:

These results indicate that rats may be an important reservoir host in the region of Mariano Melgar in Arequipa, Peru. The majority of studies about reservoir hosts for *T. cruzi* focus on guinea pigs and dogs. Here, to the best of the authors knowledge, we demonstrate the first survey of the rat reservoir in Peru. The rats were not found to be localized in one area, but were found throughout the surveyed area. The rats were all trapped in kitchens, storage areas, animal pens, and patio areas. All the rats were trapped in areas that were in close contact with the homeowners.

Out of the 34 rats surveyed 22 (64.7%) were positive for Trypanosomes. Of this sample 6 were positive by microscopy, 11 were positive by PCR (121/122 primer set), and 13 were positive by ELISA. In total, 16 out of 34 (47.0%) rats sampled were positive for *T. cruzi* by either ELISA or PCR (121/122 primer set). Since none of the 6 samples that were positive by microscopy were amplified by the 121/122 primer set *Trypanosoma lewisi*, the rat trypanosome, is suspected. In future studies we plan to analyze all samples for *T. lewisi* and *T. cruzi*.

Previously, the disease was localized toward the lower and middle areas of the surveyed area. In this study, we found positive rats in not only the middle sections of the surveyed area, but also in the highest and poorest regions of the surveyed area. None of the other sampled reservoir hosts (guinea pigs, dogs, and rabbits) in the highest section of the surveyed area were positive for *T. cruzi*. Furthermore, no triatomids were found in the houses surveyed in the upper area of the study region. This finding could indicate that the rats may be playing a significant role in transporting the disease to these upper regions.

Future studies will include a more systematic methodology for surveying the houses in this region. Currently, the selection of houses was based convenience sampling. We asked homeowners if they had had or currently had problems with rats in their households and if they replied in the affirmative, we would ask to place traps within the houses. We did not sample any houses that said that they did not have problems with rats, and we did not sample in areas outside people’s houses. In the next studies we would like to sample 25 houses from 4 sections of the study area. This methodology will provide a better understanding of the rat reservoir across and economic gradient.

The results of the study indicate that rats may be playing a more significant role in continued Chagas disease transmission than previously thought. Rats were captured within houses in close contact with the homeowners. But unlike dogs, guinea pigs, and rabbits, the rats were not bound to one house, and could freely forage between multiple houses. It is therefore possible that the rats may be transporting the disease to previously uninfected areas.

Public health interventions for Chagas disease frequently focus on vector control through insecticide sprayings. Recently, more attention has been given to reservoir hosts within houses such as guinea pigs, dogs, and rabbits. These animals are both capable of harboring the disease and serving as attractors for the triatomid vectors. However, little to no work has been done to look at rats within the houses. Our own preliminary findings suggest that the rats may be playing an important role, and possibly could serve as a new target for future Chagas disease interventions.
Works Cited:


infected by sylvatic Triatoma infestans of an endemic area of Chile. *Acta Tropica, 111*(1), 90-93. doi: 10.1016/j.actatropica.2009.02.010


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**Title Project Three:**

Oral Transmission of *Trypanosoma cruzi* in Guinea Pig Model

**Abstract:**

*Trypanosoma cruzi* is the causative agent of Chagas disease, a parasitic disease that affects 10-20 million people in Central and South America. The guinea pig is well documented as a reservoir host, yet exactly how they become infected with the parasite remains unknown. In this pilot study, we want to demonstrate possible means of transmission by examining if guinea pigs can become infected by ingestion of infected triatomid feces. Here we demonstrate an Oral mode of transmission by ingestion of contaminated alfalfa. Understanding the modes of infection in the guinea pig will enable a broader grasp of the transmission dynamics and the infectivity of Chagas Disease.

**Specific Aims:**

- Determine feeding behavior of guinea pigs, and determine if guinea pigs will ingest a triatomid
- Determine if guinea pigs will eat contaminated alfalfa.
- Determine if guinea pigs can become infected with *T. cruzi* by ingestion of contaminated
feces on alfalfa.

Introduction:

Chagas is an infectious disease caused by the flagellate parasite *Trypanosoma cruzi*. Endemic to most areas in South America, as well as regions in Central America and Mexico, the parasitic disease is highly prevalent and a continuing public health issue affecting these regions medically, economically, and socially. Chagas spreads by direct infection from the vector, through blood or organ transplants, horizontally, or congenitally from mother to child. With its multiple modes of transmission and innate sustainability by a wide variety of reservoir hosts, Chagas has affected an estimated 10-20 million people.

While infection of *T. cruzi* is well described in reservoir hosts such as guinea pigs, and dogs in Peru, the exact mode of infection from the vector remains largely unknown. Many assume that the established classical mode of transmission in which the parasite gains access to the host through mucosal surfaces or through open wounds or abrasions predominates as the mode of transmission. However, recent studies suggest that horizontal transmission via oral ingestion of infected triatomids or infected feces may be an alternative mode of transmission. In a study recently published in the Journal of Parasitology, raccoons fed nymphal stages of infected triatomids were later diagnosed as positive for *T. cruzi* infection, thus demonstrating mammalian oral transmission of *T. cruzi*. By conducting a similar study using the guinea pig, we will better be able to understand the mode of transmission that may be occurring in peri-urban areas in Peru. Horizontal transmission has been demonstrated in humans through ingestion of infected cane sugar juice as well as assia palm fruit juice. One study demonstrates in rodents, that the parasite can survive in the cane sugar juice for a full 24 hours. These studies indicate that oral transmission may play a greater role in Chagas Disease transmission.

The guinea pig is often kept within homes as a common food source in Peru, increasing vector-to-human contact. Yet, it remains unknown if the triatomids infect the guinea pig via the classical mode of transmission or rather through direct ingestion of the triatomids or infected feces. In studying this interaction we will establish a better understanding of Chagas Disease transmission. This study will help demonstrate the continued transmission of Chagas Disease in the peri-urban environment and could potentially be used to better Chagas Disease interventions within these communities.

Methods:

**Determination of Feeding Behavior:**

To determine feeding behavior, non-infected guinea pig was placed in an aquarium with non-infected triatomids. All non-infected triatomids and infected triatomids as well as guinea pigs are continuously maintained by Universidad Peruana Cayetano Heredia Arequipa Laboratory in an insecterary. A Non-infected Guinea Pig used was confirmed non-infected by serology and microscopy from blood samples. Through observation it was determined if the guinea pig ingests the triatomid bug or infected alfalfa.

**Oral ingestion of infected feces.**

Five uninfecte guinea pigs, confirmed non-infected by PCR and serology, and ten infected *T. infestens*, infected with viera strain, were used for this experiment. Infected *T. infestens* are maintained by the Universidad Peruana Cayetano Heredia Arequipa Laboratory insecterary. The ten infected *T. infestens* were examined for the presence of trypomastigotes. If
tryptomastigotes are present, the intestinal contents were collected. The infected intestinal contents of two insects were mixed with saline, approximately $1 \times 10^3$ trypanosomes per 100µL. This mixture was placed on approximately 100grams alfalfa. This alfalfa was then fed to one uninfected guinea pig fasted during the morning, under observation to confirm ingestion. The procedure was repeated for the remaining four guinea pigs.

**Positive and Negative Controls**

For comparison of the experimental group, three positive controls and two negative controls were be used. Three guinea pigs were inoculated with *T. cruzi* as positive controls. To both limit stress and pain to the animal and ensure safety to laboratory personnel, prior to inoculation the animals received an intramuscular injection of 5mg/kg xylazine and 40mg/kg ketamine for sedation. An inoculation of 0.1mL containing $1 \times 10^7$ trypanosomes per 100µL was administered intradermally. Two negative controls were used and sedated in the same manner but were inoculated with 0.1mL saline intradermally.

**Blood collection from Guinea Pigs**

Blood collection for both control and experimental groups remained the same. Blood collection from guinea pigs occurred no more than once a week. One mL of blood was be collected from either the saphenous veins. Blood collection continued for eight weeks or until the animal become positive for infection.

**Processing and analysis of blood samples:**

The blood collected was used to create thick blood smears and were examined for trypanosomes via microscopy. The buffy coat from fresh blood was also be examined via microscopy for the presence of trypanosomes. DNA was extraction using the QIAamp DNA Blood Mini Kit (QUIAGEN). PCR analysis will then be performed using *T. cruzi* specific primer set 121 and 122. Universidad Peruana Cayetano Heredia Gilman et al. protocols for PCR were used.

**Emergency euthanasia**

Any guinea pig that demonstrated clinical signs of Chagas, as determined by on-sight veterinarian, were sacrificed immediately. Animals were anesthetized with an intramuscular (IM) injection of 5mg/kg xylazine and 40mg/kg ketamine. In approximately 20 minutes, or when the animal is sufficiently sedated, 150mg/kg of sodium pentobarbital was administered intraperitoneally for euthanasia.

**Results:**

**Feeding live triatomid larval stages to guinea pig without alfalfa:**

A fasted guinea pig was placed in a small aquarium first with no alfalfa with three third-larval instar nyphs for three hours. During this time we observed the guinea pig and found that the guinea pig did not appear interested in consuming the triatomids. The triatomids did feed on the guinea pig during this time (Image 1 and 2).
Feeding live and dead triatomid larval stages to guinea pig with alfalfa:

After it was determined that the guinea pig appeared to not ingest the larval stages of the triatomids, we added alfalfa into the enclosure and place three live triatomids and two dead triatomids on top of the alfalfa (Image 3 and 4). We left the guinea pig in this environment overnight. The next morning the enclosure was examined and we found all five triatomids still within the enclosure. The guinea pig did ingest the alfalfa, but did not appear to have interest in consuming the triatomids.
Feeding dead triatomid larval instars on small amount of alfalfa:

After we determined that the guinea pig did not appear to be actively eating the dead or alive triatomids off a large amount of alfalfa we decided to embed small dead larval instar in a small amount of alfalfa. It appeared that provided that the triatomid remained embedded in the small amount of alfalfa that the guinea pig would ingest the larval stages (Image 5). If the triatomid fell off the alfalfa the guinea pig did not appear to actively seek out the triatomid to eat like it would with small remnants of alfalfa.

Feeding infected alfalfa to guinea pig:

Once we determined that the guinea pigs did not appear to be actively eating the triatomids we decided to ascertain if the guinea pigs would ingest contaminated alfalfa. Often in a natural setting the triatomids live within the guinea pig enclosures. It is therefore possible that
triatomid feces may be contaminating the alfalfa. In this experiment we infected alfalfa with non-infected triatomid feces to determine if the guinea pig would ingest the infected alfalfa. We found that the guinea pig did ingest the infected alfalfa without pause (Image 6).

**Oral Transmission of *T. cruzi* by ingestion of infected alfalfa:**

After we determined that the guinea pigs would ingest the contaminated alfalfa we proceeded to inoculate the positive controls and feed the infected alfalfa to the experimental group. By the third week of the experiment the first positive control showed parasitemia by microscopy, and by the fourth week another positive control showed parasitemia also by microscopy. None of the other samples throughout the entire experiment showed parasitemia by microscopy (Table one).

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*Blue – negative controls, Yellow- positive controls, Red- experimental group
While PCR and serological tests are ongoing we were able to process one experimental sample 1936. By the second week of the experiment the experimental guinea pig did develop infection with *T. cruzi* proving oral transmission from ingestion of infected alfalfa (Image 7). These results are preliminary and await further processing.

Image 7: PCR of experimental Guinea Pig 1936 weeks 1- 8

![Image 7: PCR of experimental Guinea Pig 1936 weeks 1- 8](image)

* 1- positive control 2- negative control 3- baseline blood draw (1936), 4- week 2 of experiment (1936), 5- week 3 (1936), 6- week 4 (1936), 7- week 5 (1936), 8- week 6 (1936), 9- week 7 (1936), 10- week 8 (1936), 11 – positive control from experiment (2033) week 8, 12- negative control from the experiment (1935) week 8

**Discussion:**

The guinea pigs did not appear to actively seek out triatomids as a food source in our behavioral experiments. When left over night in an environment that most mirrored natural conditions, the guinea pig did not consume the live or dead triatomids. We were able to feed dead triatomids to the guinea pigs while the bugs were embedded in a small amount of alfalfa. These results indicate that ingestion of triatomids most likely occurs accidentally, rather than the guinea pig actively seeking out and eating the bugs. However, our conditions do not match natural conditions of the guinea pigs in the field. Guinea pig are often housed together in large group and are fed anything from table scraps to alfalfa. Our lab guinea pig population are very
well feed with alfalfa and may not have the same pressures that field raised guinea pigs have in a more normal environment. While our experiments suggest that oral ingestion of triatomids is rare and accidental in nature, guinea pigs raised in different settings may behave otherwise.

Micheal Levy et al, in an currently unpublished work was able to ascertain via ELISA tests that many guinea pigs in Arequipa have antibodies for triatomid saliva. Indicating that the triatomids do feed off the guinea pigs. It still remains unknown if the classical mode of transmission (infection from infected feces entering a wound or mucosal surface) is the prominent mode of transmission in guinea pigs. Here we provide evidence of an alternative mode of transmission, transmission from oral ingestion of infected alfalfa. While experiments are ongoing we were able to provide evidence of infection via oral ingestion of infected alfalfa. Experimental guinea pig 1936 did develop infection in the second week of the experiment and continued to have infection in weeks thereafter. Future studies will try to better mirror natural condition to better ascertain the predominate mode of transmission.
Works Cited


