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Community Based Epidemiological Study of Chagas Disease in Rural Peru

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Abstract

COMMUNITY BASED EPIDEMIOLOGICAL STUDY OF CHAGAS DISEASE IN RURAL PERU.

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The purpose of this study was to evaluate the epidemiology of *Trypanosoma cruzi* infection, to evaluate the TESA blot, and to characterize cardiac findings of patients with Chagas disease in a rural setting near Arequipa, Peru. The study site was the town of Quequeña, Peru with a population of 774 with 236 inhabitants under the age of 18 according to the 2005 census conducted by Peru. A fumigation/insect collection campaign was done in December of 2006 to quantify household infestation levels, document housing characteristics, and GPS household locations. Of the 602 people surveyed to be living in Quequeña, blood samples were taken from 445 (73.9%), and 15 (3.37%) were positive for Chagas disease by ELISA and confirmed by immunofluorescence. The TESA blot was also performed on all positives (N=15) and a random subset of negative (N=20) blood samples with a sensitivity of 93% and a specificity of 100%. Electrocardiograms (EKGs) were performed on 37 people, 9 of whom were positive for Chagas disease and the other 28 were age and sex matched controls. All EKGs of Chagas positive patients were normal and 27 of 28 EKGs were normal in the control group. Of the 284 households in Quequeña, 242 (85.2%) were sprayed, and fifty-eight (24.0%) were infested with triatomines. Nineteen households (7.85%) harbored triatomines infected with *T. cruzi*. Of the 15 patients positive for Chagas disease, 9 lived in a house positive for triatomines (60%), of which 3 were positive for *T. cruzi* (33%). A serosurvey for Chagas disease in a rural community of Peru was successfully conducted with a prevalence of 3.4%. This information will aid the local Chagas Control Program in its estimates as it continues its fumigation campaign. In addition, the TESA blot was successfully employed in a community based setting enabling its role to be expanded in Chagas diagnosis.

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1 Introduction

Chagas disease or American trypanosomiasis, is caused by the parasitic protozoa *Trypanosoma cruzi* and is transmitted by the insect vector belonging to the subfamily triatomine with a geographic range from the southern United States to Argentina. About 13 million people are estimated to be infected with *T. cruzi* with many more at risk for acquiring the disease. Chagas disease can be transmitted four ways: by the blood sucking triatomine insect, congenital transmission, blood transfusion, and by ingesting contaminated agricultural products. The disease disproportionately affects the rural poor as the triatomine vector is domiciliated, living in and around poorly constructed homes with cracks and holes. Insecticide campaigns have proven effective in reducing the transmission of Chagas disease. Of those infected about one third go on to develop serious irreversible damage to the heart or gastrointestinal tract. Diagnosis in the acute phase relies on direct visualization of the parasite and the chronic phase relies on the detection of circulating antibodies to *T. cruzi*. Treatment of persons infected with *T. cruzi* is often ineffective and inadequate with greatest treatment benefit seen in those recently infected.

1.1 Parasite and Vector

Chagas disease is caused by the parasitic protozoa *Trypanosoma cruzi*, which is transmitted by the insect vector belonging to the subfamily Triatominae. The other member of the genus *Trypanosoma* that causes human disease is *Trypanosoma brucei*, which causes African sleeping sickness in sub-Sahara Africa (1).

The life cycle of *T. cruzi* is divided into morphologically distinct stages depending on the host and tissue location. Four stages are seen, two in the insect host

and two in the mammalian host. Triatomine insects harbor the replicative epimastigotes and shed the infective metacylcic trypomastigotes. Mammalian hosts contain the intracellularly replicative amastigotes and circulate in blood the trypomastigotes. Beginning with a blood meal from an infected mammalian source triatomine insects become infected with the trypomastigote form of *T. cruzi*. Trypomastigotes are identified microscopically by their slender appearance and undulating membrane with the kinetoplast posterior to the nucleus (see Figure 1). Kinetoplasts are interlocked DNA minicircles and maxicircles contained within the matrix of a single mitochondrion. Kinetoplast DNA (kDNA) is unique to trypanosomes and related protozoa with a structure that resembles chain mail and a singular form of replication (2, 3). Once in the midgut of the triatomine the trypomastigotes transform into epimastigotes which attach to the wall of the hindgut. Epimastigotes are replicative and are distinguished from trypomatigotes by a shortened form and location of the kinetoplast anterior to the nucleus. In the hindgut the epimastigote transforms into metacyclic trypomastigotes. Metacyclic trypomastigotes are the infective, non-replicative form, which are shed in triatomine feces (4).

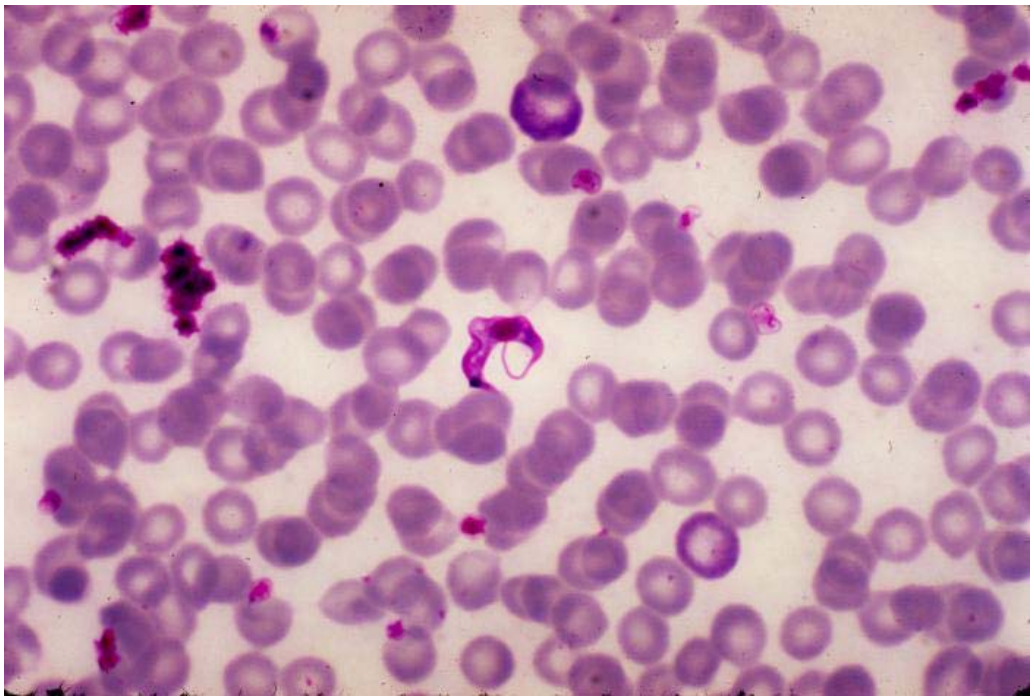


Figure 1. This is a micrograph of *Trypanosoma cruzi* in a blood smear using Giemsa staining technique (courtesy of the Public Health Image Library, CDC, http://phil.cdc.gov/phil/home.asp).

T. cruzi are classified as stercoraria because of a unique vectorial fecal transmission (1). Infection occurs when feces containing metacyclic trypomastigotes enter breaks in the skin caused by triatomine bite, micro abrasions secondary to scratching the bite, or mucous membranes (5). It is unlikely that *T. cruzi* can penetrate intact skin.

T. cruzi can be further classified as type I or type II based on analysis of randomly amplified polymorphic DNA (RAPD), rRNA 24S subunit, rRNA promoter region, and a 195 bp DNA repeat (6-9). More recently the *T. cruzi* II lineage has been further subdivided by characterization of rRNA subunits and miniexons into five different classifications designated IIA through IIE (10). There are some relevant clinical and epidemiological correlations relating to the classification into two broad groups. Type II is commonly isolated from humans and appears to be associated more with the domestic cycle whereas Type I is more often found in the sylvatic cycle (11). These two groups

also show a geographic distribution in human infection with Type I, which produces milder infections, more prevalent in Central America and northern South America, while type II, the more aggressive form, is more prevalent in South America. The evolutionary adaptation for Type II to the domestic cycle may explain why the vast majority of seropositive humans are found to be infected with this lineage. Interestingly, in an endemic zone of Bolivia both lineages were isolated from *T. cruzi* with almost the same frequency (0.67 and 0.66). However, children infected in these zones were infected with a greater frequency by *T. cruzi* II than *T. cruzi* I, 0.85 and 0.19 respectively. Clearly the role of *T. cruzi* II in the domestic cycle does not fully explain its predominace in human infection and it is postulated that specific host immunologic factors may be an important in controlling *T. cruzi* I infection (12).

The vector responsible for spread of Chagas disease belongs in the order Hemiptera, family Reduviidae and the subfamily Triatominae. There are over 130 species of triatomine with only 3 synanthropic species important in human transmission: *Triatoma infestans*, *Rhodnius prolixus*, and *Panstrongylus megistus* (1, 5). These three genera responsible for human transmission have a geographic range stretching from Mexico to Argentina. The most important vector for transmission in South America is *Triatoma infestans*, see Figure 2, and in Central America, Colombia and Venezuela *Rhodnius prolixus* (13). Transmission in Southern Peru is almost exclusively via *T. infestans* (14).

Figure 2. Picture of *Triatoma infestans.*

Triatomines are obligate hematophagous insects throughout their lifecycle. After hatching, triatomines undergo five nymphal instars over the next six months before reaching the adult stage. Triatomines can fast for up to 200 days of their twelve to eighteen month lifespan but require blood meals in order to develop. Triatomines are active at night with increased activity at dusk and dawn (14). It is at this time that they feed on their mammalian hosts (15). In the wild *T. cruzi* can maintain its life cycle among over 130 species of Triatomine and over 100 species of mammals (16). Humans are the important reservoirs among the domestic triatomine species with other domesticated animals playing a significant role. Studies done in Argentina and Venezuela have noted the seropositivity of dogs and cats living in close proximity to humans as risk factors for Chagas disease (17-19). It is notable that in the Andean regions of Peru and Bolivia guinea pigs and rabbits serve as important reservoirs of *T. cruzi*. These animals, often used as a food source, are raised in the home or in peridomestic cages, see Figure 3.

Figure 3. Peridomestic guinea pig cages next to a porous rock wall in Quequeña, Peru. The cracks in the rock wall as well as the homes are an ideal habitat for *T. infestans*.

In the domestic setting, triatomines hide inside the dwelling in cracks and holes in the wall, thatched roofs, and behind hangings on the wall. Outside the dwelling they occupy rock piles or rock fences and cages used for animal rearing. It is from these locations that the triatomine emerge at night for a blood meal from humans and other domestic animals. Often feces from the insect can be found streaked on walls and bedding in infected homes, see Figure 4.

T. infestans is so adapted to its domestic role that it has difficulty surviving outside of this specialized niche. It is thought that the natural habitat for *T. infestans* is the Cochabamba valley of Bolivia because it is the only location in which sylvatic populations can be found in rock piles associated with wild populations of guinea pigs.

Introduction of this vector into human habitats might have occurred in association with pre-Columbian domestication of the guinea pig. After adapting to a domestic environment, spread throughout South America is presumed to have occured via human migrations (20). *T. cruzi* DNA has been isolated and amplified by PCR from 4000 year old mummies on the coast of Chile where *T.infestans* is strictly domestic (21). This lends evidence to the theory of synanthropic co-migration of domesticated *T. infestans* from an original sylvatic source in Bolivia.

Figure 4. *T. infestans* excreta found on bed (left) and wall (right) of dwelling in Quequeña, Peru.

1.2 Clinical Characteristics

1.2.1 Pathophysiology

After successful inoculation into the mammalian host *T. cruzi* metacyclic trypomastigotes invade local tissues and proceed with obligate intracellular replication with subsequent local and disseminated invasion. *T. cruzi* gains access to the intracellular compartment via membrane bound vacuoles. Attachment of the parasites to the cells is glycoprotein receptor mediated. This receptor mediated attachment activates

various calcium signaling pathways that induce reorganization of the cytoskeleton with lysosomal recruitment (22). Lysosomal fusion with the parasite vacuole activates pH sensitive enzymes that release the invading parasite into the host cell cytoplasm (23). Once in the cytoplasm the trypomastigotes differentiate into amastigotes and approximately 20 hours later begin to replicate by binary fission with a doubling time of about 12 hours. 4-5 days later the host cell cytoplasm is full of replicative amastigotes and they differentiate back into trypomastigotes as the cell ruptures and spills approximately 500 parasites for each initial trypomatigote (24). These newly released parasites invade local tissues and disseminate hematogenously throughout the body with a predilection for muscles especially myocardium.

Endomyocardial biopsies done on patients with acute Chagas disease show diffuse neutrophilic and monocytic inflammatory infiltrates with scarce fibrosis and occasional intracellular amastigote nests (25). During the chronic phases of Chagas disease when parasitemia is no longer present endomyocardial biopsies reveal continued presence of the parasite as demonstrated by direct visualization of the parasite, immunofluorescent deposits, or PCR detection of *T. cruzi* DNA (26). Histopathologic changes in chronic Chagas disease include myocardial necrosis, inflammatory infiltrates, and fibrosis. Cadaveric studies show preferential fibrosis of the conducting pathways of the heart that affects the right bundle branch and the anterior division of the left bundle branch correlating well with premortem EKG findings (27). Some have proposed a largely auto-immune process to explain the effects of Chagas disease while others note the presence of parasites in the tissues affected. Although not understood completely, the pathogenesis of these lesions is thought to be due to chronic low grade infection coupled

with an adverse immunologic response by the host (28, 29). Autonomic nervous system derangements and microvascular disturbances have also been proposed and contributing mechanisms.

Gastrointestinal involvement of Chagas disease presents as one of the megasyndromes. The affected colon undergoes marked muscular hypertrophy with luminal dilation and uncoordinated peristalsis. Histopathologic studies found that chronic Chagas patients with megacolon had significantly higher numbers of natural killer cells and cytotoxic lymphocytes within enteric ganglia compared with asymptomatic Chagas patients. Megacolonic Chagas patients also displayed a 20% reduction in muscle innervation compared to asymptomatic Chagas patients and non-Chagasic controls (30).

1.2.2 Acute Chagas Disease

Acute Chagas disease occurs approximately one week after inoculation with *T. cruzi*. When infection occurs through a break in the skin a characteristic chagoma may form, which is an area of local inflammation, induration, and erythema with occasional lymph node involvement (31). When infection occurs through the conjunctiva, ispsilateral palpebral edema results in the classic sign of acute Chagas disease: Romañas sign, see Figure 5. Acute infection is normally seen only in children but can occur at any age. Systemic manifestations of acute infection are usually non-specific and easily misdiagnosed such as fever, malaise, lymphadenopathy and hepatosplenomegaly. These symptoms improve over the ensuing 6-8 weeks with complete resolution in most cases. In children, or immunosuppressed patients, some cases of acute Chagas disease can be quite severe with myocarditis or meningoencephalitis that may result in death (32, 33).

The large majority of acute Chagas disease cases go undiagnosed and untreated presumably because of mild symptoms. However, poor access to health care and diffuse non-specific symptoms are also important factors to consider.

Figure 5. This child from Panama is suffering from Chagas disease manifested as an acute infection with swelling of the right eye (courtesy of the Public Health Image Library, CDC, http://phil.cdc.gov/phil/home.asp).

1.2.3 Chronic Chagas Disease

Once the acute phase of Chagas disease has passed, patients enter into an asymptomatic stage termed indeterminate. During the indeterminate phase of Chagas disease parasites are no longer found circulating in the blood but patients do have circulating antibodies to *T. cruzi* antigens and *T. cruzi* DNA can be detected by PCR assays (34). Once patients begin to manifest symptoms of long term infection with *T. cruzi* they are considered to be in the chronic phase. Approximately one third of patients with the indeterminate form progress to the chronic phase 10 to 30 years after the initial infection. In the remainder of the patients the interaction between host immune system and innate parasite characteristics limits the tissue damage caused by the parasite and they remain in the asymptomatic indeterminate stage for life. Given the subtle nature of

the acute infection and the asymptomatic indeterminate phase most patients who have Chagas disease are unaware.

Chronic Chagas disease affects a number of organ symptoms predominately the heart, followed by large intestine and esophagus. There is a geographic pattern to which clinical forms are manifest with a dominance of megaesphagous and megacolon in the southern cone countries of South American countries whereas northern South America and Central America have relatively few cases of the megasyndromes (14). This difference is thought to be due in part to the fact that isolates from humans infected in the southern cone countries tend to be of the lineage *T. cruzi* type II and those from northern South America and Central America from type I (35).

Chagas heart disease manifests as arrhythmias, congestive heart failure, and thromboembolism. Early electrocardiographic changes in cardiac function include conduction abnormalities such as the classic finding of right bundle branch block, premature ventricular beats, and with more advanced changes atrial fibrillation, low QRS voltage and nonsustained ventricular tachycardia (36, 37). Echocardiography may detect early wall motion abnormalities and advance disease is characterized by biventricular dilation and left ventricular aneurysm formation. Right heart failure with its accompanying symptoms usually precedes the development of left heart failure. Risk stratification based on clinical, electrocardiographic and echocardiographic data has been developed to predict death due to Chagas heart disease (38). It has been shown that Chagasic patients with dilated cardiomyopathies have a much worse prognosis than patients with non-Chagasic cardiomyopathy (39). However, treatment for Chagas heart

disease is similar to treatment for other causes of congestive heart failure with pacemakers showing benefit in the management of bradyarrhythmias (40).

Chronic Chagas disease affects the enteric nervous system and such destruction gives the classic megasyndromes of megaesophagus and megacolon. Megacolonic patients present with progressive worsening constipation fecal impaction. Rarely these patients may develop sigmoid volvulus or ischemic colitits. Diagnostic studies such as barium enemas are effective in diagnosis of megacolon and almost all cases of Chagas megacolon show dilation and elongation of the sigmoid colon often with rectal involvement. Proximal colonic dilation with Chagas is rare. Conservative therapy consists of increasing fiber intake, judicious use of laxatives, and intermittent enemas. Manual disimpaction of obstructing fecalomas or surgical excision of affected bowel may be warranted in advanced cases (41).

Megaesophagus presents similar to other forms of idiopathic achalasia with progressive difficulty swallowing food and regurgitation. Some patients may persist with mild dysphagia for life while others may progress to achalasia with esophageal dilation. Barium esophogram will show a dilated esophagus with distal narrowing of the gastroesophageal junction representing the nonrelaxing lower esophageal sphincter (LES) with classic bird beak appearance. Esophageal manometry demonstrates uncoordinated peristalsis and a nonrelaxing LES. Treatment for achalasia is the same regardless of the etiology with modifications of food bolus to aid in esophageal transit. Esophagomyotomy via endoscopic balloon dilation is useful and the most severe cases may require esophagectomy (41).

1.3 Epidemiology

The WHO estimates that in 1985 when the first good epidemiological studies were done, that 25% of the inhabitants of South America, or 100 million people, were at risk for acquiring Chagas disease. The prevalence of Chagas disease was approximately 17.4 million cases with over 700,000 new cases per year (14). Vector control programs such as the Southern Cone Initiative involving Argentina, Brazil, Chile, Paraguay, and Uruguay have shown to be effective. Since 1982 these countries have seen a reduction in incidence ranging from 60% to 99% and it was estimated that in the year 2000 there were only 200,000 new cases worldwide (14). Estimated current prevalence of Chagas disease is below in Figure 6.

> 3%
- Bolivia
- Argentina
- El Salvador
- Honduras

1 – 3 %
- Paraguay
- Guatemala
- Ecuador
- Guayana
- Venezuela
- Nicaragua
- Mexico
- Brazil

< 1%
- Chile
- Belize
- Peru
- Uruguay
- Costa Rica
- Panama

(42)

Figure 6. Estimated prevalence of Chagas disease in endemic countries and map of endemic zones in Central and South America.

Transmission of *T. cruzi* largely occurs through vectoral transmission as discussed above. However, other important mechanisms of transmission include blood transfusion, congenital transmission, organ transplantation, oral transmission, and laboratory accidents. Because of migrations of rural inhabitants of endemic zones to urban non endemic zones transmission by blood transfusion has also become a concern. In Lima, where over one third of Peru's inhabitants live and is free from vectoral transmission, seroprevalence in 1993 among blood donors was 2.4% and in Santa Cruz, Bolivia it has been as high as 50% (14). Countries with no vectoral transmission of Chagas but a high number of migrants from infected countries are also at risk of transmitting the disease through blood donations especially since blood in these countries is not routinely screened for Chagas. One study in Washington D.C. done among Salvadoran and Nicaraguan immigrants found a prevalence of 5% for Chagas disease (43). Other studies done in blood donors have confirmed that infected migrants are living in the United States (44-47). It has been estimated that in the U.S. 90,000 and possibly up to 600,000 of the 12.8 million Latin American immigrants from 1981-2005 may be infected with *T. cruzi* (48).

Congenital transmission is an important mechanism of transmission in rural and urban centers. Unpublished results from my experience in Santa Cruz, Bolivia showed a seroprevalence in 2007 of 25% among expecting mothers with only 1% transmission to the infant. Other studies have shown congenital transmission rates between 1% and 7% (49, 50). Transmission by organ transplantation, laboratory and hospital accidents have been reported, as well as outbreaks due to oral transmission following ingestion of food contaminated with triatomines or their excreta (51-53) .

Epidemiological surveys conducted in Peru have shown the highest prevalence to be in the southern departments of Arequipa, Ica, Moquegua, and Tacna. Unpublished data obtained from the Chagas Control Program of the Regional Ministry of Health in Arequipa, Peru show 217 new cases of Chagas disease during 2000 through 2002 in all of Peru with 153 of those cases among the 3 southern departments of Arequipa, Tacna, and Moquegua. Good epidemiological studies from Peru are lacking and the actual number of infected persons has been estimated to be around 680,000 with 6.7 million at risk for acquiring the infection (14). Vector control has begun in the southern departments with surveillance programs in place with the hope to control vectoral spread and eliminate domestic transmission in these zones within the next few years (54). The Andean countries of Bolivia, Peru, Colombia, and Venezuela hope to replicate the success of the Southern Cone Initiative in reducing Chagas disease transmission. Significant progress has been made despite serious financial, political, and social hurdles with many more years of dedicated investment by these countries and international organizations necessary to achieve desired success.

1.4 Diagnosis and Treatment

Acute Chagas disease is diagnosed by appropriate clinical history consistent with exposure and by detecting parasites in blood. During the acute phase parasitemia motile parasites may be directly visualized by light microscopy in a drop of fresh blood. To increase the sensitivity microhematocrit tubes may be used to visualize the parasite in the buffy coat or the Strout method to increase parasite concentration. Indirect methods of parasite detection are xenodiagnosis with virgin Triatomines who are examined 1-2 months after feeding on a patient's blood for parasites in the hindgut. Another method

involves culturing the patient's blood in liver infusion tryptose (LIT) medium to check for parasite growth over the ensuing months. Both indirect methods have been shown to be effective with highly variable sensitivity based on laboratory and patient characteristics. The delay in diagnosis and difficulty in consistently conducting the indirect tests has decreased their use in routine diagnosis especially in resource poor countries. PCR diagnosis of parasite DNA has shown promise as an effective diagnostic test for acute Chagas disease for over 10 years but lack of standardization and high costs has limited the adoption of this method for routine diagnosis (34, 55, 56).

The diagnosis of chronic Chagas disease relies on the detection of circulating IgG antibodies that bind to parasite antigens. Currently there are more than 30 commercial kits available for the serological diagnosis of *T. cruzi*. The three conventional immunologic tests in use are indirect hemagglutination (IHA), indirect immunofluorescence (IIF), and the enzyme-linked immunosorbent assay (ELISA). Because the antigens for these tests are usually derived from whole parasites, numerous cross reactions with leishmaniasis, malaria, syphilis, and other diseases can occur. Numerous tests have been developed using recombinant antigens to try and decrease cross reactivity (57-60). The radioimmunoprecipitation assay (RIPA) uses radio-labeled *T.* cruzi antigens and tests the ability of patient serum to precipitate bands of 72kD and 90kD on electrophoresis indicative of a positive reaction. The RIPA has shown to be highly sensitive and specific and has been used in the U.S. as a confirmatory test but due to its high cost and laborious procedure it has not been readily adopted by other countries (61, 62). A positive result by any one of the three conventional tests should be confirmed

with a second conventional test, while a negative result needs no confirmation. There is no definitive “gold standard” diagnostic assay for Chagas disease.

An experimental assay to diagnose Chagas disease based on trypomastigote excreted-secreted antigens (TESA) has been developed (63). *T. cruzi* trypomastigotes in culture spontaneously secrete antigens known as TESA which can then be isolated and used in an immunoblot assay for Chagas diagnosis. Interestingly, sera from acute and chronic Chagas patients give two different banding patterns with immunoblot that allows distinction between the two. The chronic patients have a characteristic broad band at 150 to 160 kDa and acute Chagas patients show a ladder like pattern of 6 bands between 130 and 200 kDa (Figure 7). The TESA blot has successfully been used as a screening tool in Brazilian blood donors with inconclusive results from IIF and ELISA and was positive for 10 of 348 inconclusive sera with complete concordance with positive and negative controls(64) These results suggest that the TESA blot may be a more sensitive diagnostic tool that may aid in the diagnosis of Chagas disease. The TESA antigens have also been adapted with success in ELISA assays for Chagas diagnosis with reported sensitivity of 100% and specificity of 94% with notable cross reaction to sera from patients with leishmania (65).

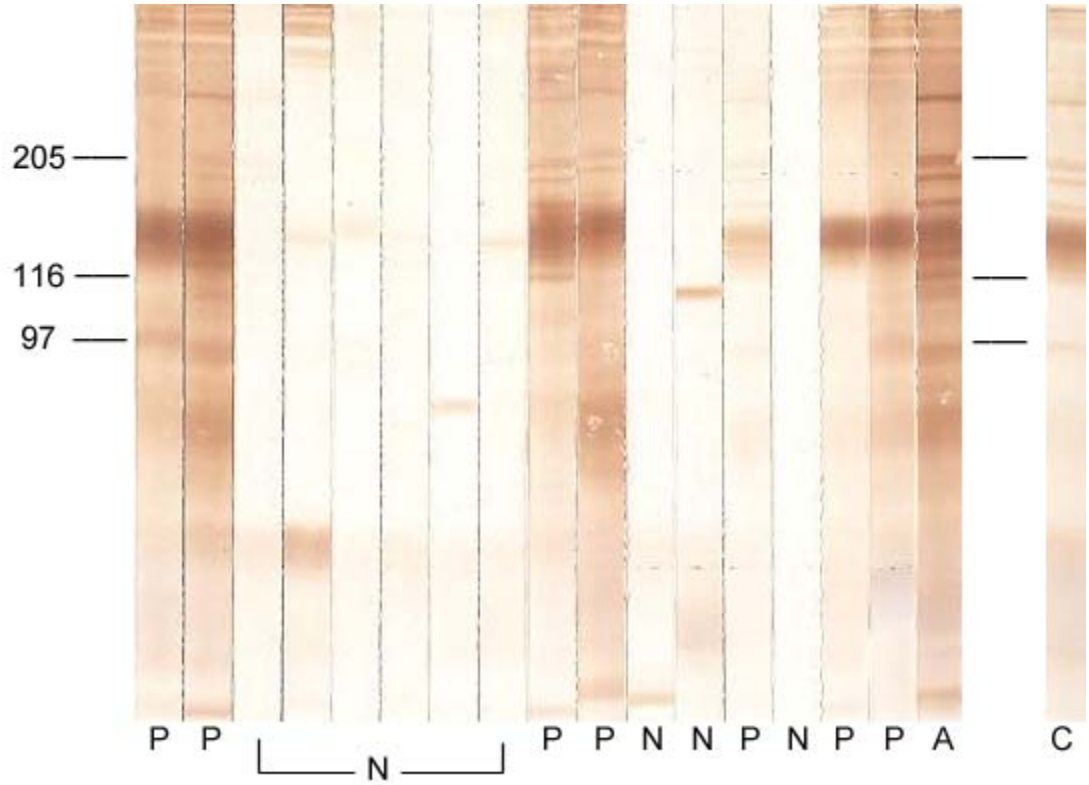


Figure 7. TESA blot showing the broad band at 150-160 kDa in chronic Chagas patients (P), and the ladder like pattern of 6 bands in acute Chagas patients (A), and Chagas negative (N) and control serum (C).

Many studies have tested PCR as a diagnostic tool for chronic Chagas disease with sensitivities reported between 50% and 100% with the majority of studies showing a sensitivity of 90% (55, 56, 66, 67). Because of the laboratory specifications, specialized personnel requirements, and expense, PCR is not likely to be useful as a large scale screening test but its value might lie in detecting cases of congenital transmission where parasitemia is no longer present, response to treatment, or confirming results when other diagnostic tests conflict (56, 68, 69).

Treatment of persons infected with *T. cruzi* is largely ineffective and often inadequate. Only two drugs have proven efficacy in the treatment of Chagas disease: nifurtimox (Lampit, Bayer 2502), and benznidazole (Rochagon, Roche 7-1051). Nifurtimox and benznidazole were introduced in 1967 and 1972 respectively and were

found to be effective for the treatment of acute Chagas infections with benznidazole being preferred due to its slightly less toxic side effect profile. Nifurtimox is given orally in a dose of 8 to 10 mg/kg/day divided into four doses and continued for 90-120 days. The dose for children and adolescents is 25-20 mg/kg/day and 12.5-15 mg/kg/day respectively and therapy is continued for the same duration as in adults. Benznidazole is also given orally in a dose of 5mg/kg/day divided into two doses for 60 days with the same dose regardless of age group. In general, the younger the patient is the more tolerant they are of treatment and less likely they are to have serious side effects. Common side effects of both drugs include maculopapular rash that can progress to stevens-johnson syndrome, anorexia with significant weight loss, peripheral neuropathy, bone marrow suppression, diarrhea, and hepatotoxicity. Side effects are the biggest reason why patients fail to finish treatment courses with most effects resolving with stopping the drug or lowering the dose. Both drugs have demonstrated mutagenic potential in numerous studies but none involving humans (70).

Treatment of chronic Chagas disease is controversial at best. Some studies have shown that recent chronic Chagas disease in children, or infection occurring within the last 10 years, responds well to treatment with benznidazole with reported conversion to seronegative status among those treated at 58% and 62% while the placebo group was 0% and 5% (71, 72). Cure was defined as conversion of serological test to negative at 3 and 4 years follow up respectively. It is recommended that all patients with recent, less than 10 years, chronic Chagas disease be treated with appropriate pharmacotherapy especially all children under the age of 12. In Peru the Chagas program will provide benznidazole free of charge to all Chagas patients 15 years of age or younger. Despite evidence for

cure no long term study has been done to assess differences in clinical outcomes among these patients.

Efficacy in the treatment of longstanding asymptomatic Chagas disease is less clear. A systematic review found only 5 randomized control trials to assess the efficacy of treatment. In all of the studies treatment of chronic Chagas patients resulted in decreased parasite load versus placebo as measured by xenodiagnosis, ELISA, or antibody titers after a few years of follow up(73). However, no study has been done to correlate this decrease in evidence of parasites with long term clinical outcome. A long term study with 6 to 18 year follow up showed that this negative serology, or "cure", is as low as 8% in treated chronic Chagas patients (74). Another randomized study demonstrated *T. cruzi* DNA detected via PCR in 100% of treated patients at 10 years follow up with no difference in EKG abnormalities in the treated and non treated groups (75). A review of the literature found no role for treatment in chronic Chagas patients with cardiomyopathy (76). A recent non-blinded, non-randomized trial found that chronic Chagas disease patients age 30-50 years old without heart failure treated with benznidazole for 30 days resulted in fewer patients with disease progression (4%) vesus (14%) in the control group as measured by a change to a more advanced Kuschnir group or death (77). Despite the limitations of this study it may open the door to selective treatment of chronic Chagas patients.

Given the common and severe side effects cause by treatment for those patients who are in the chronic phase of the disease, difficulty in assessing a cure, and no definitive study on the long term clinical benefit of treatment, the decision to treat this group of patients remains highly controversial. It is also impossible at this time to

determine which set of chronic Chagas patients will remain asymptomatic their entire lives and which patients will have clinical manifestations regardless of treatment. Despite the controversy in treating patients with chronic Chagas disease, early diagnosis enables patients to receive the appropriate screening via EKG for early heart pathology and insertion of a pacemaker or automatic implantable cardioverter defibrillator (AICD). These interventions have been shown to benefit patients with arrhythmias.

2 Study Objectives

The purpose of this study was to evaluate the epidemiology of *Trypanosoma cruzi* infection, to identify risk factors for seropositivity, to evaluate the TESA blot, and to characterize cardiac findings of patients with Chagas disease in a rural setting near Arequipa, Peru. No systematic studies have been performed in this area and reliable data about prevalence and risk factors will help determine Chagas disease burden, and help guide vector control strategies. Additional information about the effectiveness of the TESA blot in a community screening will shed light on its value in the diagnostic armamentarium for Chagas disease.

Specific objectives of this study were the following:

1. What is the prevalence of seropositivity to *T. cruzi* in a population living in rural Chagas-endemic communities near Arequipa, Peru?
2. How does the TESA blot compare to traditional Chagas diagnostic methods?

This work will aid Peru and the Andean countries as they continue their campaign to eradicate vector transmission of Chagas disease by contributing valuable up to date information on prevalence and risk factors for contraction of Chagas disease in a rural environment. The clinical information provided in this study can shed light on regional, differences in cardiac pathology. The study is in alignment with the WHO's Millennium Development goals of combating disease in developing countries that contributes to morbidity and mortality.

3 Methods

3.1 Study Population and Sites

The study site is the town of Quequeña, Peru, elevation of 2300 m (7,545 ft), located about 25 km south of Arequipa. The village has a population of 774 with 236 under the age of 18 according to the 2005 census conducted by Peru. It is a rural, agricultural town that is isolated from the city of Arequipa, population 850,000, by distance and geography. There is no continuity of the sprawling slums of Arequipa with the town of Quequeña. Its economic survival is based almost exclusively upon agriculture and animal husbandry. In the community there is a school for primary education and students must travel to Arequipa for secondary education. A local health post is open 5 days a week in the mornings staffed by a nurse and two assistants. Minor check ups, vaccinations, and some medications are available at the health post. A nurse midwife and general physician attend at the clinic one day a week. No acute, overnight, or obstetrical care is available. Quequeña as well as other rural towns have suffered population declines in recent decades as many rural inhabitants have migrated to the cities because of terrorism or economic opportunities. Very few immigrants live in Quequeña so the majority of infected persons probably contracted Chagas in Quequeña. See appendix 2 for satellite images of the study site.

The majority of homes are of poor construction and many date to the first half of the 20th century. The community infrastructure consists of electricity and plumbing that brings water from local tanks into most homes but no sewer system exists. The water was unchlorinated during the duration of this study. Many homes also raise guinea pigs, rabbits, dogs, chickens, sheep, and cattle in close proximity to their dwellings which

supply an ideal habitat and blood meals for *T. infestans* while providing an important reservoir for *T. cruzi* infection. Community members recognize *T. infestans* by the local name of "chirimacha" and are often aware of the infestation but knowledge of *T. cruzi* infection by *T. infestans* was limited. *T. infestans* excreta could be found on the walls and bedding of homes prior to fumigation campaigns.

In the community of Quequeña an extensive entomologic and ecologic survey was conducted in December of 2006 by Michael Levy. Entomological collection was carried out in coordination with the Arequipa Regional Ministry of Health Vector Control Program's insecticide spraying campaign. Entomological surveillance included timed collection of *T. infestans* (30 minutes by two collectors for a total of one person-hour) in houses and peridomestic animal corrals after application of deltamethrin (K-Othrine) insecticide by the Ministry of Health vector control team. 296 houses were GPS-mapped in Quequeña. The *T. infestans* collected were analyzed for the presence of *T. cruzi* in the hindgut.

3.2 Community Based Survey

All community based work was done in coordination and with the support of the Ministry of Health and the regional Chagas Disease Control Program directed by Dr. Juan Cornejo. Local support was obtained by working closely with the local health post, government and religious leaders. Numerous community meetings were conducted to notify the community of the objectives and design of the study. A meeting was held at the local primary school with parents and teachers to detail the inclusion of children in the study as most cases of Chagas disease are contracted during childhood. In addition to explaining the purpose of the study at each meeting, a presentation designed by the

Chagas Control Program was given to educate the community about Chagas transmission, detection, and prevention.

After community education programs were completed, a house to house census of the town was done with 284 inhabited houses and 602 residents surveyed. Two weekends were programmed for community wide serosurveys where residents came to the health post or church and after informed consent 5cc of blood was obtained. After parental consent was obtained one day was spent in the primary school conducting the serosurvey with parents present. As outlined in the consent form, participation was voluntary and any resident who declined to participate could contact the Ministry of Health hospital for evaluation of Chagas disease. In order to facilitate community participation, individuals who were unable to come to the scheduled serosurveys were visited in their homes up to 4 times by study nurses early in the morning or evening.

3.3 Case Control Study

All seropositive community residents and two age and sex matched community controls were invited to participate in a nested case-control study to evaluate risk factors for Chagas and EKG differences. All participants were administered a questionnaire and a 12 lead EKG was done with a portable EKG machine at the local health post. The EKG's were read by a local cardiologist who was privy only to patient age and their unique laboratory code.

3.4 Epidemiologic Data Collection and Analysis

Data collected by Michael Levy and team in December of 2006 includes *T. infestans* field data, and GPS location of all houses. Census information, serosurvey, laboratory analysis, and case-control study was performed by myself. All questionnaires

were translated by a certified Spanish translator and administered by a local nurse fluent in the language. Data analysis was done using SPSS statistical software.

3.5 Specimen Collection and Laboratory Testing

All blood specimen collection was done using new material donated by the Centers for Disease Control and Prevention (CDC) of the United States. Only study nurses drew samples and they followed universal precautions and standard sterile technique. Blood was obtained using Vacutainer blood collection needles, 21 G x 1.25 inches and 5 mL Vacutainer red top plastic serum tubes from BD (#368607, #367814). Samples were kept on ice, stored at -4°C overnight, and processed the next morning into aliquots of serum and clot that were then stored at -20°C. One aliquot of specimens were given to the Ministry of Health's diagnostic laboratory for quality control and the other was sent overnight to the laboratory of Dr. Robert Gilman at the Universidad Cayetano Heredia in Lima, Peru for laboratory testing.

All samples were screened by commercial ELISA kit Chagatek following the manufacturer's instructions (bioMérieux: distributed by Quimica Suiza, Lima, Peru). All positives were confirmed with indirect immunofluorescence (IIF) as well as a group of negatives. Specimens were considered positive when fluorescence was observed with a dilution of 1:16 or higher. The TESA blot was performed as described previously (63) on all positive samples and a subset of negative samples.

3.6 Treatment of Seropositive Children

In accordance with the guidelines of the Peruvian Ministry of Health all seropositive children under 15 years of age would be treated with benznidazole for 60

days (10 mg/kg/d for children less than 10 years old; 5-7 mg/kg for children 10-15 years old per Roche Brazil) (78). No children in this age group were seropositive.

3.7 Informed Consent and Confidentiality

Appendix 1 shows the consent forms in English that were used for this study. Consent forms were translated into Spanish by a certified translator and consent forms were read and explained by study nurses fluent in Spanish. Consent was obtained by signature or fingerprint. Each individual was assigned a unique laboratory code that was affixed to all laboratory samples. Names of participants were entered into the census database along with their unique code to provide a key. All other databases used the code numbers only. All databases are only available to study investigators and computers are secured with passwords. Hard copies of questionnaires are locked in a file cabinet in Lima, Peru.

3.8 IRB Approval

The amendment for this study was approved by the Johns Hopkins Bloomberg School of Public Health Institutional Review Board on April 10, 2007 (IRB# H22.05.01.26.A1). Approval was also obtained from the IRB of A.B. PRISMA on April 3, 2007 (IRB# CE152.07) in Lima, Peru.

4 Results

Of the 602 people surveyed to be living in Quequeña, blood samples were taken from 445 (73.9%) with 237 female and 208 male. Table 1 shows the gender breakdown by decade for participants in the study.

Table 1. Age and sex of participants.

Age (years)	Sex		
	F	M	Total
<10	31	33	64
10 - 19	52	45	97
20 - 29	39	30	69
30 - 39	38	30	68
40 - 49	29	30	59
50 - 59	21	8	29
60 - 69	12	15	27
70 - 79	11	12	23
80+	4	5	9
Total	237	208	445

Mean 31.93 years
Median 29 years
Range 2 - 83 years

Of the 284 households in Quequeña, 242 (85.2%) were sprayed, and fifty-eight (24.0%) were infested with triatomines. Nineteen households (7.85%) harbored triatomines infected with *T. cruzi*. This signifies that 41.1% of participants in the study lived in a household where the insect vector was present, and 11.9% lived in a household with *T. cruzi* infected triatomines, see table 4. 3 households refused to participate in the spraying campaign, 28 households were abandoned or closed, 2 were vacant lots, and 9 households were public spaces that were sprayed but not surveyed. 95% of participants in the study gave basic information regarding their housing status, i.e. electricity, potable water, sewer, and material of floor. These housing characteristics are shown in relation to harboring *T. infestans* in Table 2 and in relation to vectors positive for *T. cruzi* in Table 3.

Table 2. Housing characteristics and *T. infestans* status

		T. infestans status					
		Positive		Negative		Total	
		Number of patients	%	Number of patients	%	Number of patients	%
Electricity	Yes	162	38.2%	220	51.9%	382	90.1%
	No	18	4.2%	24	5.7%	42	9.9%
	Total	180	42.5%	244	57.5%	424	100.0%
Potable water	Yes	170	40.1%	209	49.3%	379	89.4%
	No	10	2.4%	35	8.3%	45	10.6%
	Total	180	42.5%	244	57.5%	424	100.0%
Sewer	Yes	0	.0%	79	19.1%	79	19.1%
	No	175	42.3%	160	38.6%	335	80.9%
	Total	175	42.3%	239	57.7%	414	100.0%
Material of Floor	Cement	39	9.2%	85	20.1%	124	29.3%
	Dirt	138	32.6%	153	36.2%	291	68.8%
	Other	3	.7%	5	1.2%	8	1.9%
	Total	180	42.6%	243	57.4%	423	100.0%

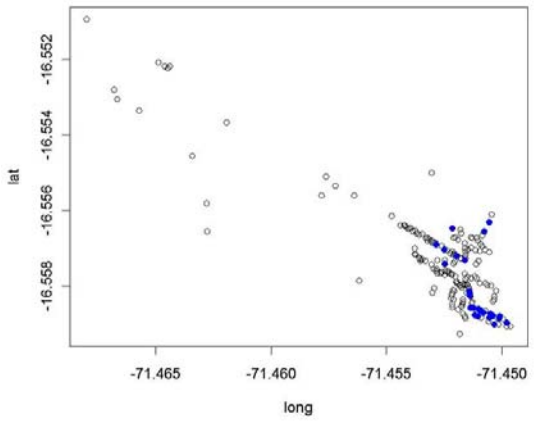
Table 3. Housing characteristics and *T. cruzi* status

		T. cruzi status					
		Positive		Negative		Total	
		Number of patients	%	Number of patients	%	Number of patients	%
Electricity	Yes	50	27.8%	112	62.2%	162	90.0%
	No	0	.0%	18	10.0%	18	10.0%
	Total	50	27.8%	130	72.2%	180	100.0%
Potable water	Yes	46	25.6%	124	68.9%	170	94.4%
	No	4	2.2%	6	3.3%	10	5.6%
	Total	50	27.8%	130	72.2%	180	100.0%
Sewer	Yes	0	.0%	0	.0%	0	.0%
	No	50	28.6%	125	71.4%	175	100.0%
	Total	50	28.6%	125	71.4%	175	100.0%
Material of Floor	Cement	18	10.0%	21	11.7%	39	21.7%
	Dirt	29	16.1%	109	60.6%	138	76.7%
	Other	3	1.7%	0	.0%	3	1.7%
	Total	50	27.8%	130	72.2%	180	100.0%

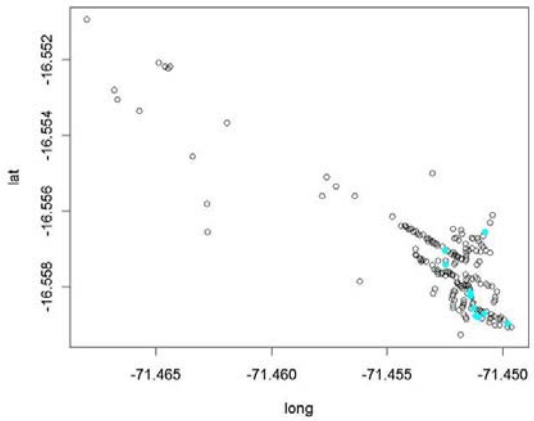
Table 4. Presence of insect vector and *T. cruzi* compare with age and sex.

		T. infestans status				*T. cruzi* positive *T. infestans*			
		Negative		Positive		Negative		Positive	
		N	N %	N	N %	N	N %	N	N %
Sex	F	145	32.6%	92	20.7%	214	48.1%	23	5.2%
	M	117	26.3%	91	20.4%	178	40.0%	30	6.7%
	Total	262	58.9%	183	41.1%	392	88.1%	53	11.9%
Current Age q 10 years (Banded)	<10	39	8.8%	25	5.6%	56	12.6%	8	1.8%
	10 - 19	54	12.1%	43	9.7%	84	18.9%	13	2.9%
	20 - 29	35	7.9%	34	7.6%	58	13.0%	11	2.5%
	30 - 39	45	10.1%	23	5.2%	60	13.5%	8	1.8%
	40 - 49	35	7.9%	24	5.4%	55	12.4%	4	.9%
	50 - 59	22	4.9%	7	1.6%	28	6.3%	1	.2%
	60 - 69	14	3.1%	13	2.9%	24	5.4%	3	.7%
	70 - 79	15	3.4%	8	1.8%	20	4.5%	3	.7%
	80+	3	.7%	6	1.3%	7	1.6%	2	.4%
	Total	262	58.9%	183	41.1%	392	88.1%	53	11.9%

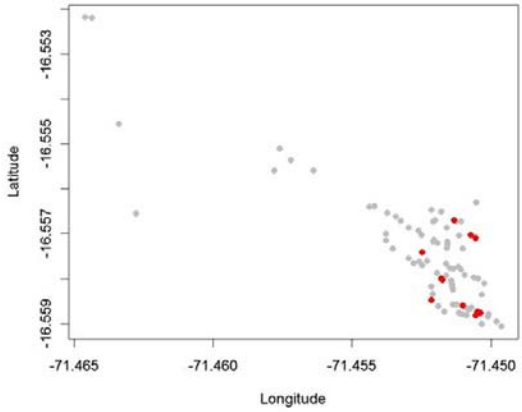
Figure 8 shows each household as a circle with its spatial distribution determined by a global position system (GPS). Three figures display the location of households with *T. infestans*, vectors positive for *T. cruzi*, or households with Chagas positive inhabitants. Of the 15 patients positive for Chagas disease, 9 lived in a house positive for triatomines (60%), of which 3 were positive for *T. cruzi* (33%). 2 of those 3 patients who inhabited a *T. cruzi* positive household shared the same house. Rough clustering of the insect vector, *T. cruzi* households, and Chagas patients exists as seen by the figure below.



(a) *T. infestans* positive households



(b) *T. cruzi* positive households



(c) Chagas positive inhabitants

Figure 8. GPS location of households in Quequeña, each circle represents a household. Blue circles represent triatomine positive households (a), teal circles represent households with *T. cruzi* infected triatomines (b), and red circles represent households with *T. cruzi* positive inhabitants (c).

15 (3.37%) patients were positive for Chagas disease by ELISA and confirmed by immunofluorescence, see Figure 9 for ELISA results with OD cutoff values. The TESA blot was also performed on all positives and a random subset of negative blood samples. In this community based survey the TESA blot had a sensitivity of 93% and a specificity of 100%, see Table 5. All negative results of the TESA blot matched with the ELISA and IIF results. All 14 positives by TESA were also positive by ELISA, with one ELISA positive not being detected by the TESA blot.

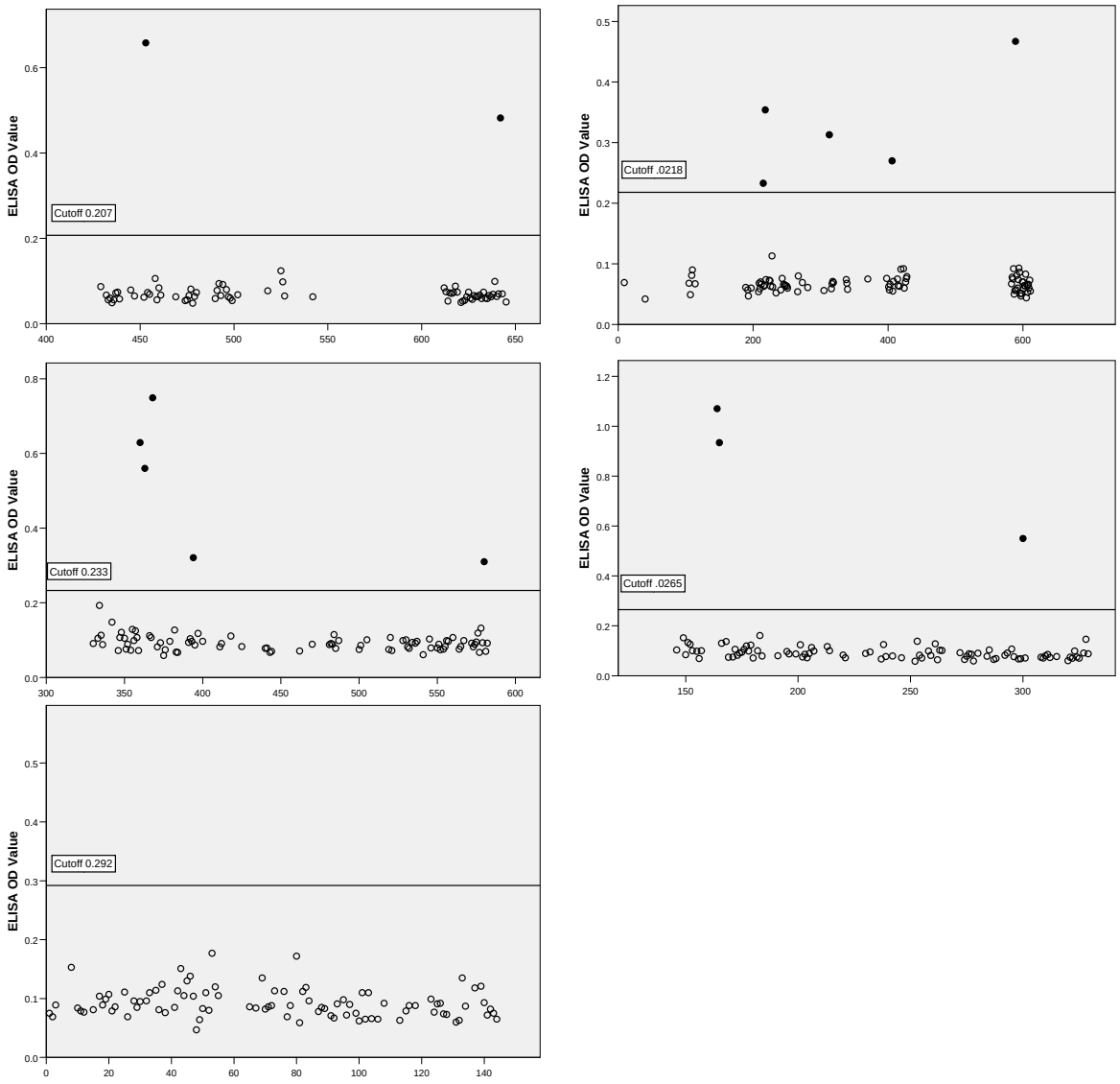


Figure 9. ELISA results and OD cutoff values. 15 total samples were above the cutoff value.

Table 5. Sensitivity and specificity of the TESA blot and immunofluorescence (IIF).

Assay	Positive	Negative	Total	Sensitivity	Specificity	PPV	NPV
ELISA	15	430	445				
IIF	15	19	34	100%	100%	100%	100%
TESA-Blot	14	20	34	93%	100%	100%	83%

Serostatus for Chagas disease compared with age and sex variables is shown in Table 6 and Figure 10 displays percent seropositive by decade of life.

Table 6. Serostatus compared with sex and age by decade.

		ELISA result					
		Negative		Positive		Total	
		N	N %	N	N %	N	N %
Sex	F	228	51.2%	9	2.0%	237	53.3%
	M	202	45.4%	6	1.3%	208	46.7%
	Total	430	96.6%	15	3.4%	445	100.0%
Current Age	<10	64	14.4%	0	.0%	64	14.4%
	10 - 19	97	21.8%	0	.0%	97	21.8%
	20 - 29	64	14.4%	5	1.1%	69	15.5%
	30 - 39	64	14.4%	4	.9%	68	15.3%
	40 - 49	55	12.4%	4	.9%	59	13.3%
	50 - 59	29	6.5%	0	.0%	29	6.5%
	60 - 69	27	6.1%	0	.0%	27	6.1%
	70 - 79	22	4.9%	1	.2%	23	5.2%
	80+	8	1.8%	1	.2%	9	2.0%
	Total	430	96.6%	**15**	**3.4%**	445	100.0%

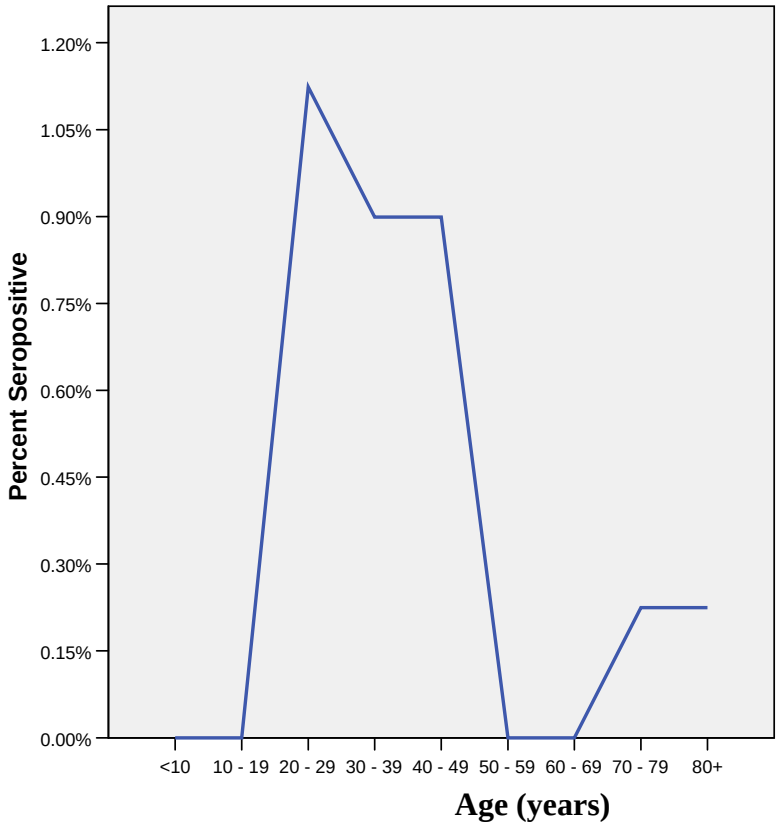


Figure 10. Percent seropositive for Chagas disease by age.

Electrocardiograms (EKGs) were performed on 37 people, 9 of whom were positive for Chagas disease and the other 28 were age and sex matched controls. All EKGs of Chagas positive patients were normal and 27 of 28 EKGs were normal in the control group, see Table 7. The lone abnormal EKG belongs to a 74 year old Chagas negative male who displayed occasional premature ventricular contractions.

5 Discussion

My study was a successful serosurvey for Chagas disease in a rural village of Peru in coordination with the regional Ministry of Health Chagas Control Program insecticide campaign. The local government has allocated sufficient funds to begin insecticide campaigns in many regions surrounding Arequipa, Peru but lacks the resources to conduct detailed epidemiological surveys or laboratory analysis of the inhabitants. It was important to conduct the serosurvey in coordination with the insecticide campaign to ensure that the vector transmission cycle was broken before determining the burden of disease. All data from my project was shared with Dr. Juan Cornejo, Director of the Chagas Control Program in Arequipa, Peru.

I found that the prevalence of Chagas disease in this rural village to be 3.4% of those sampled. This is consistent with previous estimates in other rural sites in Arequipa (personal communication with Cornejo, J.). Of note was an absence of infected persons in the age range of 2-20 years old and 50-70 years old. This is especially unusual because children in Quequeña recall being bitten by the insect vector and there is ample evidence of recent triatomine excreta on the walls and bedding of children indicating contact between the vector and children. 42% of children lived in houses where the insect vector was present and 13% where the vector was positive for *T.* cruzi. There have been no previous insecticide campaigns and speaking with people from the village only one family had independently fumigated their house. No major events occurred twenty years ago that would explain the negative findings in children.

Other rural studies in Brazil have shown an increase in prevalence beginning at birth, peaking around 20 years of age with a gradual decline due to excess mortality in middle

age (79). In rural Guatemala the same pattern was seen with the peak about 30 years of age (80). A recent study conducted in a peri-urban zone among children of Arequipa found a prevalence rate of 5.3% with clusters of infected children loosely clustered around *T.* cruzi positive vector infested homes (81). They also demonstrated that a child's risk of being seropositive increased by 20% per year of age and by 4% per vector found inside the home.

One possibility that would reduce the exposure time of teenagers is the lack of secondary education in the village of Quequeña, meaning all students must travel to Arequipa to complete their studies. Many live in Arequipa during the school week and spend the weekends at home with family. This would reduce their exposure to the vector but only during their teenage years and does not explain fully the lack of seropositive children.

Given the rustic nature of the housing in Quequeña it was not surprising to find that 24% of households harbored the insect vector and 7.85% were positive for *T. cruzi*. There exists a correlation between being infested with triatomines and basic measurements of household services. 38.2% of households positive for triatomines had electricity while 52.9% of households negative for triatomines had electricity. This was also true of potable water (40.1% vs. 49.3%), and sewer (0.0% vs. 19.1%). There was no correlation between type of floor. This same correlation held true for presence of *T. cruzi* and electricity (27.8% vs. 62.2%), and potable water (25.6% and 68.9%). Clusters of vector infested and *T. cruzi* positive homes were in the "older" part of town and can be seen on the GPS maps. This part of town contains homes that are in a more dilapidated state with an environment more suitable to triatomines.

There is a risk for reinfestation with *T. infestans* and this has been documented to occur in other rural sites. However, because of the isolated nature of Quequeña reinfestation is likely to occur much slower than in a peri-urban community. The community has established a vigilance campaign to have community members notify the health post if they find evidence of reinfestation. A study from Argentina found reinfestation of a rural community two years after spraying from a pig pen that was missed in the initial campaign(82). The vector was found in households 450 meters surrounding the putative site. *T. infestans* has been shown to be able to spread via walking up to 42 meters and spread beyond is thought to occur to occasional flight(83). If there was a reinfestation site like this in Quequeña it would put the majority of the community at risk depending on its location. However, a study in the Bolivian highlands (2,750 m asl) showed no evidence of genetic spread among isolated triatomine populations suggesting that at this altitude walking is the only method of dispersion(84).

I also showed that no patients with Chagas disease had any evidence of EKG abnormalities. EKG abnormalities, classically right bundle branch block, can be used to screen for disease progression and future arrhythmia treatment. Because only one third of Chagas patients go on to develop clinical disease my lack of positive cases makes it probable that it was due to pure chance. This lack of clinical presentation may also be due to a less virulent strain of the parasite or the host immune response in the community (85).

The diagnosis of Chagas disease was done according to established criteria as all positives were confirmed by a different assay. In my study all serum samples were

screened by ELISA and confirmed by indirect immunofluorescence (IIF). There was complete concordance between the two tests.

I was also the first person to test the TESA blot in a community setting. Previously it has been validated as a sensitive and specific test in serum banks but has never been employed in a community based setting (63, 64). In this study the TESA blot had a sensitivity of 93% and a specificity of 100% proving that it can be successfully employed in community surveys. The advantage of the TESA blot is that is can distinguish between an acute and chronic infection which enables the clinician to begin treatment without reservation in the acute patients. Results are available in a few hours with pre-made immunoblot strips. No expensive microscope or ELISA reader is required for diagnosis and it does not cross react with leishmaniasis(63, 64).

The disadvantage to the TESA blot is that it is unavailable commercially although similar systems exist. In order to produce the assay a laboratory must have active *T. cruzi* cultures and the ability/equipment to perform western blots. If this infrastructure exists it would be much cheaper to produce locally than to purchase commercial systems. Once the immunoblots are made they can be used with relatively few reagents in a more isolated environment. My experience is that reading of the strips is more qualitative than quantitative and can often lead to a questionable result that requires repeat or alternative testing.

In summary, my study was able to gather important clinical information from a rural site endemic for Chagas disease. This clinical information will help the local Chagas Control Program as it continues its vector control campaign by allowing them to more accurately predict disease burden in such settings. I was able to successfully employ the

TESA blot as a diagnostic test in a community based setting which will allow its expanded use as a Chagas diagnostic assay.

6 Appendix 1

6.1 Consent Form for Chagas' Serosurveys

Fleisch-Kincaid Reading Level: 7.9 (in MS Word)

Please ask our study staff to explain any words or information you do not understand. If you agree to be in the study, we will give you a copy of this consent form.

Introduction

Asociación Beneficia PRISMA, the Regional Office of the Peruvian Ministry of Health in Arequipa, Johns Hopkins University, USA, and the Centers for Disease Control and Prevention, USA, are doing a research study in Perú to learn more about an illness called Chagas' disease. This illness causes fevers, swelling in the face or eyes, weight loss and weakness. Many years later, it can cause heart disease, difficulty swallowing, and death. Chagas' disease is caused by a parasite carried by the chirimacha, a large insect. We are asking you/your child to take part in this study because there are chirimachas and people with Chagas' disease living in your district.

Purpose

We wish to learn more about why some people get Chagas' disease and others do not. This information will help to plan ways to stop the spread of Chagas' disease in the future.

Blood test.

For the above purpose, we will take about 1 teaspoon (about half a teaspoon for children under 5) of blood from your/your child's arm. We will do blood tests for Chagas' disease. We will give you the results of the Chagas' tests after 1 month.

Follow-up if Chagas' blood test is positive.

If the first Chagas' blood test is positive, we will perform a second test on the same blood that we collected to make sure that the result is right. The first test is very sensitive. That means it can sometimes be positive in people without Chagas' disease. The second test is stricter. If both blood tests are positive, we will give you a letter to go to the Ministry of Health clinic for treatment.

If both blood tests are positive:

1. We will visit you to give you the results and ask you to be another study. We will also ask some people with negative results to be in this second study. It is up to you whether you/your child will be in this study. .
2. For children younger than 15 years old with positive blood tests, we will write a letter for you to take your child to the nearest health post or district hospital to get adequate treatment. The drug to treat Chagas' disease is given for free by the Ministry of Health. For people older than 15 years old who are positive for infection, we will refer you to a doctor for a check-up to see if you have damage to your heart from Chagas disease. If necessary, we will help with the costs of transportation.
3. The treatment with antiparasitic medicine is not given to people older than 15 years.

Benefits.

1. This study will help your child to get antiparasitic treatment free of cost if we find your child has Chagas' disease. The treatment will be given by doctors under the supervision of the Ministry of Health. If the Ministry does not have medicine available, the project will buy the medicine (Nifurtimox or Benznidazole) so that the Ministry doctors can treat your child. For people older

than 15 years old who are positive for infection, we will refer you to a doctor for a check-up to see if you have damage to your heart from Chagas disease.
2. The results of the study will help us plan better programs to find, treat, and prevent Chagas' disease for your community and other communities in Perú.

Risks and discomfort.
You/your child will feel a small sting like a pin prick when we take the blood sample. You/your child may get a small bruise where the needle goes in but this will go away soon. Rarely taking blood may cause infection, but we will try to prevent this by using sterile needles and cleaning the skin before taking the blood sample.

Alternatives to this study.
You/your child can be tested for Chagas disease at the district hospital.

Being in the study is voluntary.
It is up to you whether you/your child will be in the study. You/your child may decide not to take part, or to quit the study at any time. This would not affect your/your child's care or any other benefits.

Compensation.
If you are hurt as a result of being in this study, the study staff will help you to receive treatment at the closest District Hospital. This treatment will be free of charge. Johns Hopkins University, the CDC, and A.B. PRISMA do not normally pay for harm done to you as a result of being in a research study. However, by signing this consent form and agreeing to be in this study, you are not giving up any of your rights.

Confidentiality.
Your name/your child's name will be kept private to the extent allowed by law. We will record your name and address on a list, so that we can tell you the test results. The forms that record study information will have a code number, which will be linked to your/your child's name. Only the health workers doing our study will have access to the list of names. The list will be destroyed once the study is done.

Questions.
If you have questions about the study, please call Dr. Juan Cornejo, Chagas' Disease Program, Arequipa Region, Ministry of Health (54 28 1536) or Dr. Robert Gilman (01 464-0221).

Human subjects contact. If you have questions about your rights as a research subject, or if you believe you have been harmed by being in the study, please contact the head of the ethics committee of one of the 2 local institutions working on the project: Dr. Ricardo López Ingunza, President of the Ethics Committee of the Instituto Nacional de Salud (telephone: 01-4719920, extension 175); and/or Dr. Salomón Zavala, President of the Ethics Committee of A.B. PRISMA (telephone 464-0490, extension 246). Both committees will be responsible to ensure your rights as a participant.

Name of participant (print)

_____

I have read this consent form or someone explained it to me. I freely agree to be in/(to have my child be in) the study.

_____ ___/___/___
Signature or fingerprint of participant *Date*
Required for participants ≥ 18

_____ ___/___/___
Signature or fingerprint of parent/guardian *Date*
Required for participants < 18

_____ ___/___/___
Signature of witness *Date*

STORAGE OF BLOOD SAMPLES

If you agree, we will store the sample after the study is over (in about 2 years). We will use the sample to try out future tests for Chagas' disease or other diseases to see if they work. We may also perform studies on the DNA in the blood clot. After our study ends, we will take your code number off the sample when we store it. After that there will be no way to connect you to the blood sample. This means we will not be able to report the results of future tests to you. We may also look in your sample for blood factors that make people more likely to get sick with Chagas' disease, but we will only do this after we take your code number off the sample. We will not do HIV testing on the sample. If you are willing to have us test your sample now, but you do not want us to store the sample, you can still take part in the study. We will record your wishes about whether we can store you/your child's sample on this form.

I give permission to store my/my child's blood sample.

_____ ___/___/___
Signature or fingerprint of participant *Date*

_____ ___/___/___
Signature of witness *Date*

6.2 Consent Form for Chagas' Case-Control Studies

Fleisch-Kincaid Reading Level: 8.0 (in MS Word)

Please ask our study staff to explain any words or information you do not understand. If you agree to be in the study, we will give you a copy of this consent form.

Introduction

Asociación Beneficia PRISMA, the Regional Office of the Peruvian Ministry of Health in Arequipa, Johns Hopkins University, USA, and the Centers for Disease Control and Prevention, USA, are doing a research study in Perú to learn more about an illness called Chagas' disease. This illness causes fevers, swelling in the face or eyes, weight loss and weakness. Many years later, it can cause heart disease, difficulty swallowing, and death. Chagas' disease is caused by a parasite carried by the chirimacha, a large insect. We are asking you/your child to take part in this study because there are chirimachas and people with Chagas' disease living in your district.

Purposes

1. We wish to learn more about why some people get Chagas' disease and others do not. This information will help to plan ways to stop the spread of Chagas' disease in the future.
2. We would like to learn how the disease affects the heart . We hope this information will help people to get better treatment in the future.

What we will ask you to do as part of this study

We are asking two groups of people to be in this study. First, we are asking all people whose blood test was positive for Chagas' disease. Second, we are asking some people with negative blood tests to take part in order to compare their results to those of people with a positive.test. If you agree, we will do the following:

1. We will ask you some questions about you and the members of your household, their health and past illnesses, and about your house, land, and animals. All of this will take from 30 minutes to one hour. You do not have to answer any questions you do not want to.
2. We will collect insects from inside and outside your house, and from your animals' housing. We will use a small machine like a radio to show the exact location of your house on a map. If we already did these things in the past, we ask your permission to use this information in this study.
3. We will arrange for you/your child to have a test called an electrocardiogram (EKG), at the local health post. For this test, the nurse will place several sticky pads on you/your child's chest, arms, and legs that are attached to wires to an EKG machine. This machine measures the electrical signals in the heart and can show certain problems of heart function. This will take about ten minutes.
4. We will arrange for you/your child to have a test called an echocardiogram, at the hospital in Arequipa. For this test, the doctor will use a small microphone to see your heart. This works like radar and does not hurt. It allows the doctor see the size and shape of your heart and how well it is working. This will take about 30 minutes. We will arrange for transportation to and from the hospital.

Benefits.

1. This study will help your child to get antiparasitic treatment free of cost if we find your child has Chagas' disease. The treatment will be given by doctors under the supervision of the Ministry of Health. If the Ministry does not have medicine available, the project will buy the medicine (Nifurtimox or Benznidazole) so that the Ministry doctors can treat your child.
2. For adults with infection, we will help you to be examined by a heart doctor who will

recommend whether you need any treatment.
3. The results of the study will help us plan better programs to find, treat, and prevent Chagas' disease for your community and other communities in Perú.

Risks and discomfort.
The heart tests may cause some minor inconvenience or discomfort, but will not be painful. They will not cause you any harm.

Alternatives to this study.
You may go to the district hospital for health care if you believe you have Chagas disease. Perú has national guidelines for treatment of Chagas' disease. The study will not change the treatment if you/your child is infected with Chagas' disease.

Being in the study is voluntary.
It is up to you whether you/your child will be in the study. You/your child may decide not to take part, or to quit the study at any time. This would not affect your/your child's care or any other benefits.

Compensation.
If you are hurt as a result of being in this study, the study staff will help you to receive treatment at the closest District Hospital. This treatment will be free of charge. Johns Hopkins University, the CDC, and A.B. PRISMA do not normally pay for harm done to you as a result of being in a research study. However, by signing this consent form and agreeing to be in this study, you are not giving up any of your rights.

Confidentiality.
Your name/your child's name will be kept private to the extent allowed by law. We will record your name and address on a list, so that we can tell you the test results. The forms that record study information will have a code number, which will be linked to your/your child's name. Only the health workers doing our study will have access to the list of names. The list will be destroyed once the study is done.

Questions.
If you have questions about the study, please call Dr. Juan Cornejo, Chagas' Disease Program, Arequipa Region, Ministry of Health (54 28 1536) or Dr. Robert Gilman (01 464-0221).

Human subjects contact. If you have questions about your rights as a research subject, or if you believe you have been harmed by being in the study,please contact the head of the ethics committee of one of the 2 local institutions working on the project: Dr. Ricardo López Ingunza, President of the Ethics Committee of the Instituto Nacional de Salud (telephone: 01-4719920, extension 175); and/or Dr. Salomón Zavala, President of the Ethics Committee of A.B. PRISMA (telephone 464-0490, extension 246). Both committees will be responsible to ensure your rights as a participant.

Name of participant (print)

_____

I have read this consent form or someone explained it to me. I freely agree to be in/(to have my child be in) the study.

_____ ___/___/___
Signature or fingerprint of participant *Date*
Required for participants ≥ 18

_____ ___/___/___
Signature or fingerprint of parent/guardian *Date*
Required for participants < 18

_____ ___/___/___
Signature of witness *Date*

6.3 Survey Assent Form for Children 7-15 Years Old

Fleisch-Kincaid Reading Level: 5.3 (MS Word)

We would like to learn more about an illness called Chagas' disease. It causes fevers, swelling in the face or eyes, weight loss, heart problems, difficulty swallowing and weakness. Also this study will help us plan better programs to find, treat and prevent the illness.

If you agree, we will do a blood test. We will take one teaspoon of blood from your arm to do a test for Chagas' disease. This means you will feel a small stick. If this test is positive, we will make sure you are not sick and we will send you to the doctor for medicine.

If the Chagas' disease blood tests are positive, we will send you to the doctor for evaluation and treatment with medicines. The purpose of these medicines is to get rid of the Chagas' infection.

Your parents have said it is all right for you to be in the study. You do not have to join this study if you do not want to. If you do not, it will not affect anything at home or in your health care. You can quit the study at any time. If you do decide to join, you will help us to help the people in this town protect themselves against Chagas' disease.

We will record your name and address, so that we can tell your parents/guardians the results of our tests. We will not give the results of your tests to anyone but you, your parent/guardian and your doctor. Please ask us if you have any questions. We are happy to answer them at any time.

Name of participant (print)

_____

I have read this consent form or someone explained it to me. I freely agree to be in the study.

_____ ___/___/___

Signature or fingerprint of child *Date*

_____ ___/___/___

Signature of witness *Date*

6.4 Case-Control Assent Form for Children 7-15 Years Old

Fleisch-Kincaid Reading Level: 5.3 (MS Word)

We would like to learn more about an illness called Chagas' disease. It causes fevers, swelling in the face or eyes, weight loss, heart problems, difficulty swallowing and weakness. Also this study will help us plan better programs to find, treat and prevent the illness.

We will ask all children with a positive Chagas test to take part in this study. We will also ask some children with negative Chagas tests to take part. This is so we can compare the results of the children with positive tests to the results of the children with negative tests. This helps us understand why some children get Chagas and others do not.

If you agree, we will do two tests to check how your heart works. The first test is the electrocardiogram. For this test, we will put small sticky pads on your chest to see how your heart is beating. The second test is the echocardiogram. For this test we will use a small microphone on your chest so the doctor can see how your heart looks. These tests will not hurt. We will also ask some questions about your health, where you sleep, and what animals your family has.

Your parents have said it is all right for you to be in the study. You do not have to join this study if you do not want to. If you do not, it will not affect anything at home or in your health care. You can quit the study at any time. If you do decide to join, you will help us to help the people in this town protect themselves against Chagas' disease.

We will record your name and address, so that we can tell your parents/guardians the results of our tests. We will not give the results of your tests to anyone but you, your parent/guardian and your doctor. Please ask us if you have any questions. We are happy to answer them at any time.

Name of participant (print)

_____

I have read this consent form or someone explained it to me. I freely agree to be in the study.

_____ ___/___/___

Signature or fingerprint of child *Date*

_____ ___/___/___

Signature of witness *Date*

7 Appendix 2 Satellite Imagery

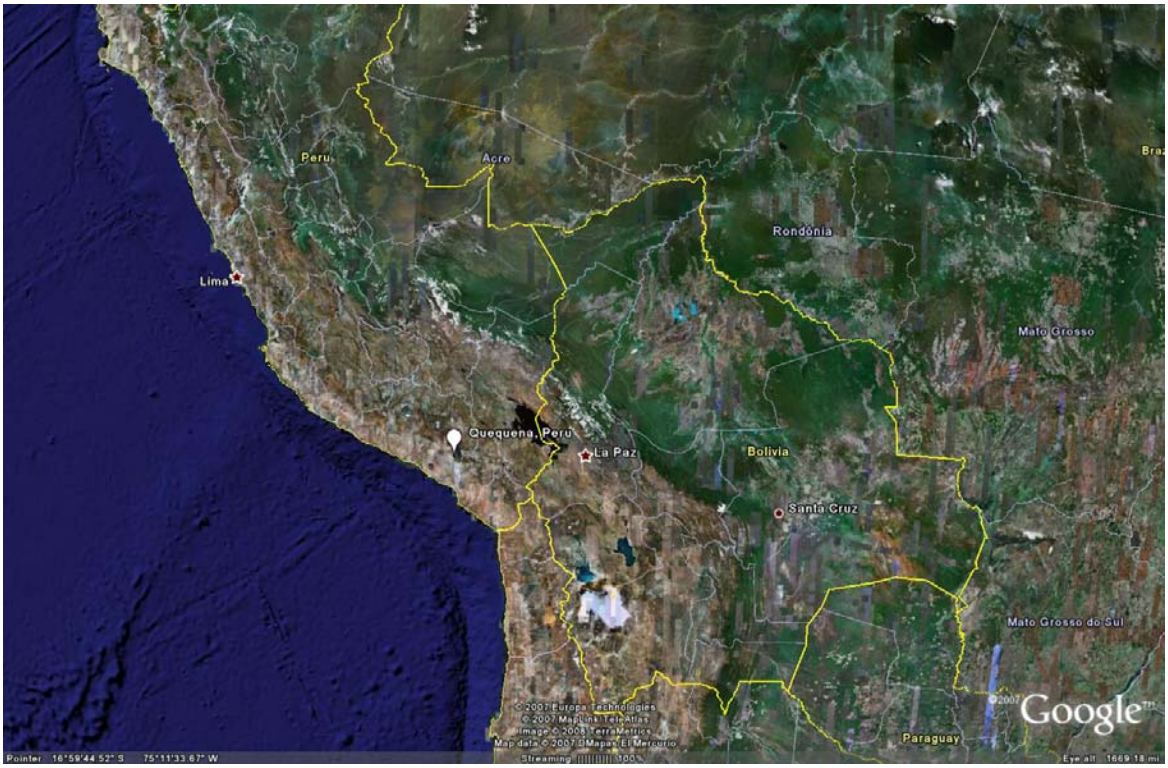


Figure 11. Satellite image of Peru showing Quequeña in the South of the country near Bolivia.

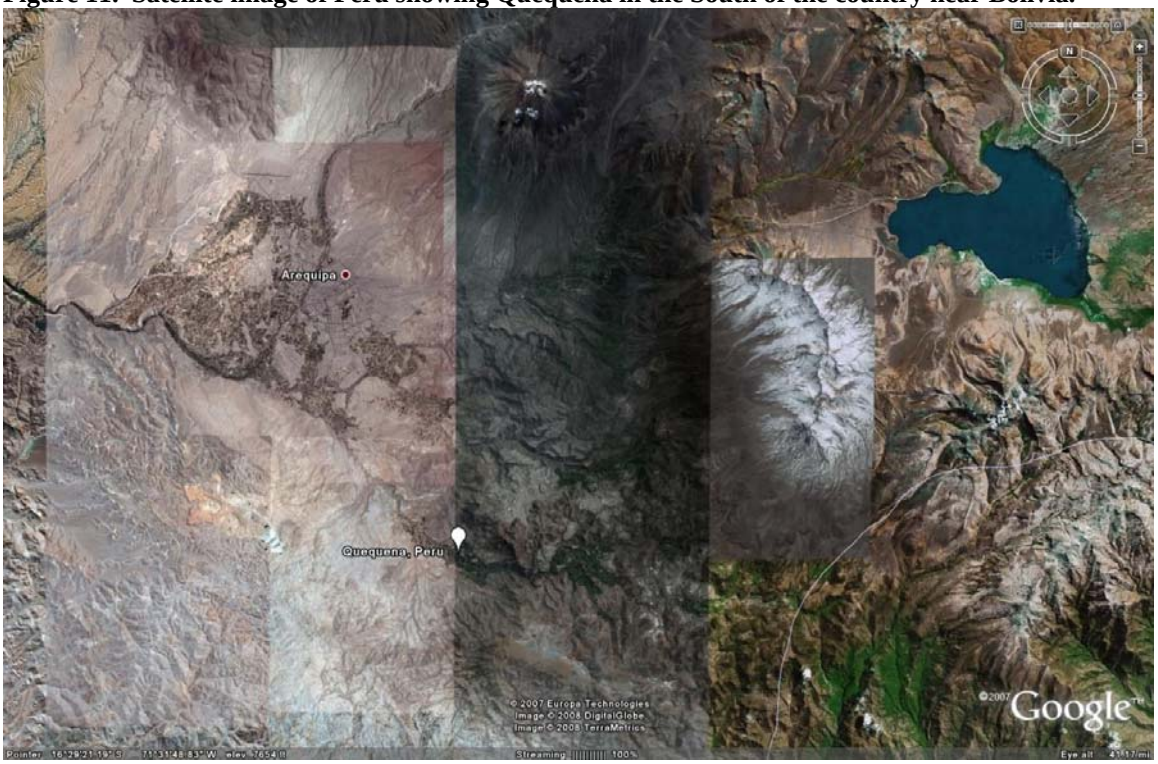


Figure 12. Satelitte image showing location of Quequeña relative to Arequipa. Arequipa lies at the base of an active volcano (snow capped peak, top).

Figure 13. Satellite image of Quequeña illustrating the agricultural community in isolation.

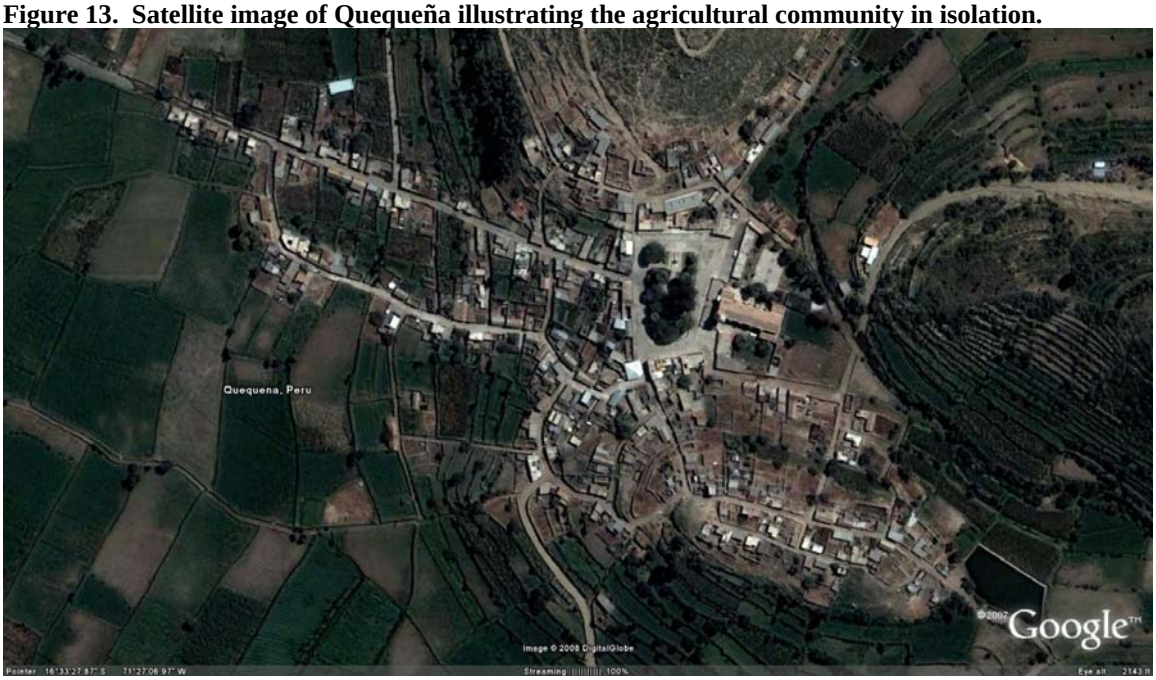


Figure 14. Close up satellite imagery of Quequeña with town square and church (center).

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