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Short Report: Detectable *Trypanosoma cruzi* Parasitemia during Pregnancy and Delivery as a Risk Factor for Congenital Chagas Disease

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Abstract. Vector control has led to a drastic decrease in the prevalence of acquired Chagas disease in Latin America, thus redirecting attention to congenital Chagas disease. We report results of a longitudinal study of 359 pregnant women in Yacuiba in southern Bolivia, of whom 147 (40.9%) were infected with *Trypanosoma cruzi*, to evaluate the relationship between the patency period of the parasitemia and the risk of congenital infection. Maternal infection was assessed by using *T. cruzi*-specific serologic tests, and parasitemia in mothers and newborns was diagnosed by using microscopic examination of blood in heparinized microhematocrit tubes. Parasitemia was present in 28.6% of the infected women. Its prevalence increased during the third trimester, then decreased at delivery. The likelihood of congenital infection was significantly correlated with the parasite density in the mother's blood. The risk of transmission increased during the third trimester of pregnancy and could explain premature births or low-weight newborns for infected mothers.

Chagas disease, a major parasitic endemic disease caused by *Trypanosoma cruzi*, is a public health priority in Latin America, particularly among rural populations with low economic status. In spite of the effectiveness of vector control in the past two decades,[1] several countries, including Bolivia, are still highly endemic for this disease. However, the dramatic decrease in vectorial transmission shows the roles of blood transfusion and congenital transmission in the development of Chagas disease. The latter factor became a major concern in non-endemic countries where *T. cruzi*-infected women of childbearing age are living, both within Latin America and in countries with heavy immigration from the disease-endemic countries.

The transmission rate, i.e., the prevalence of infected women who transmit the parasite to their child, seems rather constant at approximately 5%,[2] which at least partly explains the significant correlation between the prevalence of congenital Chagas disease and the general prevalence of the parasite in the population. In addition to the level of endemia, i.e., *T. cruzi* prevalence in the population as a whole,[3] immunologic factors specific to the mother and fetus support the transmission of the parasite.[4] The association between congenital transmission and high *T. cruzi* parasitemia levels in the mother at delivery was observed by several authors.[3,5,6] Moreover, Torrico and others[2] showed that a high *T. cruzi* parasitemia level in the newborn leads to more severe clinical attacks. However, the stage of pregnancy when parasite transmission occurred and its effects on the development of the pregnancy remained undocumented.

We conducted a longitudinal study involving 147 pregnant women with positive serologic results for Chagas disease in the city of Yacuiba, in the southern Bolivian Chaco, a dry steppe at a low altitude. The women were followed-up during prenatal consultations to evaluate the relationship between the patency period for the parasitemia and the risk of congenital infection.

The study was conducted during June 2004–November 2005 in the public maternity hospital of the city of Yacuiba (83,500 inhabitants), county town of Gran Chaco, Department of Tarija, in southern Bolivia, on the border of Argentina. The city is situated within a highly disease-endemic county, where previous studies have estimated the Chagas disease prevalence at 42%.[3] However, health authorities have initiated regular indoor insecticide spraying operations against vectors of the disease (*Triatoma infestans*), and the town of Yacuiba appears to be free of vector infestation. Written informed consent was obtained from each mother before she entered the study. The protocol was reviewed and approved by the Bolivian Ministry of Health.

At the first prenatal visit, maternal blood samples were collected in 600-μL Microtainer® tubes with lithium heparinate and separate gel (Becton Dickinson, Franklin Lakes, NJ). Maternal infection was assessed with *T. cruzi*-specific serologic tests: indirect hemagglutination test (HAI Chagas; Polychaco SAIC, Buenos Aires, Argentina) and enzyme-linked immunosorbent assay (first-generation; Wiener Laboratories, Buenos Aires, Argentina) for confirmation. In the case of a discrepancy between the test results, a second enzyme-linked immunosorbent assay with recombinant antigens (third-generation; Wiener Laboratories, Rosario, Argentina) was performed. A second serologic determination was performed at delivery to diagnose any newly acquired infection during pregnancy.

We collected maternal blood at each prenatal visit and newborns' cord blood at delivery to search for *T. cruzi* parasites. For cesarean births, newborns' blood was collected by finger or heel puncture. Parasitemia in mothers and newborns was diagnosed using microscopic examination of the buffy coat from four heparinized microhematocrit tubes (50 μL of blood/75-μL capacity tube, total = 200 μL) centrifuged for 5 minutes at 12,000 × *g*.[7] The interface of the buffy coat was examined at 100× and 400× magnifications. Parasite density was examined by counting the parasites in each microhematocrit tube.[2]

A team from the Laboratory of Entomology of the Bolivian Ministry of Health in La Paz and the Health District of Yacuiba in charge of insecticide-spraying operations visited the houses of all mothers who were positive for parasitemia during pregnancy to search for *T. infestans* in the home.

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Whenever possible, congenitally infected newborns were treated for 60 days with benznidazole (10 mg/kg/day). The results of these studies were communicated to each mother, who was also informed that her newborn would be re-examined for additional parasitologic and serologic controls.

Results were expressed as the mean ± SD or percentage of variables measured in the mothers or newborns. Mann-Whitney nonparametric tests were used to compare means. Chi-square or Fisher's exact tests were used to compare proportions.

A total of 359 pregnant women were followed-up during the investigation, and 147 (41%) had positive serologic results for Chagas disease. These 147 women had an average of 5 prenatal visits (range = 2–9 visits). The mean ± SD age of Chagas-positive women was 25.2 ± 6.4 years (range = 15–46 years), and the mean ± SD age of noninfected women was 23.1 ± 5.7 years (range = 14–43 years) ($P = 0.001$). The microhematocrit method estimated a parasitemia prevalence of 28.6% among the parasite-positive pregnant women at any point during pregnancy. The prevalence of maternal parasitemia was significantly higher during the third trimester of pregnancy than during the first two trimesters ($P = 0.002$ and $P = 0.05$, respectively) and decreased at delivery ($P = 0.002$) (Table 1). The risk of congenital transmission followed a similar pattern, which peaks during the third trimester of pregnancy (Table 1). Entomologic investigations found no vectors (*T. infestans*) in the houses of parasitemic mothers.

The frequency of parasite transmission to the child was significantly correlated with parasite density in the mothers, regardless of the stage of pregnancy (Table 2). However, the correlation was highest during the third trimester of pregnancy ($P < 0.001$). At this stage of pregnancy, all of the women were sampled the same number of times: mean ± SD = 2.9 ± 0.8 for the women whose parasitemic result was positive and 2.4 ± 0.8 for the other women. The mean ± SD parasite density was significantly higher ($P < 0.001$) in the mothers of infected newborns (26.4 ± 22.3 parasites/mm^3) than in the mothers of non-infected newborns (3.5 ± 8.4 parasites/mm^3).

Most studies prior to the current study were limited to transversal investigations at delivery. A follow-up investigation of women infected with *T. cruzi* during pregnancy enabled us to evaluate the relative risks of parasite transmission from mother to child in relation to maternal parasitemia and stage of pregnancy.

Taking into account the mean ± SD age of Chagas disease–positive pregnant women (25 ± 6 years) and the seroprevalence (41%) observed in the study area, we observed that most Chagas disease–positive women had the chronic phase of the disease, in which the parasite is not easily detectable by conventional techniques. According to Portela-Lindoso and Shikanai-Yasuda,[8] during the chronic phase, the sensitivity of a unique xenodiagnosis using 40 nymphs of *T. infestans* ranges between 9% and 36%, and the sensitivity of a 30-mL blood cultivation ranges between 26% and 55%. Although the microhematocrit method is generally considered poorly sensitive because it examines a relatively small amount of blood (200 µL), the parasitic density in our study participants was high enough to be detected in nearly 30% of infected women because of repeated sampling during pregnancy (5 examinations with an average or 1.5 mL of blood/examination). In their study that involved 90 parasite-positive women who were examined 3 times, Storni and Bolsi[9] found a higher proportion of positive xenodiagnosis results among 50 pregnant women (66%) than among 40 women who were not pregnant (30%).

The nature of maternal parasitemia during pregnancy has been poorly investigated. We showed a significant increase in maternal parasitemia during the third trimester of pregnancy (22.4%), followed by a notable decrease at delivery (8.8%). Menezes and others[10] conducted 4 xenodiagnoses during pregnancy in each of 119 Chagas-positive women and found a non-significant increase in the number of positive xenodiagnosis results during the second (19%) and third (21%) trimesters than in the postpartum period (13%). Increasing parasitemia at the end of pregnancy was also reported by Storni and Bolsi.[9]

The detectable maternal parasitemia levels during pregnancy did not seem to be related to prolonged exposure to vectors because no *T. infestans* was captured in the houses of the parasitemic women. The relationship between maternal parasitemia and repeated inoculations with *T. cruzi* by vectors appears to be more complex than previously thought.[11]

To our knowledge, parasite density during pregnancy has not previously been measured. However, it can now be estimated by using the microhematocrit method. The results of this study showed maternal parasite density directly predicts the risk of *T. cruzi* congenital transmission, particularly during the third trimester (Table 2). Bern and others[6] also showed that parasite loads measured by quantitative polymerase chain reaction were higher in mothers of congenitally infected children than in the mothers of non-infected children. Similar effects were observed in cases of maternal-fetal transmission of other parasites or viruses. For example, the maternal plasmatic density of human immunodeficiency virus 1 (HIV-1) RNA, particularly at the end of the pregnancy, is directly related to the risk of HIV-1 transmission to the fetus.[12] Similarly, a significant correlation was observed between the density of *Toxoplasma gondii* in the placentas of primary infected mothers and congenital infection of their newborns.[13]

Detection of circulating parasites in a large number of women before delivery suggested that immunologic tolerance reactivates the parasitemia. Hermann and others compared the immunologic response of infected women who

TABLE 1

Prevalence of parasitemia and relative risk of congenital *Trypanosoma cruzi* transmission according to the stage of pregnancy, Bolivia

Stage of pregnancy	Prevalence of parasitemia, %	Relative risk of congenital transmission (95% confidence interval)
First trimester (n = 62)	4.8	0
Second trimester (n = 129)	13.2	3.3 (0.6–16.6)
Third trimester (n = 134)	22.4	20.8 (2.6–166)
Delivery (n = 147)	8.8	4.1 (0.9–19.2)
Total (n = 147)	28.6	15 (1.9–121)

TABLE 2

Prevalence of infected newborns classified by maternal parasite density at various stages of pregnancy, Bolivia

Stage of pregnancy	% (N) of congenital cases by level of maternal cumulative parasite densities		
	0 parasites/mL	1–10 parasites/mL	> 10 parasites/mL
All pregnancy	0.9 (1/106)	4.5 (1/22)	26.3 (5/19)
Third trimester	1.0 (1/104)	8.7 (2/23)	50.0 (4/8)
Delivery	3.7 (5/134)	0 (0/9)	66.6 (2/3)

transmitted *T. cruzi* to their children and those who did not at delivery and just after delivery.[5] Congenital transmission was associated with high parasite loads at delivery, and a defective immunologic response associated with a poor production of interferon-γ in the mothers who transmitted the parasite to their children, in contrast to higher monocyte activation in the mothers who did not transmit the parasite to their children.[5]

The immunologic response of the fetus and genetic capacity to resist the infection also play an important part in infection susceptibility and congenital parasite transmission. Normally, in uninfected newborns born to infected mothers, the specific immunologic response is characterized by the proliferation and activation of T lymphocyte, which produce interferon-γ, which limits multiplication of the parasites and the morbidity and mortality of the congenital infection.[4] Activation of T and B lymphocytes is also associated with excretion of specific IgM and IgA against *T. cruzi* and the activation of monocytes that produce inflammatory cytokines, which result in parasite antigens passing through the placenta and the modulation of specific and non-specific immunologic response in the fetus.[14,15] Our results suggest that the risk of transmission is higher during the third trimester than at the delivery, which could explain certain premature births or low-weight newborns.

The current recommendation for the control of vertical transmission and diagnosis of congenital cases is based on serologic screening of all pregnant women before delivery and searching for parasites and specific antibodies in all children born to infected mothers from birth to the age of 12 months. However, most studies emphasized that 80% of children are lost to follow-up after the age of 6 months and less than one in two congenital cases is correctly identified and treated.[3,16,17] Studies indicated the difficulty in convincing mothers to bring their infants back for follow-up and the poor competency of primary care systems in performing routine follow-up activities and keeping track of the children.

In this context, delivering safe and well-tolerated trypanocidal drug during pregnancy could help to avoid transmission *in utero* of *T. cruzi* and prevent vertical transmission. However, benznidazole and nifurtimox, the two anti-*T. cruzi* drugs available, have side effects and their tolerance during human pregnancy is unknown. Use of preventive chemotherapy, such as intermittent preventive treatment for malaria during pregnancy and the prevention of mother-to-child transmission of HIV by antiretroviral drugs, during pregnancy and especially during the third trimester could help reduce the risk of congenital transmission of *T. cruzi*. Such preventive chemotherapy with benznidazole or nifurtimox during pregnancy has been consistently suggested by the World Health Organization since 1987.[18,19]

Our study confirmed that parasite prevalence increases throughout pregnancy and is highest during the last trimester before decreasing at delivery. Thus, the risk of congenital transmission is higher during the last trimester of pregnancy and decreases at delivery. It is therefore essential to maintain the systematic serologic screening for *T. cruzi* infection in pregnant women and serologic and parasitologic diagnosis of congenital Chagas disease in infants up to the age of 12 months born to serologically positive women whenever possible. Alternative strategies, such as intermittent preventive treatment, should also be considered.

Moreover, *T. cruzi* congenital transmission is significantly related to increasing maternal parasitemia, which depends on the immunologic state of the pregnancy. Dosages of cytokines and anti-*T. cruzi* immunoglobulins during the third trimester could confirm the influence of specific and nonspecific immunologic responses of the mother and child on the congenital risks of *T. cruzi* parasite transmission and its morbidity.

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