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Maternal infection and congenital transmission of *Trypanosoma cruzi* in Santa Cruz de la Sierra, Bolivia

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An abstract of a thesis submitted to

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Abstract

Maternal infection and congenital transmission of *Trypanosoma cruzi* in

Santa Cruz de la Sierra, Bolivia

By Jessica Tarleton

Introduction: The parasite *Trypanosoma cruzi* is the causative agent of Chagas disease, which is a major cause of morbidity and mortality in Latin American countries. Although aggressive vector control has reduced the spread of the disease, congenital (mother-to-child) transmission perpetuates the disease in populations. Bolivia has the highest prevalence of *T. cruzi* infection in the world, with an estimated 1500 new congenital infections each year.

Objectives: First, we sought to identify factors associated with risk of *T. cruzi* infection in pregnant women in Santa Cruz de la Sierra, Bolivia. Second, we examined demographic, epidemiologic, and biologic risk factors for congenital transmission from *T. cruzi*-infected women to their infants. Third, we examined the sensitivity, specificity, and feasibility of different approaches to maternal and infant diagnosis.

Methods: Women presenting for delivery at Hospital Japonés in Santa Cruz de la Sierra, Bolivia were asked to participate. Cord blood was taken, and characteristics of the delivery and neonatal exam were recorded. Diagnosis of mother and infant involved a combination of standard serologic tests, microhematocrit, and an experimental rapid diagnostic test. Infants of infected mothers followed up until 9 months to rule out congenital infection.

Results: Of 467 women, 97 (20.8%) were infected with *T. cruzi*, and 7 (7.2%) of these transmitted the parasite to their infants. Lower levels of education, older age (OR 1.070, 95% CI 1.036, 1.105), and years spent in an infested house (OR 1.021, 95% CI 1.002, 1.041) were associated with maternal infection. Premature rupture of membranes is marginally associated with congenital transmission (p=0.0725). The majority of infants were diagnosed by micromethod by 30 days of age, but only 25% of infants of infected mothers completed 9 months of follow up.

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INTRODUCTION

The global health community considers Chagas disease one of the "neglected tropical diseases" because it affects some of the world's most marginalized communities. The parasite *Trypanosoma cruzi* causes Chagas disease and is transmitted by insect vectors found in the Americas and has its biggest impact in Latin America. The parasite induces an acute infection that may be asymptomatic or characterized by non-specific symptoms, such as fever, lymphadenopathy and hepatosplenomegaly that usually goes unrecognized. In the absence of successful antitrypanosomal treatment, the individual continues in a state of chronic, life-long infection. Usually, people infected with *T. cruzi* experience no signs or symptoms for many years. However, around 20 to 30% of people infected with *T. cruzi* eventually develop potentially devastating disease. The infection may cause serious heart damage, resulting in conduction abnormalities, heart failure, and sudden death. Less commonly, the parasite causes denervation and consequent dilation of the esophagus or colon, resulting in serious digestive disease.[1]

Chagas disease is strongly associated with poverty, transmitted by an insect that thrives in poor housing conditions and perpetuated by lack of access to medical care. Unfortunately, it also *contributes to* poverty, and as a major cause of heart failure and other manifestations, is responsible for 426,000 disability-adjusted life-years lost (DALYs) per year worldwide.[2] Chagas disease has been called "the hidden affliction" for its ability to silently chronically infect its hosts while causing progressive damage to the heart, digestive system, and other organs.[3] Its silent nature also extends to the way it can pass from mother to child (congenital infection), in many instances without a woman's awareness that she herself carries the infection.

The WHO estimates more than 1.8 million women of reproductive age are living with Chagas disease in the world, resulting in 14,385 cases of congenital transmission a year.[4] With increased worldwide migration, congenital cases of Chagas disease have been detected in places like Switzerland, Spain, and the United States.[5-7] The Southern Cone Initiative and other vector control programs in the Americas have seen great success in reducing vector-mediated and transfusion-related new *T. cruzi* transmissions; however, congenital transmissions have not reduced proportionally, to the point that congenital transmissions are estimated to have eclipsed acute vector-borne transmissions in the formerly endemic nation of Argentina by ten-fold.[8] While mechanisms are in place to immediately interrupt vector borne and blood and tissue transplant-mediated disease, the establishment of comprehensive and sustainable prenatal screening remains elusive, and silent congenital transmissions from asymptomatic mothers to asymptomatic babies perpetuate the disease in endemic and non-endemic populations around the world.

Worldwide, a trend exists for the migration of rural populations to more urban areas for economic opportunity and increased access to resources. At the same time, diseases that are usually prevalent in more rural areas become urban problems, especially those conditions that are chronic, such as *T. cruzi* infection. The urbanization of Chagas disease results in a high prevalence of infection in areas in which active vector transmission is very low or does not exist at all. Therefore Chagas disease control priorities in urban areas must focus on (1) primary prevention of non-vector-borne *T. cruzi* transmission, i.e., congenital and blood product-related; and (2) secondary prevention, or ameliorating the impact of a parasite that has already been transmitted.

Currently, the optimal diagnosis of congenitally-transmitted *T. cruzi* involves several steps. First, the mother is tested for *T. cruzi* infection. Currently-available drug therapy is not recommended during pregancy due to lack of safety data. Second, the newborns of women with *T. cruzi* infection are examined for parasites immediately after delivery, while the family is in contact with the healthcare system. However, several studies have shown that parasite detection at birth misses a third or more of congenital infections.[9-11] Therefore, adequate diagnosis of congenital infection requires testing the infant several more times after birth. In resource poor areas, many infants are lost to follow-up before completion of the battery of diagnostic tests to rule out congenital transmission. It has been shown that the earlier in a person's life treatment is started, the more effective it is and the less adverse events are related to the drug treatment.[12]

Increased information along the steps of this process could help identify women and children at risk more easily and streamline the process of congenital diagnosis. Our study population, women presenting for delivery in a large public hospital in Santa Cruz de la Sierra, Bolivia (population 2 million), had a prevalence of *T. cruzi* infection of 29% in 2006-2007 data.[9] The first objective of this study is to identify demographic and epidemiologic features that help identify mothers who have *T. cruzi* infection in this urban area where the vector transmission is uncommon. These features are likely to be different in different regions of the world and even within Bolivia itself. The second objective is to explore whether demographic, epidemiologic, or obstetric features help predict which women will transmit *T. cruzi* infections to their infants.

The ideal process for diagnosis of all congenital infections is still unclear: how many follow-up diagnostic tests should be done, which ones, and at what time periods

they should be used? Many different diagnostic tests exist, with varying amounts of sensitivity and specificity at different times in an infant's first year, as well as varying degrees of reliance on operator expertise. Contributing to that information, a third objective of this study, may aid in the optimization of this process is an ongoing goal of our group and a goal of this specific study.

In summary, this study seeks to play a role in improving detection of congenitally-transmitted *T. cruzi* infection. Early detection in children leads to early and more successful treatment. As congenitally-transmitted infections become a higher proportion of all new infections, improving detection and treatment of these infections will play a larger role in preventing Chagas disease.

REVIEW OF THE LITERATURE

Prevalence of *Trypanosoma cruzi* infection in pregnant women in Bolivia

Bolivia has the highest estimated prevalence of *Trypanosoma cruzi* infection of any country in the world, at 6.7% overall.[4] Chagas disease accounts for an estimated 428 disability-adjusted life-years lost per 100,000 population in Bolivia—again, higher than any other country in the world.[2] In 2005, PAHO estimated that 229,000 women of reproductive age were living with Chagas disease in Bolivia, causing approximately 1,500 congenital infection a year.[4] However, in some departments, the population prevalence, and hence prevalence among pregnant women, has been found to be much higher. In recent studies from Bolivia, the prevalence in pregnant women ranged from 17.3% to 70.5% (see Table 1). The studies represented here do not cover areas of Bolivia in which a low prevalence of infection and vector density can be expected, such as La Paz province and the rest of the Andes and Altiplano in the west of the country (see Map). Torrico et al. found that the prevalence in women presenting for delivery in a hospital in Cochabamba significantly declined in the 5-7 years between his two samples.[13] The city of Santa Cruz de la Sierra is reported to be free of vector transmission, and the high seroprevalence is mainly attributed to immigration from the surrounding rural areas.

The area of Bolivia called the Gran Chaco has the greatest prevalence of *T. cruzi* infection and levels of vector infestation. For various reasons, including vector resistance to insecticides and failure to improve the commonness of substandard housing, efforts at eliminating *T. cruzi* transmission have been largely unsuccessful.[14] Many of the

populations that have been studied that have a high prevalence of *T. cruzi* infection, such as those found in Table 1, are either located in or migrated from the Gran Chaco. The Chaco encompasses southern part of the department of Santa Cruz (not including the city of Santa Cruz de la Sierra), eastern Tarija, and eastern Chuquisaca, as well as parts of northern Paraguay and Argentina (see map in Figure I).

Risk factors for maternal infection

Several studies have shown that older age is associated with maternal infection (see Table 2),[13, 15-17] although some have not found such an association.[10] Any association with older age is likely due to both longer exposure to vectors as well as decreasing prevalence over time as vector control measures become more successful. Azogue et al. found that in 910 pregnant women in Santa Cruz, coming from a rural area, living in a valley region, and lower levels of education were associated with maternal infection.[17] In addition to studies that address risk factors for infecton in pregnant women specifically, risk factors for infection are likely the same as risk factors for the population at large.

Rates of and risk factors for congenital transmission

The rate of congenital transmission to infants from women with *Trypanosoma cruzi* infection is usually reported between 5-10% (see Figure 3). Although some studies report a lower incidence of congenital transmission in mothers infected with *T. cruzi*, neonatal testing is generally more likely to under-detect than over-detect neonatal

infection, and is highly dependent on type and number of diagnostic modalities used, type and number of specimens tested, and the length of infant follow-up.

A number of risk factors for congenital transmission from infected mothers have been studied. Both Bittencourt and Torrico et al. found the transmission of the parasite to a baby was associated with younger maternal age.[13, 18] Bittencourt postulates that this was because parasitemia wanes with older age. Hermann et al. found that congenital transmission was associated with lower levels of IFN-γ production in *T. cruzi*-specific T-cells, which in turn is associated with younger age and lower parity.[19] Most authors, however, have found no association between age and congenital transmission.[20] Some of the same studies that find an association between younger age and congenital transmission also find an association between fewer previous pregnancies and transmission,[13, 19] but in Torrico, et al., when the association with primiparity is controlled for age, it becomes nonsignificant.[13]

Congenital transmission is more likely when maternal parasitemia is higher by a number of different measures. In two different studies, maternal parasites were detected more frequently by hemoculture in women who transmitted *T. cruzi* than in women who did not.[19, 21] Salas et al. found this to be true also when parasitemia was assessed by direct microscopy of the buffy coat.[20] Bern et al. found that the risk for congenital transmission was higher in women when parasites can be detected by qualitative PCR, and also transmission is also associated with higher levels of parasitemia by quantitative PCR.[9]

Risk for maternal-fetal transmission is also increased in conditions that are associated with increased levels of parasitemia, such as acute infection during pregnancy and *T. cruzi* infection in the context of HIV infection.[22] Of the eight cases reported in the literature of acute infection during pregnancy before 1995, five (63%) of these resulted in an infected baby, much higher than the average transmission found in chronically infected mothers.[18, 23] Salas found that of 4 women acutely infected during pregnancy, 2 transmitted the parasite to their babies.[20] HIV was a risk factor for congenital transmission in another study by Scapellato of 94 infants of mothers with Chagas disease, where 3 out of 3 women with both Chagas disease and HIV transmitted *T. cruzi* to their infants.[24]

Mothers in areas with higher levels of vector infestation are more likely to have positive hemoculture results, indicating higher levels of parasitemia.[25] Torrico et al. found that higher levels of vector infestation are associated with poor outcomes in congenitally-infected babies. [25] Infected babies born to infected mothers who lived in areas with high levels of vector infestation were more likely to have low Apgar scores, low birth weights, prematurity, respiratory distress, and mortality than infected babies whose mothers lived in areas with fewer vectors. However, vector infestation was not associated with higher levels of congenital transmission. Torrico et al. postulates that these findings may be related to repeated infection of the mothers before and/or during pregnancy. This theory is supported by evidence that cardiac pathology appears to be more severe in patients who live in continuously exposed areas compared with patients who live in non-endemic areas.[26]

Pregnancy produces a unique immunologic state driven by the female body's requirement to tolerate the foreign antigens of the fetus. The immunologic changes of pregnancy have been shown to change the maternal body's susceptibility to some infectious diseases in general and *Trypanosoma cruzi* in particular. Mothers who transmit *T. cruzi* produce less of the pro-inflammatory cytokines interferon (IFN)-γ and tumor necrosis factor (TNF)-α production in response to parasite antigens.[19, 21, 27] Hermann et al. also demonstrated that the immune cells of mothers of infected infants have lower levels of activation markers than those who do not transmit.[19] Congenital transmission is also associated with lower maternal production of the anti-inflammatory cytokine interleukin (IL)-10 after delivery.[19] Alonso-Vega et al. similarly showed decreased IFN-γ production in mothers who transmit, and also showed differences in production of IL-10 and IL-2.[21] Interestingly, maternal infection in the absence of congenital transmission is associated with changes in the fetal/infant immune system in a number of ways,[27-28] with one example being that uninfected infants of *T. cruzi*-infected mothers have Th1-polarized immune responses to vaccines compared to infants of uninfected mothers.[29]

Congenital transmission has also been shown to be associated with other factors surrounding the pregnancy and birth of the child. Congenital transmission is associated with lesser gestational age at birth.[15, 18, 30] Bittencourt et al. found in studies from the 1970s that the highest incidence of congenital transmission was found in babies born or miscarried between 22 and 26 weeks.[18] Related to this, congenital infection was found to be associated with low birth weight in some studies.[30] However, not all studies showed this association.[31-32]

Torrico et al. also showed that congenital infection is associated with premature rupture of membranes (PROM) during delivery.[13] PROM is defined as rupture of the membranes of the amniotic sac before labor, or the onset of contractions. In a full-term pregnancy (≥37 weeks), rupture of membranes before labor begins can be a normal consequence of physiologic weakening of the membranes in anticipation of labor. PROM occurs in about 1/3 of preterm births (<37 weeks of gestation), and risk of PPROM is increased with intrauterine inflammation, chorioamnionitis, and specific infections such as chlamydia.[33-35] In both term and preterm, PROM can carry increased risk of perinatal infection and *in utero* umbilical cord compression.[36]

Effects of *T. cruzi* infection on maternal and child health

Conflicting reports have been published about the significance of *Trypanosoma cruzi*, the causative agent of Chagas disease, in spontaneous abortion and stillbirths. In 1983, Hernandez-Matheson et al. found the incidence of spontaneous abortion in women with *Trypanosoma cruzi* infection to be about twice that of uninfected women. [37] While some studies have linked maternal Chagas infection with a history of previous spontaneous abortions,[13] others have found no such association.[16] Interestingly, while at least one study concluded that maternal *T. cruzi* infection is not a risk factor for adverse pregnancy outcome or poor neonatal health in the absence of congenital transmission,[13] in a mouse model of acute *T. cruzi* infection, fetal death has been observed even in the absence of congenital infection.[38] Recently, Brutus et al. found that pregnant women

with Chagas disease experienced significantly shorter periods of gestation in their pregnancies, that is, their babies were born earlier.[31]

Aside from the few studies that have shown that maternal infection in the absence of congenital transmission plays some role in pregnancy outcome, there little in the literature that suggests that maternal infection without transmission has an impact on the baby. Brutus et al. found that uninfected babies of *T. cruzi*-infected mothers had no difference in weight, length, or hemoglobin than babies of uninfected women.[31] Similarly, Torrico et al. observed no impact of maternal infection on uninfected babies in factors such as Apgar scores, gestational age, birth weight, birth length, and head circumference.[13]

The vast majority of neonates born with *T. cruzi* infection show no clinical signs or differences from uninfected babies at birth.[20, 39-40] The most common signs when signs are present are hepatomegaly and splenomegaly.[9, 11, 13, 16, 41-43] Torrico et al. found that infected infants showed statistically significantly lower Apgar scores, more prematurity, lower birth weight, shorter length, and smaller head circumference. Mortality from congenital Chagas disease as well as the incidence of prematurity, low birth weight, and low Apgar scores were significantly decreased in babies born in 1999-2001 compared to those born in 1992-94. In this study, they also demonstrated more respiratory distress, anasarca, and petichiae in neonates with *T. cruzi* infection. In the blood, infected infants showed lower leukocyte and neutrophil counts.[13] Myocarditis, megaesphagus, megacolon, microcephaly, meningoencephalitis, edema, and hemolytic anemia are other, more rare manifestations of congenital Chagas disease at birth.[18, 43] Pneumonitis with

respiratory distress has been reported and was thought to be a result of parasite circulation in the amniotic fluid.[44]

Diagnosis of congenital Chagas disease

The diagnosis of congenital Chagas disease infection is challenging, as newborns with the parasite are often asymptomatic, circulating parasitemia is not always apparent, and antibody detection cannot be done before 6 months of age because the presence of maternal antibodies in the newborn gives these tests a false positive. The WHO recommends that babies of infected mothers are tested with a conventional antibody detection test at 8 months of age.[45] Any of the traditional serologic tests can be used as they are in adults: enzyme-linked immunosorbent assay (ELISA), indirect hemagglutination (IHA), and indirect immunofluorescence (IFA).[45] Maternal IgG crosses the placenta, so detection of IgG at birth is only specific for maternal infection. By 8-9 months in an uninfected baby, *T. cruzi*-specific IgG should become undetectable as the maternal immunoglobulin is cleared from the neonate.

The Strout and microhematocrit methods use hemoconcentration followed by direct microscopy to detect parasites. These methods detect circulating parasitemia in the neonate and are the standard way that neonates in endemic areas are diagnosed at birth or within the first months of life. PCR can also detect parasites by amplifying target parasite DNA sequences found in human blood, but again, this method depends on circulating parasitemia. Finally, xenodiagnosis and hemoculture methods detect parasites by cultivating those found in patient blood either in the lab or in a vector. However,

these methods take a relatively long time (weeks or months), specific laboratory expertise, and in the case of xenodiagnosis, a live insect colony.

A variety of studies have been done to compare the sensitivity and specificity of these methods in detecting congenital infection. Performing micromethod on umbilical venous blood at birth is an easy, non-invasive way of attempting to ascertain the infection status of a newborn.[31] However, congenital diagnosis by this method is only 50% sensitive or less with one specimen and must be followed up with other tests in order to diagnose most infections,[41] and sensitivity may improve in the first few months of life, since parasitemia in infants peaks around 1-3 months of life after congenital transmission.[9, 46] Each method is contingent upon the time it is used (i.e., at birth, within 6 months, or after birth). PCR has been found to be one of the most sensitive methods of diagnosis. Altech et al. found it to be 80.3% sensitive and 97.8% specific overall, when compared with microhematocrit in the first 6 months and standard serologic tests from 6-12 months. However, the average age of diagnosis was 8.5 months.[39] Diez et al. demonstrated that PCR was at least if not more sensitive than microhematocrit in the first 9 months of life.[47] PCR may also be the best method of diagnosis in laboratories in non-endemic areas or those that that are otherwise not familiar with more *T. cruzi*-specific methods like microhematocrit and xenodiagnosis.[48] In the study by Bern et al., PCR detected congenital infection in most neonates before micromethod or serologic tests.[9]

However, PCR is certainly not sufficient. More commonly, health programs and studies use a combination of three or more diagnostics, both antibody and parasite detection, and over the first year of life of a child born to an infected mother. Mora et al. combined PCR, hemoculture, and the microhematocrit method on umbilical venous blood

at birth and found 90% sensitivity when compared with serologic tests later in the first year of life of the child, although in this population with active vector transmission, transmission to the infant by vector during the first year is possible. He found that of 27 cases diagnosed by a combination of the three, 8 were positive by microhematocrit, 18 by hemoculture, and 15 by PCR. Hemoculture was more sensitive than PCR, but PCR provides more immediate results. [11] A cohort in Buenos Aires demonstrated that a combination of micromethod in the first year with standard serologic tests from 6-12 months diagnosed 68.9% of infected babies before 5 months with the remaining third diagnosed between 6 and 12 months. Half the infected babies were diagnosed by microhematocrit in the first month.[10] A monitoring program in northwest Argentina found that 81% of infected neonates were diagnosed within the first month by the microhematocrit method, with the remaining diagnoses occurring by microhematocrit or standard serologic tests at 3 or 6 months.[16] However, this study suffered from high losses to follow up at 3 and 6 months, increasing the possibility infections that could have been detected at 3 or 6 months were missed.

Programmatic and treatment considerations

In many published studies in the resource-poor areas in which Chagas has its greatest impact, children are often lost to follow-up by 6 or 8 months, if not sooner. This amount of loss in later time points tends to make the proportion of infections diagnosed at birth and early in infancy artificially high. It also highlights a very important obstacle in translating these studies into congenital Chagas disease detection programs. In several

published studies of the success of monitoring programs for congenital Chagas disease, as many or more than half of children of *T. cruzi*-infected mothers are lost to follow-up before they have been adequately screened.[9-10, 16] Mora et al. recommend that one 12-month follow-up encounter would be the best congenital diagnosis strategy balancing cost and timing of diagnostic tests, but recognizes that "in the province of Salta [Argentina] and many other areas, a major difficulty for medical control of newborns and infants is the failure of mothers to respond to health center recalls for other than urgent matters…[which] is the major impediment for serological control of congenital Chagas disease.[11] Program evaluations in regions in which congenital Chagas detection programs exist would be helpful in identifying how important a role loss to follow-up plays in a programmatic setting. If any single conclusion can be drawn from the body of literature comparing diagnostics for congenital Chagas it is that no single method and no single time point is currently sufficient for diagnosing congenitally-transmitted Chagas disease.

Treatment of congenitally-acquired *T. cruzi* infection is recommended for all infected children. Benznidazole and nifurtimox, trypanocidal drugs that are used for adult infection as well, work well in pediatrics, and may even have greater efficacy and less adverse drug reactions in children than in adults. Early treatment of congenitally acquired *T. cruzi* infection is thought to prevent early consequences of infection, chronic Chagas disease later in life, adverse drug events, and decreased later drug efficacy.[45, 49]

METHODS

All women who presented for delivery during the day at the Hospital Japonés in Santa Cruz de la Sierra, Bolivia, were approached for enrollment. Vector-borne transmission in the city is reported to be rare or absent. However, the city has been growing at a rapid pace for the last few decades as migrants from the surrounding rural areas with very active vector-borne *T. cruzi* transmission. Our group revealed 29% prevalence in pregnant women presenting for delivery in 2006-2007.[9] Hospital Japonés is a large public hospital with around 3,000 deliveries a year, serving as a referral center for surgical deliveries from health centers throughout Santa Cruz. The study protocol was approved by the Ethics Board of the Hospital Japonés, Johns Hopkins University (Baltimore, MD), and the Centers for Disease Control and Prevention (Atlanta, GA).

Trained study nurses explained the protocol, and informed consent was obtained from volunteers. Study nurses were present during the day and captured all scheduled Cesarean sections as well as spontaneous vaginal deliveries. Because scheduled Cesarean sections occurred mostly during the day and spontaneous vaginal deliveries continued at approximately the same pace through the day and night, we oversampled women who delivered by Cesarean section. Those who agreed to participate gave demographic and epidemiological information to the study nurses according to a standardized questionnaire. The attending obstetrician and pediatrician recorded information about the birth and neonatal exam. The information was transcribed by the study nurses from the medical chart into the standardized study questionnaire.

Umbilical cord blood was obtained during delivery and tested with *Trypanosoma* Detect (InBios International) and Polychaco indirect hemagglutination (IHA) as rapid

diagnostic tests (RDTs) of maternal infection with *T. cruzi* by trained study laboratory technicians in the Hospital Japonés. Umbilical cord blood was also examined by the microhematocrit method to diagnose congenital transmission, as described previously.[2-3] If either RDT was positive, women and their infants continued in the study; if both were negative, their participation ended.

In women with positive results by at least one RDT and a 10% sample of women with negative RDT results, women were asked to provide peripheral venous blood for serologic tests. Maternal serum was tested with three conventional serologic tests in a central study laboratory in Lima, Peru: Chagatek enzyme-linked immunosorbent assays (ELISA) by bioMérieux Laboratories, Chagatest Recombinante ELISA by Wiener Laboratories, and kit by Lemos Laboratories. IHA dilutions of 1:16 were considered positive. Women who had positive results by two or more conventional serologic tests were considered to have confirmed *T. cruzi* infection. Infected women received electrocardiograms to diagnose Chagas cardiomyopathy, and were referred for treatment if cardiomyopathy was diagnosed.

Infants of women with any positive RDT were seen by a pediatrician at 30 days, 6 months, and 9 months. Blood samples were taken at each time point and examined by micromethod, IHA, and ELISA as shown in Table 4. If a child was positive by any one of these tests, the child was considered to have *T. cruzi* infection and was referred for treatment with benznidazole and close follow-up by the designated pediatrician of the Bolivian Chagas Disease Control Program.

Statistical analyses were performed in SAS software for Windows. Categorical variables were compared by the χ^2 test or the Fisher exact test as appropriate. Continuous variables were analyzed using the Kruskal-Wallis test. Demographic information, obstetric history, and neonatal outcomes at findings at delivery were analyzed for associations with maternal infection in a cross-sectional fashion. After delivery, neonates of infected mothers were followed as a cohort until infection was detected or until 9 months of age. In addition to demographic information, maternal test results were also analyzed as risk factors for congenital *T. cruzi* infection.

RESULTS

Characteristics of the population

Four hundred and seventy three (473) women were enrolled in the study. Four hundred and sixty seven (467) women had at least 2 serologic tests by which they could be classified as infected or uninfected, and the remaining 6 were excluded from analysis. Ninety-seven women out of 467, or 20.8% of women presenting for delivery, were seropositive by at least 2 conventional serologic tests (see Figure 2). Seven cases of neonatal infection were detected, representing 6.9% transmission, including all infants that were born into the study even if they did not complete follow-up. Infants were delivered by Cesarean section in 62% of cases. The type of delivery in terms of Cesarean section versus vaginal delivery was not associated with congenital transmission (see Table 5).

The age of the women presenting for delivery ranged from 13 to 46, with a mean of 25.6. Pregnant women with *T. cruzi* infection were significantly older than women without infection (see Figure 3 and Table 6). One hundred and eighty nine (189) women had lived in houses in which insect vectors for Chagas disease (the "vinchuca") were seen. Of those that had ever lived in such a dwelling, the mean number of years spent living there was 20.0 years. Women presenting with *T. cruzi* infection spent an average of 12 years of their lives in areas where the vector for Chagas disease is found, which is significantly greater than pregnant women without infection (see Table 6). Neither maternal age nor years spent in a dwelling in which vectors were found predicted which infected mothers would transmit Chagas disease. In a logistic model including age and

years in a house with insects with outcome maternal infection, age remains significantly associated with maternal infection ($p<0.0001$) and years in a house with insect also remains significantly associated with maternal infection ($p=0.0311$). Level of education was also associated with maternal infection, with infected mothers having significantly lower levels of education than uninfected mothers (see Figure 4 and Table 8).

Three quarters of all women presenting for delivery reported having been tested for Chagas disease previously. Eighty-three percent of infected women said that they knew they had Chagas disease before presenting for delivery. Only 3 women reported having been treated for Chagas disease in the past; these three women had negative serology. One quarter of all women said that they had a family member with Chagas disease (see Table 9). More infected women than uninfected women had a family member with Chagas disease ($p<0.0001$).

Women had a mean parity of 2.87, including the current pregnancy (see Table 10). *T. cruzi*-infected women had an average of one child more than *T. cruzi*-uninfected children, which was statistically significant ($p=0.0003$). This association was no longer significant when controlling for the influence of age ($p=0.1248$). No elements of the obstetric history were associated with transmission of *T. cruzi* from infected mothers to their babies.

Delivery and neonatal examination

Although gestational age at delivery as a continuous variable was not associated with maternal or infant infection (see Table 11), infected mothers of uninfected infants experienced less preterm delivery (i.e., delivery before 37 weeks of gestation) than

uninfected mothers (see Table 12). Preterm delivery was not associated with infant infection.

Seventy women (15.4% of all women) experienced premature rupture of membranes (PROM) and 17 of these (3.7% of all women) could be classified as preterm premature rupture of membranes (PPROM), i.e., PROM before 37 weeks of gestation. There was a trend toward more PROM in women who transmitted *T. cruzi* to their infants compared to infected mothers who had uninfected babies (Fisher's exact p=0.0526, see Table 13). PROM was not increased in infected women with uninfected infants compared to uninfected women. One infected mother and none of the mothers of infected infants experienced PPROM, and PPROM was not associated with maternal or infant infection (see Table 14). Infected women that transmitted Chagas disease to their neonates tended to have a longer time between rupture of membranes and delivery, but the difference was not statistically significant, and no association was seen when only vaginal deliveries were considered (see Table 10).

At one minute, uninfected babies born to infected mothers had significantly lower Apgar scores than uninfected babies born to uninfected mothers (p=0.0150), but this was not true at 5 or 10 minutes (see Table 15). Weight of the baby, weight of the placenta, and head circumference did not significantly differ between infected babies, uninfected babies born to infected mothers, and uninfected babies born to uninfected mothers. Uninfected babies born to infected mothers were significantly longer than uninfected babies born to uninfected mothers (p=0.0329). One *T. cruzi*-infected neonate was born with respiratory distress, and vesicular breath sounds, and nasal flaring were observed.

No hepatomegaly or splenomegaly were reported in any neonates. There were no significant differences between groups in terms of other neonatal physical findings.

Diagnostic tests

One hundred seven women were positive by one out of two rapid diagnostic tests performed on cord blood: 84 by both InBios and IHA, 3 by IHA only, and 20 by InBios only. Together, the two rapid diagnostic tests detected 100% of maternal infections in comparison to a subset of 33 RDT-negative samples that were confirmed by maternal peripheral venous blood IHA, ELISA, and IFA. Three RDT-positive women were later determined to be negative by confirmatory serologic tests for a specificity of 98.4% (see Table 16). The quantitative results of maternal serologic tests were not significantly associated with congenital *T. cruzi* transmission, although there is a trend toward increased absorbance in the Chagatek ELISA test ($p=0.0526$, see Table 17). There was also a trend toward increased IHA dilutions in cord blood and serum and congenital transmission, but these were not statistically significant ($p=0.1005$ and $p=0.1319$, respectively).

All neonates of RDT-positive women, as well as one for whom screen data is missing, went into the neonatal follow-up protocol, and a total of 7 (7.2%) were diagnosed with congenital Chagas disease. One of these was diagnosed at birth by micromethod visualization of parasites within the cord blood. The majority of congenital diagnoses were made at the 30 day time point (see Table 18). The infant diagnosed at 9 months had missed follow-up time points at 30 days and 6 months, making it possible that the infection could have been diagnosed at an earlier time point. A total of 81

infants, 73.6% of those born to RDT-positive mothers, were lost to follow-up by 9 months. If every infant had appeared for each follow-up and a proportional number were diagnosed, approximately 3 congenital infections were missed because of loss to follow-up, and congenital transmission could have been as high as 10%.

CONCLUSIONS AND RECOMMENDATIONS

The prevalence of Chagas disease in pregnant women continues to be high in this urban population without recent vector transmission. However, it is encouraging that the prevalence that has been reported in the literature has been declining, from 51% in 1985[30] to 20-30% in 2006-2010.[9]

The association between age and maternal infection has been demonstrated in many previous studies, and our data supports this association again.[13, 15-16] One might conclude that more years of age simply provide more possible time of exposure to the vector. However, in multivariate analysis, both age and years spent in an infested house were associated with maternal infection. An explanation of the independent associations of age and time spent in a house with vectors could be that older women were present in areas with vector transmission at a time when prevalence was higher and becoming infected was more likely, and could be an indication that public health measures are working. The large number of women who had lived in houses with vectors highlights that many women have lived outside of Santa Cruz for long periods of time, likely having been born in rural areas and moving to the city later in life, and that as a consequence large populations of *T. cruzi*-infected women may be living in urban centers. Urban areas are not exempt from the consequences of Chagas disease by virtue of lacking vector transmission, and in fact, they may carry much of the burden. This suggests that urban areas that support populations of migrants from rural areas should be focal points for congenital *T. cruzi* interventions.

We also found that education is associated with maternal infection with *T. cruzi*. It is likely that education is a proxy for poverty, both current as well as past: since women are more likely to have been infected before they came to the city of Santa Cruz, the economic situation of the family may be more important than that which the women currently experience. However, education affects how people view their health and interact with the healthcare system, and it would be useful to know if maternal education and knowledge about Chagas disease plays a role in maternal participation in congenital Chagas disease screening programs. A survey of maternal knowledge about Chagas disease could also help target educational materials during prenatal care and delivery.

It is impressive that 83% of women presenting for delivery who were infected with *T. cruzi* knew already that they were infected. Hopefully, this is a sign that the integration of maternal screening during prenatal care is working. Screening for Chagas disease is supposed to be a normal part of prenatal care in the department of Santa Cruz, and prenatal visits are generally very well attended because of financial incentives provided by the national government. In the province of Santa Cruz, 97.7% of women received prenatal care and 84.3% attended four or more prenatal care visits according to the 2008 Demographic and Health Survey.[50] These prenatal visits could provide a time not only to screen mothers for *T. cruzi* infection but to educate those mothers who are infected about the importance of strict adherence to diagnosis and treatment plans for their infants.

We also found that a large proportion of women had a family member with Chagas disease, and that having a family member with Chagas disease is associated with the maternal infection. From this study, we might characterize pregnant women with

Chagas disease in this population as being older, originating from a place other than the city of Santa Cruz de la Sierra and from a place where vectors inhabit, having a family history of Chagas disease, and also being aware of their own infection. None of the demographic or epidemiologic factors that we examined were associated with transmission of *T. cruzi* from infected mother to baby, including age, which has been shown in some studies to be associated with congenital transmission.[13, 18]

Our study population had a 62% rate of Cesarean section, but this is unlikely to be a representative sample of Santa Cruz or even the Hospital Japonés. As previously mentioned, because of study nurses' presence during the day and the predominance of Cesarean births during the day, we oversampled Cesarean deliveries. Notably, however, the department of Santa Cruz has a very high rate of Cesarean section deliveries at 36.1%. In comparison, the department of La Paz delivers 13.4% of infants by Cesarean section, and Bolivia as a whole delivered 18.6% of babies by Cesarean section in the same time period.[50]

The incidence of congenital transmission in our study was 6.9% of babies born to *T. cruzi*-infected mothers. This is comparable to the last study in this population, in which 6.5% of babies born to infected mothers were infected. However, assuming the infants lost to follow-up are the same as those who appeared for their follow-ups and that a proportional number were infected, we may have missed around 3 infant infections, and the congenital transmission rate could have been as high or higher than 10%

Comparing different rates of congenital transmission across studies is difficult because there is no standard way of diagnosing and representing congenital transmission.

That is, differences between rates of transmission in studies are more likely due to differences in study protocols than to biologic factors like parasite strain or maternal health. Several studies have shown that with currently available diagnostics, testing only at birth may miss many congenitally-transmitted *T. cruzi* infections, so studies that test only once or even twice could publish rates of congenital transmission that are artificially low.[9-10] Detection of parasitemia is likely to be most sensitive from 1-3 months after congenital transmission,[9, 46] and serologic tests may be more sensitive than microhematocrit after 6 months, so rates of congenital transmission are more likely to reflect a given protocol's ability to detect congenital infection than a true measure of how often *T. cruzi* is transmitted from mother to child. One must also be careful to understand, if sufficient information is given, the way rates of congenital transmission are calculated. Some studies, like Muñoz et al., calculate rates of transmission by only including all children that completed the full period of follow-up. Using different denominators in different papers can vastly affect the appearance of rates of transmission: if our study had calculated transmission considering only infants that had followed up to 9 months, we could have reported our congenital transmission rate as 24%.

This study once again demonstrates that obstetric history, including fertility, is not associated with either maternal infection or incidence of congenital transmission. Our data also suggest that Cesarean section does not appear to alter the risk of *T. cruzi* transmission from mother to baby. This is consistent with the idea that congenital infection usually occurs transplacentally during gestation rather than during delivery, as is the case for infections such as HIV.

Our data suggesting an association between premature rupture of membranes and congenital transmission support the observations of the same thing in Torrico et al.[13] Our data also suggest that congenital transmission is associated with a longer time between membrane rupture and delivery in both Cesarean section and vaginal deliveries ($p=0.0725$), but not when only vaginal deliveries are considered. These observations could suggest that intrauterine infection may provoke PROM, or that transmission of *T. cruzi* is increased during prolonged rupture of membranes. If transmission of *T. cruzi* is increased during rupture of membranes, one may observe that Cesarean section is protective against congenital transmission; with almost half of the infected babies having been born by Cesarean section, we did not observe this in our study. With only 7 infected infants, the power to draw any conclusions of this kind is difficult, so an increased sample size or better follow-up, assuming this would improve detection, could help affirm or refute these observations. Examining the association in only vaginal deliveries eliminates from consideration Cesarean sections, in which in many cases membrane rupture occurred intraoperatively only seconds before delivery and for which the definition of PROM is irrelevant. However, it also eliminates from consideration those deliveries in which PROM may have occurred and then Cesarean section came subsequently because of complications, and these may in fact be the deliveries that are at even higher risk of congenital transmission. Future studies could improve upon the definition of this variable by collecting information on the indication for Cesarean section so that these situations could be considered separately.

Interestingly, maternal infection without transmission is associated with more full term deliveries than in uninfected women, indicating that maternal infection is a

protective factor in preterm delivery. If anything, one might guess that maternal infection might trigger preterm delivery, as occurs in some other maternal infections.[33] Our survey did not address any other predictors of preterm delivery that could be confounding this paradoxical observation such as smoking, short time interval between pregnancies, low BMI, the presence of other infections (in the reproductive tract or elsewhere), or general maternal health,[51] that would be less likely to be present in women infected with *T. cruzi*. In short, the association between preterm birth and *T. cruzi*-uninfected mothers is difficult to explain.

We found a statistical difference in Apgar scores at one minute between uninfected babies of infected mothers and uninfected babies of uninfected mothers, which may indicate that maternal infection can negatively impact uninfected babies. However, for several reasons, it is unlikely that this finding has any significance. First, the difference between Apgar scores of uninfected babies from infected versus uninfected mothers is only 0.26, which has little medical significance. Second, at five and ten minutes, no statistical difference existed between these groups, so if lower Apgar scores at 1 minute did indicate a difference in neonatal well-being, the difference attenuated over the first 10 minutes. Third, Apgar scores are generally meant to indicate a need for neonatal resuscitation and not to predict outcomes.[52] Although Apgar scores may predict mortality in the neonatal period, in the absence of mortality, they are not associated with long term neurologic outcomes.[53] In summary, it is not clear what, if any, significance the finding of a difference in Apgar scores in uninfected babies of infected compared to uninfected mothers has in this population.

Likewise, it is unclear that a statistical difference of just over half a centimeter in length of babies of uninfected mothers versus uninfected babies of infected mothers has any real meaning, especially in the absence of a difference in birth weight or head circumference. With only 7 infected neonates, detection of any difference in clinical findings between infected and uninfected neonates is hard to ascertain. We detected no hepatomegaly or splenomegaly at birth, the most common manifestations of congenital *T. cruzi* infection. One infant diagnosed with *T. cruzi* infection at 9 months had respiratory distress at birth, and another was found to have low birth weight. Without more information, we cannot say that these manifestations were a consequence of infection of mothers and babies. However, studies in the future may want to pay attention to the potential differences in neonatal health of babies born to infected mothers in the absence of congenital transmission.

In terms of diagnostics for maternal infection, the combination of InBios rapid serologic test and the IHA showed excellent sensitivity and specificity for diagnosing maternal infection. Improvement on rapid tests could improve detection of mothers at risk for transmitting *T. cruzi*, especially in the context of systems that have low to no attendance in prenatal care, where results could be immediately available. Interestingly, there is a trend towards greater absorbance values in the diagnostic Chagatek ELISA kit in women who transmitted *T. cruzi* compared to infected women who do not ($p=0.0526$), although such a difference does not exist in the Wiener ELISA kit or any other quantitative diagnostic test. The implications of this finding, if real, are that women who transmit *T. cruzi* have higher levels of antibodies to epimastigote lysate, against which the test was designed, and that the Chagatek ELISA kit has the ability to discriminate

between these levels of antibody. Future studies should attempt to affirm these findings and determine the significance of antibody levels, both general and specific, against *T. cruzi* in congenitally-transmitted infections.

In terms of neonatal diagnosis, 4 out of 7 infants were diagnosed at 30 days by micromethod. This is consistent with the data that shows parasitemia is highest between 1-3 months in infants that acquire *T. cruzi* via congenital transmission.[9, 46] Two out of seven infants were diagnosed at 6 months or after by standard serologic tests. If more infants had come to 6 and 9 month visits, more may have been diagnosed by serologic tests as well. As other studies that have used multiple methods and multiple time points to diagnose congenital infection have shown, no single method or time point is sufficient to diagnose congenitally-transmitted *T. cruzi*. When conventional diagnostic methods are used, our data still support the use of micromethod at one or more time points before 6 months and at least one serologic test after 6 months to reliably diagnose infection. The last study in this population examined infants of infected mothers at birth and then 7, 21, 30, 90, 180, and 270 days and found a rate of congenital transmission of 6.5%.[9] With the assumption that the population did not change significantly in the two years between studies, reducing the number of follow-up visits did not negatively impact detection of congenital infection. However, follow-up of infants in the previous study was 58%, where in the current study only 25% completed follow-up, which could influence the probability of detection, and could indicate that while more intensive follow-up may not be required for optimal detection of congenital infection, it may improve adherence of mothers to the diagnostic and treatment plans of their infants.

Several studies used only micromethod at one or two time points to diagnose congenital infection, but this is likely to miss a third to a half of congenital infections.[20, 31] The comprehensive evaluation of the timing and type of diagnostic methods used to detect congenital infection could be improved by achieving much greater rates of follow-up as well as continuing to follow infants who have already been diagnosed. While it would be convenient to continue test already-diagnosed infants to study the natural course of congenital infection for one year, it is unethical not to treat these infants at the time they are diagnosed.

As has been mentioned extensively throughout this paper, loss to follow-up in our cohort as well as others' cannot be underestimated as a limitation to research of this kind. Although a research setting such as ours probably differs from a public health intervention, if loss to follow-up is also a problem for public health programs as well, it greatly limits the impact the program might have. Our setting-- in a central, public, tertiary care center—is much different from what might occur when a family encounters their primary care physician in a community health center. In our study, women may have been reluctant to bring their children to follow-up appointments if no other services are provided, the hospital is far away from their home, and their child is not sick. If testing of infants of infected mothers could be combined with regularly-attended pediatric visits in community health centers, this could improve follow-up. The vaccine coverage overall for children in Bolivia is 78.6% and 83.2% in the department of Santa Cruz,[50] so the majority of infants do encounter the health care system during infancy. If testing for *T. cruzi* is also offered at this time to infants at risk, the coverage of congenital *T. cruzi* diagnosis is likely to be much better. In a research context, the setting of a community

health center where families seek regular care might improve follow-up and thus the strength of conclusions that can be drawn.

This study has some important limitations, many of which have already been mentioned. Regardless of the trend, the prevalence of maternal *T. cruzi* infection in this study may not accurately represent that of Santa Cruz de la Sierra as a whole, since it is an obstetric referral center that handles sicker patients with complicated deliveries. As a public hospital, it tends to take poorer patients, who may have a higher prevalence of Chagas disease than the population of Santa Cruz as a whole; on the other hand, the hospital serves as a primary care center for many residents of the center of the city, while immigrants from rural areas with higher vector transmission may tend to settle and seek care on the periphery of the city. Also, with only 7 infected infants, we did not have enough statistical power to make many conclusions about risk factors for congenital transmission. Again, the high loss to follow-up rate prevents us from drawing many conclusions about the optimal timing of diagnostics during infancy.

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TABLES AND FIGURES

Figure 1: Map of the departments of Bolivia

Table 1. Prevalence of *Trypanosoma cruzi* infection in pregnant women in Bolivia in the literature, 1992-2007

Lead author (Year of publication)	Year(s) of study	Prevalence in Pregnant Women, % (n)	Department
Torrico (2004)	1992-94	27.6 (1606)	Cochabamba
"	1999-2001	17.3[a] (3879)	Cochabamba
Salas (2007)	2003-4	42.2 (2712)	Tarija
Brutus (2010)	2004-5	40.9 (359)	Tarija
Chippaux (2009)	2004-7	70.5 (459)	Tarija
"	2007	23.5 (1030)	Santa Cruz
Bern (2009)	2006-7	29 (530)	Santa Cruz

[a]The difference between prevalence in 1992-94 versus 1991-2001 was statistically significant in the original publication ($p<0.001$).[13]

Table 2. Factors associated with maternal *T. cruzi* infection in the literature

Lead author (Year of publication)	Year(s) of study	Country	Factors associated with maternal infection
Azogue (1993)	1998-99	Bolivia (Santa Cruz)	Living in valley region (vs. plains or altiplano); originating from rural areas (vs. urban); level of education
Blanco (2000)	1992-94	Argentina	Older maternal age
Torrico (2004 and 2006)	1992-94; 1999-2001	Bolivia (Cochabamba)	Older maternal age; greater parity[a]; history of spontaneous abortions[a]; level of triatomine infestation
Brutus (2008)	2002-04	Bolivia	Older maternal age
Muñoz (2009)	2005-07	Latin American women in Spain	Coming from Bolivia; history of living in a mud house; history of living in a rural area

[a]Not significant after controlling for age.

Table 3: Rates of and factors associated with congenital transmission in the literature

Lead author (Year of publication)	Location	Rate of congenital transmission	Length of follow-up after birth	Diagnostic methods	Factors associated with congenital transmission
Azogue (1985)	Bolivia (Santa Cruz)	11.2%[b]	None-birth only	Strout method	None identified
Azogue (1991)	Bolivia (Santa Cruz)	18.5%[c]	2 months	Strout method, xenodiagnosis, placental pathology[d]	Female sex of the infant
Nisida (1999)	Brazil	5.2%[f]	None, birth only	Microhematocrit, QBC, artificial xenodiagnosis	Maternal HIV infection
Blanco (2000)	Argentina	7.1%	1 year	Microhematocrit, IHA, ELISA	None identified
Torrico (2004 and 2006)	Bolivia (Cocha-bamba)	4.9% (1992-94) 5.9% (1999-2001)	1 month	Microhematocrit, hemoculture	Younger maternal age; fewer previous pregnancies; PROM
Mora (2005)	Argentina	9.6%	11.3±2.1 (SD) months	Microhematocrit, IHA, ELISA	Not reported
Salas (2007)	Bolivia (Tarija)	6%	1 month	Microhematocrit	n/a[a]
Brutus (2008)	Bolivia (Tarija)	5.2%	None, birth only	Microhematocrit	None identified
Bern (2009)	Bolivia (Santa Cruz)	6.5%	9 months	Microhematocrit, PCR	Positive maternal PCR, level of parasitemia by qPCR
Muñoz (2009)	Latin American immigrants in Spain	7.3%[e]	8 months	ELISA, PCR	None identified

De Rissio (2010)	Argentina	6.1%	1 year	Microhematocrit, IHA, IFA, ELISA	None identified
Brutus (2010)	Bolivia (Tarija)	5.8%	None, birth only	Microhematocrit	Level of parasitemia ("parasite density") of mother
Scapellato (2009)	Argentina	13.8%	6 months	Buffy coat microscopy, IHA, IFA, ELISA	HIV

QBC= quantitative buffy coat, qPCR= quantitative PCR, PROM= premature rupture of membranes during delivery

[a]Salas et al. only examined associations between *T. cruzi* infected and uninfected babies, and not infected babies versus uninfected babies of infected mothers, which would identify what factors are different between infected women who transmit *T. cruzi* and those who do not.

[b]In the original publication, the identification of parasites in placentas were considered a positive diagnosis of congenital transmission of *T. cruzi*. This has been shown to be a very non-specific test, as parasites may be found in placentas of infants later found to have no sign of *T. cruzi* infection.(Ref)

[c]This study was conducted with infants weighing ≤2500 g only; therefore, this does not represent the probability of transmission in all infants.

[d]Although placental pathology does not provide a reliable diagnosis of congenital Chagas disease, all infants in which parasites were found in the placenta were eventually confirmed positive by the other diagnostic methods.

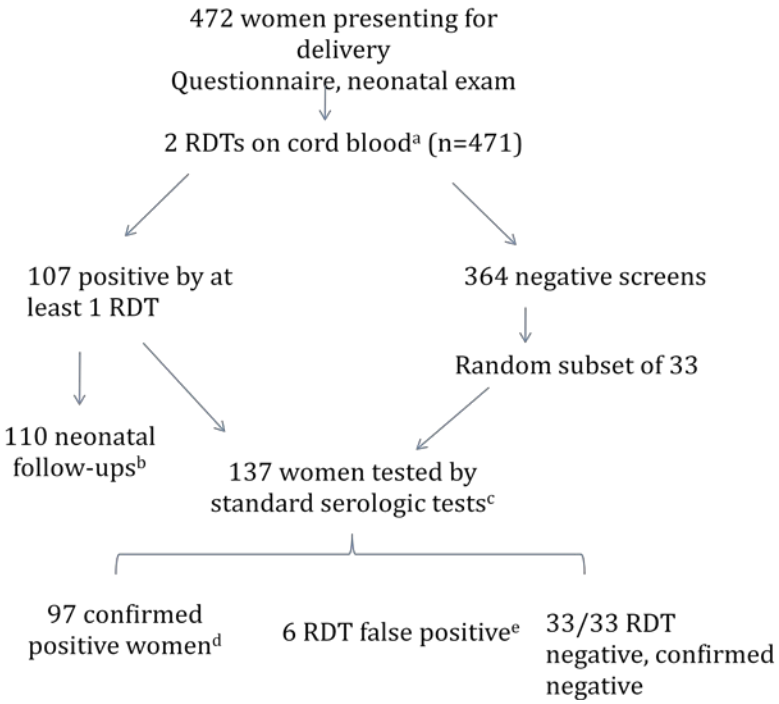
[e]Only includes infants that completed 8 months of follow-up.

[f]Selection criteria of these women is not clearly stated, but 53.6% of mothers had symptomatic Chagas disease (48.8% cardiomyopathy and 8.8% digestive disease), so this maternal population is not representative of all pregnant women from Sao Paolo.

Table 4. Diagnostic tests performed during infant follow-up

Infant follow-up time point	Micro-method	IHA	ELISA
30 days	X		
6 months	X	X	
9 months	X	X	X

Figure 2: Schematic of maternal diagnostic testing

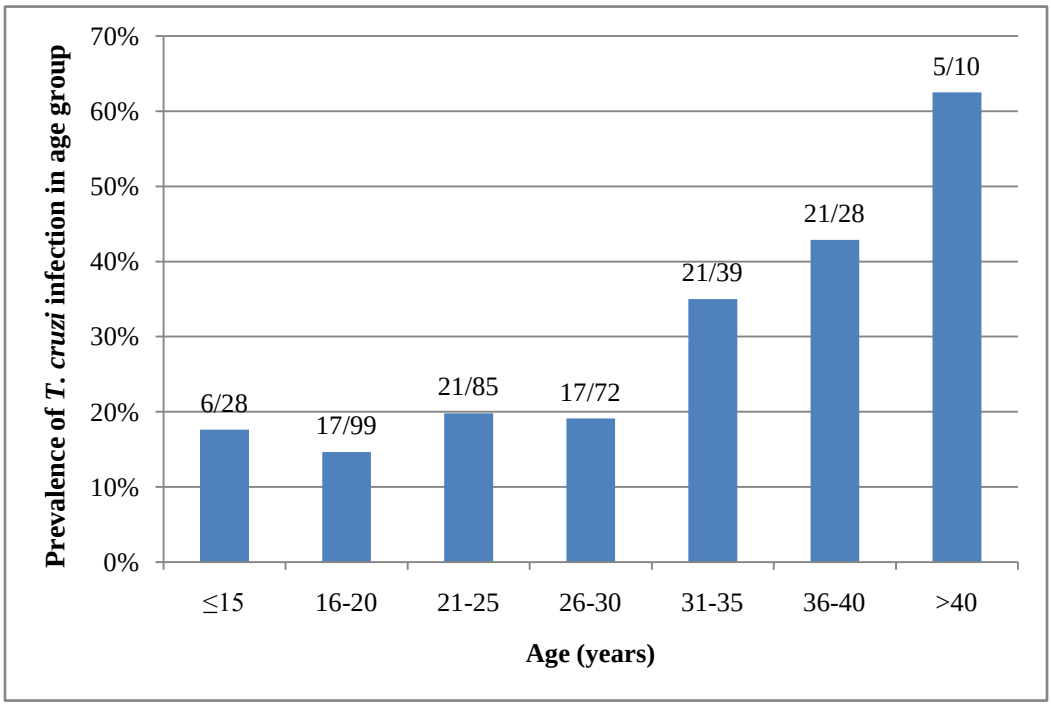


NOTE: [a]RDT=rapid diagnostic test. RDTs here are InBios rapid test and IHA on cord blood. [b]Includes infants of one woman missing both RDTs and 2 sets of twins of RDT-positive mothers. [c]Includes one woman missing both RDTs and excludes 4 women who had <2 serologic tests. [d]"Confirmed positive" is defined as positive by 2 or more standard serologic tests including IHA, IFA, or ELISA on maternal serum. [e]"False positive" is defined as positive by at least one RDT and negative by 2 or more standard serologic tests including IHA, IFA, and ELISA.

Table 5. Type of delivery is not associated with congenital transmission. (Fisher's exact p=0.2946)

	Uninfected mothers (% of row)	**Infected mothers with uninfected babies (% of row)**	**Infected mothers with infected babies (% of row)**	**Total**
Vaginal birth	133 (76.4)	37 (21.3)	4 (2.3)	174
Cesarean section	231 (81.6)	49 (17.3)	3 (1.1)	283
Total	364	86	7	457

Figure 3: Prevalence of *T. cruzi* infection increases with age.



NOTE: Numbers above each bar represent the number of *T. cruzi*-infected women over the total number of women in each category. Total sample size=462.

Table 6. Maternal age and years spent in a house with insect vectors are associated with maternal infection.

	Uninfected mothers	**Infected mothers**	**OR[b] (95% CI)**
Mean maternal age (median) (n=449) Range: 11-46 Mean: 25.8 Median: 24.4	24.8 (23.6) (n=354)	29.4 (29.0)[a] (n=95)	1.070 (1.036, 1.105)
Mean years spent in a house with insect vectors (median) (n=450) Range: 0-43 Mean: 8.28 Med: 0	7.12 (0) (n=355)	12.5 (12)[a] (n=95)	1.021 (1.002, 1.041)

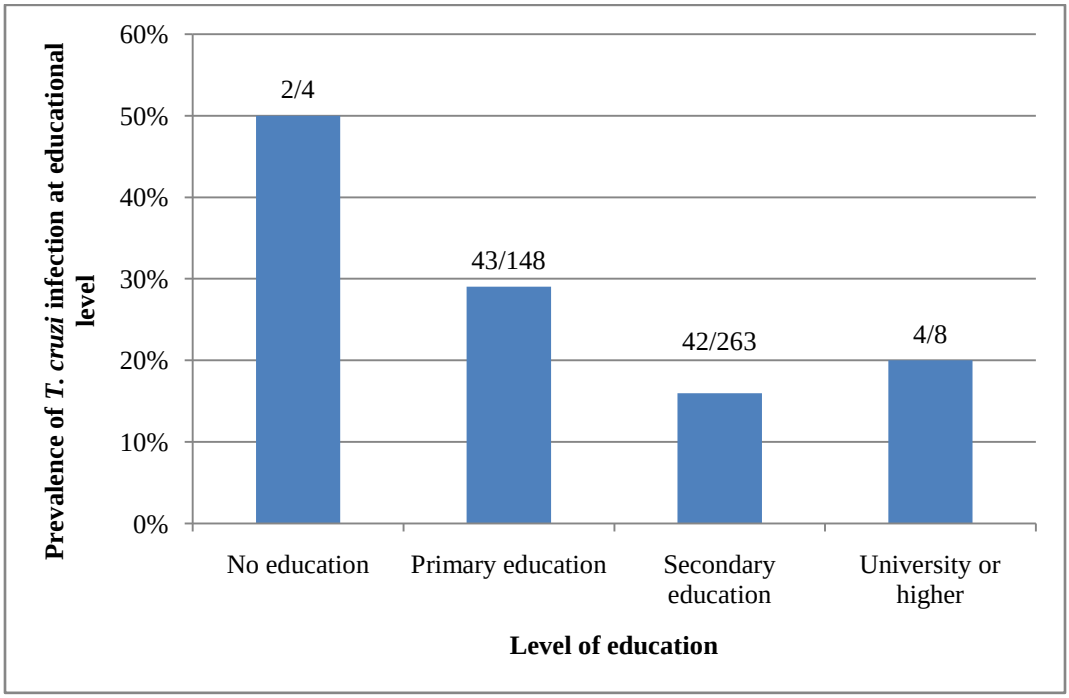
NOTE: [a]The difference between infected mothers and uninfected mothers is statistically significant (<0.0001) when each factor is considered independently. [b]OR in a model that includes both age and years spent in a house with insect vectors.

Table 7. Maternal age and years spent in a house with insect vectors are not associated with congenital transmission.

	Infected mothers with uninfected babies[a]	**Infected mothers with infected babies[b]**	**P-value**
Mean maternal age, years (median) (n=95)	29.4 (30)	29.4 (31)	0.9943
Mean years spent in a house with insect vectors (median) (n=95)	12.4 (11.5)	13.9 (20)	0.7048

NOTE: [a]n=88; [b]n=7

Figure 4: Women with *T. cruzi* infection have lower educational level than those without infection.



NOTE: Numbers above each bar represent the number of *T. cruzi*-infected women over the total number of women in each category. Total sample size=435.

Table 8. Level of education is associated with maternal *T. cruzi* infection (Fisher's exact p= 0.0059).

	Number (%) of infected mothers	**Number (%) of uninfected mothers**	**Total (% of all mothers)**
No education	2 (2.2)	2 (0.6)	4 (0.9)
Primary education	43 (47.3)	105 (30.5)	148 (34.0)
Secondary education	42 (46.2)	221 (64.2)	263 (60.5)
University or higher	4 (4.4)	16 (4.7)	20 (4.6)
Total	91	344	435

Table 9. Having a family member with Chagas disease is associated with maternal infection. Data is from the volunteer's response to the question, "Does anyone in your family have Chagas disease?" Women infected with *T.* cruzi were more likely to report having family members with known Chagas disease ($p<0.0001$).

	Infected mothers	**Uninfected mothers**	**Total**
Number of women that have family members with Chagas disease (% of column)	42 (44.7%)	71 (20.1%)	113 (25.3%)
Number of women that do not have family members with Chagas disease (% of column)	52 (55.3%)	282 (79.9%)	334 (74.7%)
Total	94	353	447

Table 10: Obstetric history among uninfected versus infected mothers.

	Uninfected mothers	**Infected mothers**	**P-value**	**P-value when controlled for age**
Mean parity (median) n=449 Range: 1-13 Mean: 2.87 Med: 2	2.63 (2) (n=354)	3.78 (3) (n=95)	0.0002	0.1248
Mean spontaneous abortions (median) n=447 Range: 0-2 Mean 0.23 Med 0	0.22 (0) (n=352)	0.31 (0) (n=95)	0.2667	n/a
Mean stillbirths (median) n=447 Mean 0.09 Med 0	0.07 (0) (n=352)	0.16 (0) (n=95)	0.0871	n/a
Mean spontaneous abortions plus stillbirths (median) n=447 Mean 0.32 Med 0	0.28 (0) (n=352)	0.46 (0) (n=95)	0.0609	0.4667
Mean live births (median) n=449 Mean 1.52 Med 1 Range 0-11	1.33 (1) (n=354)	2.23 (2) (n=95)	0.0003	0.1713

Table 11. Characteristics of the pregnancy and labor of *T. cruzi*-infected mothers

	Uninfected mothers	**Infected mothers with uninfected babies**	**Infected mothers with infected babies**
Mean length of time between rupture of membranes and birth in all births, hours (median) n=464 Range: 0.0-40.40 Mean 3.33 Med 0.40	3.38 (0.01) (n=365)	2.94 (0.01) (n=88)	5.55 (0.30)[a] (n=7)
Mean length of time between rupture of membrane and birth in vaginal births only, hours (median) n=41 Range: 0.01-40.0 Mean: 6.25 Median: 1.70	6.29 (6.85) (n=130)	6.18 (1.70) (n=37)	6.96 (6.85) (n=4)
Gestational age of the pregnancy at delivery, weeks.days (median) n= 464 Range: 30.0-43.0 Mean: 39.0 Median: 39.0	39.0 (39.0) (n=368)	39.1 (39.5) (n=89)	38.4 (39.4) (n=7)

NOTE: [a] Kruskal-Wallis p for the difference between length of time between rupture and birth in all births is 0.0725. All other differences in this table are not significant ($p>0.10$).

Table 12: Term birth is associated with maternal infection with *T. cruzi*. Premature birth is defined as birth before 37 weeks of gestation. Fisher's exact p (including all categories)= 0.579. Fisher's exact two-sided p comparing infected mothers of infected babies with infected mothers of uninfected babies= 0.5294. Mantel-Haenszel Chi-square p comparing uninfected mothers with infected mothers of uninfected babies=0.0233.

	Uninfected mothers (% of column)	**Infected mothers of uninfected babies (% of column)**	**Infected mothers of infected babies (% of column)**	**Total (% of column)**
Premature delivery	77 (19.5)	9 (9.6)	1 (14.3)	87 (17.5)
Term delivery	318 (80.5)	85 (90.4)	6 (85.7)	409 (82.5)

Table 13. Premature rupture of membranes may be associated with congenital infection with *T. cruzi*. Premature rupture of membranes is defined as rupture of membranes before the onset of contractions. Deliveries for which no time of onset of contractions was noted or were noted to have had a scheduled Cesarean section were excluded. Fisher's exact p (including all categories)= 0.0683. Fisher's exact two-sided p comparing infected mothers of infected babies with infected mothers of uninfected babies= 0.0529. Mantel-Haenszel Chi-square p comparing uninfected mothers with infected mothers of uninfected babies=0.2346.

	Uninfected mothers (% of column)	**Infected mothers of uninfected babies (% of column)**	**Infected mothers of infected babies (% of column)**	**Total (% of column)**
Number of women with PROM	57 (16.0)	10 (11.0)	3 (42.9)	70 (15.4)
Number of women without PROM	300 (84.0)	81 (89.0)	4 (57.1)	385 (84.6)

Table 14. Preterm premature rupture of membranes (PPROM) is not associated with maternal and infant infection with *T. cruzi*. Preterm premature rupture of membranes is defined as rupture of membranes before onset of contractions in a pregnancy of 37 weeks or less gestation. Deliveries for which no time of onset of contractions was noted or were noted to have had a scheduled Cesarean section were excluded. Fisher's exact p (including all categories)= 0.4003. Fisher's exact two-sided p comparing infected mothers of infected babies with infected mothers of uninfected babies= 1. Fisher's exact two-sided p comparing uninfected mothers with infected mothers of uninfected babies=0.1532.

	Uninfected mothers (% of column)	**Infected mothers of uninfected babies (% of column)**	**Infected mothers of infected babies (% of column)**	**Total (% of column)**
Number of women with PPROM	16 (4.5)	1 (1.1)	0 (0)	17 (3.7)
Number of women without PPROM	341 (95.5)	90 (98.9)	7 (100)	438 (96.3)

Table 15. Neonatal exam findings according to maternal and neonatal *T. cruzi* infection

	Babies of uninfected mothers	**Uninfected babies of infected mothers**	**Infected babies**
Mean Apgar score at one minute after birth (median) n=454 Mean: 7.91 Med: 8 Range: 3-10	7.96[a] (8) (n=362)	7.73 (8) (n=86)	7.83 (8) (n=6)
Mean Apgar score at five minutes after birth (median) n=457 Mean: 8.96 Med: 9 Range: 5-10	8.98 (9) (n=363)	8.90 (9) (n=87)	9.00 (9) (n=7)
Mean Apgar score at ten minutes after birth (median) n=290 Mean: 9.71 Med: 10 Range: 8-10	9.71 (10) (n=234)	9.68 (10) (n=53)	9.67 (10) (n=3)
Mean weight of the placenta, grams (median) n=447 Mean: 670.0 Med: 650 Range: 65-7500	659.8 (650) (n=354)	714.7 (640) (n=86)	635.7 (600) (n=7)
Mean weight of the baby at birth, grams (median) n=466 Mean: 3266 Median: 3305 Range: 1270-5150	3258 (3300) (n=367)	3307 (3400) (n=92)	3093 (2900) (n=7)
Mean head circumference of the baby at birth, cm (median)	34.2 (34.0)	34.4 (34.0)	36.5 (34.0)

n=459 Mean: 34.3 Median: 34.0 Range: 28-55	(n=363)	(n=89)	(n=7)
Mean length of the baby at birth, cm (median) n=461 Mean:50.2 Median: 50 Range: 38-60	50.1 (50)[b] (n=364)	50.7 (51) (n=90)	49.5 (50) (n=7)
Mean heart rate of the baby at birth, beats per minute (median) n=463 Mean: 149.7 Median: 150 Range: 60-198	149.8 (150) (n=367)	149.6 (150) (n=89)	146.1 (150) (n=7)
Mean respiratory rate of the baby at birth, respirations per minute (median) n=460 Mean: 53.6 Med: 52 Range: 15-160	53.8 (52) (n=364)	53.2 (52) (n=89)	49.0 (50) (n=7)

NOTE: [a]The difference between Apgar scores at one minute of babies of uninfected mothers and uninfected babies of infected mothers is statistically significant (p=0.0150).

[b]The difference between the length of babies of uninfected mothers and uninfected babies of infected mothers is statistically significant (p=0.0359). Other neonatal findings did not differ significantly among the three groups.

Table 16. Sensitivity and specificity of rapid diagnostic tests (RDTs) on cord blood

Seropositive is defined as having ≥2 positive standard serologic tests (IHA, ELISA, IFA) on maternal serum. Seronegative is defined as having <2 positive standard serologic tests on maternal serum. Not all women had results for both RDTs. κ statistic for agreement between standard serologic tests and InBios rapid test=0.9637 (95% CI: 0.932, 0.9954)

	Seropositive women	**Seronegative women**	**Sensitivity**	**Specificity**
InBios +	94	6	**InBios alone**	
InBios-	2	364	97.9%	98.4%
IHA +	82	1	**IHA alone**	
IHA -	4	369	95.3%	99.7%
InBios+ or IHA+	94	3	**InBios[a] and IHA together**	
InBios- and IHA-	0	364	100%	98.4%

Table 17. Maternal serology among transmitting mothers versus non-transmitting mothers

	Infected mothers with infected babies	**Infected mothers with uninfected babies**	**P-value**
Mean IHA dilutions in cord blood of infected mothers (median) n=90 Median: 512 Range: 0-1024	694.9 (1024) (n=7)	416.4 (256) (n=83)	0.1005
Mean IHA dilutions in serum of infected mothers (median) n=92 Median: 384 Range: 0-1024	694.9 (1024) (n=7)	443.5 (256) (n=85)	0.1319
Mean Chagatek absorbance[a] in infected mothers (median) n=97 Mean: 0.8487 Med: 0.8979 Range: -0.1457 to 1.283	1.0015 (0.992) (n=7)	0.8368 (0.8948) (n=90)	0.0526
Mean Wiener absorbance[a] in infected mothers (median) n=97 Mean: 2.2159 Med: 2.4289 Range: -0.3014 to 2.6646	2.2928 (2.3564) n=7 Med 2.3564	2.2100 (2.4292) n=90 Med 2.4292	0.6963

[a]Absorbance is defined as sample absorbance minus cut-off absorbance.

Table 18. Neonatal diagnosis and losses to follow-up

Only one infant missed scheduled time points but appeared for subsequent time points, and this infant was diagnosed with *T. cruzi* infection at 9 months. Results were not available for all diagnostic methods in all infants at 6 and 9 months. Micromethod at 30 days, 6 months, and 9 months was done on infant peripheral venous blood. [a]Number lost to follow-up at time point includes infants that were present at previous time point but did not appear for any subsequent follow up. Total lost to follow-up includes all infants that had been lost to follow up at this time point.

Time point	Birth (n=110)	30 days (n=82)	6 months (n=32)	9 months (n=22)
Diagnostic methods used	Cord blood micromethod	Micromethod	Micromethod IHA	Micromethod IHA ELISA IFA
Number diagnosed with *T. cruzi* infection at time point (Method)	1 (micromethod)	4 (micromethod)	1 (IHA)	1 (IFA and ELISA)
Number lost to follow-up at time point (Total lost to follow-up) [a]	n/a	26 (26)	45 (71)	10 (81)