Influence of Pregnancy on *Trypanosoma cruzi* Parasitemia in Chronically Infected Women in a Rural Bolivian Community

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Abstract. To determine the role of pregnancy on *Trypanosoma cruzi* parasitemia, a matched cohort study was carried out in a rural Bolivian community comparing parasite rates in gravidae, puerperae, and non-pregnant infected women. A selection of 67 chronically infected women, who delivered between March 2004 and May 2005, were initially evaluated during the third trimester of pregnancy and again after delivery. They were matched for age, parity, and location with 104 seropositive non-pregnant women, who likewise had submitted blood for microscopic examination for *T. cruzi* parasites in June 2005. Seroreactive pregnant women had a higher rate of *T. cruzi* parasitemia (14.9%) than matched non-pregnant infected women (2.9%; $P = 0.004$). After delivery, parasitemia significantly decreased during puerperium (1.5%) compared with the period of pregnancy (14.9%; $P = 0.03$). This study showed an increase of parasite loads in maternal peripheral blood, during the third trimester, and a significant decline after delivery.

INTRODUCTION

During the acute phase of Chagas disease, *Trypanosoma cruzi* parasites are detected in the peripheral blood of the host for a few days to weeks after infection, regardless of the mechanism of transmission. With the subsequent development of an immune response, the patient enters the chronic phase of the disease, and parasites are no longer directly detected in the bloodstream.¹ In fact, parasitemia may be low or high in different individuals during the chronic phase of Chagas disease and is variable and fluctuating over time.^{2,3} In particular, individuals with an impaired cell-mediated immune response may experience an exacerbation of chronic infection and increased parasite loads.¹ Using various parasitological methods, a growing number of studies has detected *T. cruzi* parasites in the bloodstream of chronically infected pregnant women.^{4–6} In a cohort of Bolivian women chronically infected by *T. cruzi*, nearly 30% of the women presented with parasites in the bloodstream, and parasitemia tended to increase from the first to the third trimester and then decrease at delivery.⁷

During pregnancy, a systemic shift toward Th2 dominance and suppression of cell-mediated immunity may contribute to increased susceptibility to intracellular pathogens. Indeed, the hormonal environment of pregnancy contributes to local suppression of cytotoxic T lymphocyte responses at the maternal-fetal interface and the release of Th2 stimulating cytokines, which activate B lymphocytes and stimulate antibody secretion.⁸ In areas with high rates of *Plasmodium falciparum* transmission, the incidence of clinical malaria attacks and parasite densities increase during pregnancy and the first days of puerperium in comparison with the periods before pregnancy and 2–12 months after delivery.⁹ In one matched cohort study, pregnancy was associated with an increased prevalence of intestinal helminth infection (*Ascaris lumbricoides* and *Trichuris trichiura*) in a sub-Saharan African community, but several other studies failed to show any influence of pregnancy on helminth prevalence or parasite densities (i.e., intes-

tinal helminth egg counts or *Wuchereria bancrofti* microfilarial loads).^{10–13}

In a sample of Chagas-positive Argentinean women, a higher rate of parasitemia was observed in pregnant women than in their non-pregnant counterparts.¹⁴ Unfortunately, control women were not age- or location-matched with pregnant women, and thus, confounding effects may not have been adequately controlled in the Argentinean study. Pregnancy-related parasitemia clearly is a concern because it is associated with congenital transmission.⁶ However, whether all chronically infected women or only chronically infected pregnant women can harbor high parasite loads in the blood is an unresolved issue. Therefore, we sought to determine if pregnant women are more susceptible than non-pregnant women to *T. cruzi* parasitemia. This matched cohort study aimed to compare the prevalence of *T. cruzi* parasitemia in gravidae, puerperae, and non-pregnant infected women in a rural Bolivian community.

MATERIALS AND METHODS

Study design and population. The study took place in the municipality of Carapari (800–1300 meters high, 9,035 inhabitants in 2001 according to the National Institute of Statistics), department of Tarija, in the southern Bolivian Chaco region. Chagas disease, locally transmitted by *Triatoma infestans*, is highly endemic in this rural community, with a prevalence of 64% in women of childbearing age.¹⁵ The National Chagas Control Program (NChCP) and non-governmental organizations (NGOs) are currently implementing vector control programs (indoor insecticide spraying and home improvement) in the municipality. The Chaco region is also endemic for *Plasmodium vivax* and *Mansonella ozzardi*.^{16,17}

In March 2004, the research team started a house-to-house screening system for congenital Chagas disease in this rural area and recruited two nurses who realized blood sampling and served as field investigators. They visited all of the houses in the 77 villages of the municipality of Carapari and assessed all women of childbearing age using a standardized questionnaire. The census of the women of childbearing age was updated monthly to identify all ongoing pregnancies. Between March 2004 and May 2005, 214 pregnant women were examined for *T. cruzi* infection at home by field investigators during their third trimester of pregnancy.

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In June 2005, we designed a matched cohort study to assess the pattern of *T. cruzi* parasitemia in relation to pregnancy. We selected all the women in puerperium living in 17 easily accessible villages, reflecting the different levels of endemicity existing in the municipality.¹⁵ Puerperae were matched for age (± 5 years), parity (0–1, 2–3, > 3), and location (same village during pregnancy) with up to three non-pregnant women seropositive for anti-*T. cruzi* antibodies, and who likewise submitted blood for microscopic examination for *T. cruzi* parasites. On the basis of the study census, periodically updated, non-pregnant women were recruited from homes closest to those of the puerperae.

From March 2004 to May 2005, the observed prevalence of *T. cruzi* parasitemia (measured by the microhematocrit method) was 10% (95% confidence interval [CI]: 5–15) among the cohort of infected pregnant women, and 1% prevalence was assumed in non-pregnant controls. A minimum sample size of 61 pairs of participants was chosen to detect a 9% difference between the two groups with a power of 0.8 at a two-sided significance level of $\alpha = 0.05$. The study protocol was in accordance with the ethical principles outlined in the Declaration of Helsinki and was approved by the Ministry of Health and Sports of Bolivia. All participants provided written informed consent.

Data collection and analysis. At enrollment, all of the women provided social and demographic information by questionnaires. To confirm non-gravid status for the non-exposed women, urine human chorionic gonadotropin (HCG) tests were available.

Chagas infection in women was first assessed using a rapid diagnostic test (Chagas Stat-Pak, Chembio Diagnostic System, Medford, NY) immediately performed on whole blood according to manufacturer's recommendations. Second, a blood sample was collected in 600- μ L Microtainer tubes with lithium heparinate and separate gel (Becton Dickinson, Franklin Lakes, NJ). After centrifugation, the Microtainer tube was frozen at -20°C until an enzyme-linked immunosorbent assay (ELISA) test for confirmation was carried out (Wiener Laboratory, 1st generation, Rosario, Argentina). When discrepancies arose between these two tests, a second ELISA with recombinant antigens (Wiener Laboratory, 3rd generation) was performed for definitive diagnosis, and the corresponding woman was not included in the analysis. Parasites of *T. cruzi* and *M. ozzardi* in mothers' peripheral blood were directly diagnosed in the field by microscopic examination of the buffy coat from four heparinized microhematocrit tubes (75 μ L) centrifuged for 5 min at 12,000 g. The interface of the buffy coat was observed at 100 $\times$ and 400 $\times$ magnifications by the same laboratory technician (MR) for the entire duration of the study (March 2004 to June 2005).

The results of these studies were communicated to each mother. All *T. cruzi*-positive women were referred to the health service for case management.

Data were entered and analyzed using Stata/MP 11 (StataCorp, College Station, TX). Matched groups were compared for *T. cruzi* parasitemia and pregnancy status, taking into account matching variables (including age, gravidity, and residence). Potential risk factors for parasitemia (e.g., household characteristics and vector exposition) were also evaluated. In matched analyses, a conditional logistic regression model of 67 groups estimated the odds of *T. cruzi* parasitemia, given the matching variables and pregnancy status.¹⁸

RESULTS

Of 214 evaluated pregnant women, 131 *T. cruzi*-infected women delivered in the municipality between March 2004 and May 2005. Of this sample, 67 pregnant women (exposed) were selected for the matched cohort survey. On the basis of the study census, 321 women were identified in the vicinity of the puerperae; 276 (86%) consented to be screened for *T. cruzi* infection in June 2005, and 164 (59%) were seropositive for Chagas disease. Among these, 104 infected non-pregnant women (non-exposed) were finally selected because they met the matching criteria on age, parity and place of residence.

Baseline characteristics of age, parity, and *T. cruzi* exposure (i.e., percentages of insecticide sprayed, renovated dwellings, or the proportion of *T. infestans*-infected houses) were similar between the two groups (Table 1). Pregnant women had not lived as long in the municipality as non-pregnant women (10.7 versus 15.7 years, $P = 0.005$), and pregnant women lived more frequently in precarious dwellings than non-pregnant women (16.9 versus 6.7%, $P = 0.04$).

The presence of triatomine vectors inside the houses was statistically associated with *T. cruzi* parasitemia in pregnant women (rate ratio [RR] 4.1 95% CI = 0.9–17.9; $P = 0.04$) and in the entire population (RR 4.8 95% CI = 1.1–20.8; $P = 0.02$). No relationship was observed between the presence of triatomine vectors and pregnancy status. Furthermore, none of the other variables listed in Table 1 (i.e., household characteristics) influenced *T. cruzi* parasitemia in pregnant women or the population as a whole (data not shown).

TABLE 1
Characteristics of pregnant and non-pregnant *Trypanosoma cruzi*-infected participants

	<i>T. cruzi</i> -infected		<i>P</i> value χ^2 test
	Pregnant women (exposed)	Non-pregnant women (non-exposed)	
Baseline characteristics <i>N</i>	67	104	
Age groups			
≤ 20 years, <i>N</i> (%)	20 (29.8)	25 (24.0)	0.68
21–30 years, <i>N</i> (%)	25 (37.3)	44 (42.3)	
31–45 years, <i>N</i> (%)	22 (32.8)	35 (33.6)	
Number of pregnancies			
0–1, <i>N</i> (%)	18 (26.9)	32 (31.1)	0.62
2–3, <i>N</i> (%)	27 (40.3)	34 (33.0)	
> 3 , <i>N</i> (%)	22 (32.8)	37 (35.9)	
Time of residency			
≤ 5 years, <i>N</i> (%)	27 (41.5)	27 (26.0)	0.10
6–15 years, <i>N</i> (%)	15 (23.1)	28 (26.9)	
≥ 16 years, <i>N</i> (%)	23 (35.4)	49 (47.1)	
Precarious dwelling, <i>N</i> (%)	11 (16.9)	7 (6.7)	0.04
House with tap water, <i>N</i> (%)	52 (77.6)	85 (82.5)	0.43
House with electricity, <i>N</i> (%)	35 (52.2)	45 (43.7)	0.27
Renovated house, <i>N</i> (%)	20 (29.8)	31 (29.8)	0.99
House sprayed with insecticides, <i>N</i> (%)	56 (84.8)	89 (85.6)	0.90
House infected with triatomines, <i>N</i> (%)	33 (49.2)	57 (56.4)	0.36
Household with known cases of Chagas disease, <i>N</i> (%)	29 (70.7)	30 (68.2)	0.80
<i>T. cruzi</i> parasitemia, <i>N</i> (%)	10 (14.9)	3 (2.9)	0.004
Microfilariemic, <i>N</i> (%)	8 (11.9)	11 (10.6)	0.78

Comparison between gravid and non-gravid women.

Pregnant women also had a higher rate of *T. cruzi* parasitemia than non-pregnant women (14.9 versus 2.9%, respectively, RR 5.2 95% CI = 1.5–18.1; $P = 0.004$, Table 1). Mean parasite densities were also higher for the 10 pregnant parasitemic women (45 parasites/mL, range 5–230) than for the 3 non-pregnant parasitemic women (10 p/mL, range 5–15). Because of the reduced sample size, this difference did not reach statistical significance. In contrast, pregnant women had a frequency of *M. ozzardi* infection similar to that of non-pregnant women (11.9 versus 10.6%, respectively; $P = 0.78$).

The 67 pregnant women were also surveyed in June 2005 during puerperium. In contrast to the period of pregnancy (14.9% positive for *T. cruzi*), only one woman was positive for *T. cruzi* parasites (1.5%) 8 months after delivery. This showed a 10-fold decrease in the frequency of parasite detection in puerperae compared with pregnant women (odds ratio [OR] 10.0 95% CI = 1.5–64.2; McNemar's exact test, $P = 0.004$, Table 2).

Risk factors for *T. cruzi* parasitemia. A conditional logistic regression model for *T. cruzi* parasitemia of the 67 pregnant/non-pregnant matched groups was conducted. The model controlled for the influence of age, parity, and location (matching variables). Of the 67 groups, 54 were dropped because they resulted in zero outcomes. Among the remaining 13 groups, pregnancy status was an independent risk factor significantly associated with *T. cruzi* parasitemia ($P = 0.01$, Table 2). Taking into account the presence of triatomine vectors inside the houses ($P = 0.03$) did not change the existing relationship between pregnancy and parasitemia ($P = 0.01$), but given limited sample size, it decreased the precision of the estimates (confidence intervals of the odds ratios, data not shown).

DISCUSSION

Our data showed a significantly higher proportion of patient parasitemia in *T. cruzi*-infected pregnant women in the third trimester compared with non-pregnant infected women. Furthermore, pregnancy status is likely to be a significant risk factor for *T. cruzi* parasitemia, according to conditional logistic regression, indicating that pregnant women have a 5-fold higher rate of parasitemia during the third trimester when compared with age-, parity-, and location-matched non-pregnant women. In addition, our study also showed a significant decrease of *T. cruzi* parasitemia in puerperae relative to the level during pregnancy. However, we did not observe any change in the prevalence of *M. ozzardi* related to pregnancy.

TABLE 2

Analysis of 67 matched groups—rate ratio (RR) for *Trypanosoma cruzi* parasitemia

	Risk of <i>T. cruzi</i> parasitemia			
	% (n/N)	RR	95% CI*	P value
Conditional logistic regression				
Pregnant vs. non-pregnant women	14.9 (10/67) 2.9 (3/104)	5.2	1.4–19.2	0.01
McNemar's exact χ^2				
Pregnant women vs. puerperae	14.9 (10/67) 1.5 (1/67)	10.0	1.5–64.2	0.004

* CI = confidence interval.

Only two other studies have attempted to assess the relationship between pregnancy and *T. cruzi* parasitemia. The risk of parasitemia was two times higher (RR 2.2 95% CI = 1.3–3.7) in 50 pregnant women compared with 40 non-pregnant control women 16–40 years of age in a study in Argentina.¹⁴ Similarly, another study in Brazil showed a significantly lower proportion of *T. cruzi* parasitemia in 119 women in puerperium compared with the period of pregnancy.¹⁹ To search for *T. cruzi* parasites, both studies used xenodiagnosis with seven to 10 nymphs of *T. infestans*. In the Argentinean study, non-pregnant women were not matched with pregnant women, and the selection criteria were not indicated. However, the results of these two studies are in accordance with our results, and both confirm a pregnancy-induced increase of *T. cruzi* parasitemia and a decline in the parasite load after delivery.

The methodological limitations of our study include the single blood sampling and the technique used to search for *T. cruzi* parasites. In the chronic phase of Chagas disease, peripheral parasitemia is fluctuating and intermittent. Thus, serial blood sampling, whenever possible, is preferable, to show the presence of parasites.³ The parasitemia of 202 chronically infected patients was studied for ~13 years by repeated xenodiagnosis. The level of parasitemia oscillated and tended to decline over time, and the proportion of patients with high parasitemia was ~5–10%.² However *T. cruzi* parasites showed no circadian rhythm and no periodicity over 13 consecutive days, indicating that blood examination may be valid at any time.²⁰

During the chronic phase of Chagas disease, xenodiagnosis (10–70% sensitivity) and blood cultures (25–95% sensitivity) improve the detection of parasitemia.²¹ Unfortunately, there is no parasitological method with 100% sensitivity during the chronic phase of the disease, even including molecular tests using polymerase chain reaction.²² The use of the microhematocrit method was initially proposed for the detection of African trypanosomes and was validated for acute phases and congenital forms of Chagas disease.^{23–25} The microhematocrit is less sensitive than blood culture or xenodiagnosis but is simpler and faster to detect high parasite loads.

The challenge in the study of relationships between pregnancy and infectious diseases is the selection of an appropriate control group.⁸ In this study, subjects were matched for age, parity, and place of residence. As already indicated, parasitemia tends to decline over time and therefore with age.² By matching for age, we aimed to control time effects in the study. Parity-dependent effects of malaria during pregnancy are well documented, and primigravid women are at higher risk for malaria infections and low birth weight babies.²⁶ Similarly, primigravidae show high susceptibility to congenital transmission of *T. cruzi*, justifying matching pregnant and non-pregnant women for parity.²⁷ Peak parasitemias after reinfection challenges in dogs and mice have been observed.^{28,29} Therefore, controlling the level of exposure to vectors and of reinfection (i.e., the place of residence) appeared to be necessary in this study. Retrospectively, because women living in houses infested with *T. infestans* presented higher rates of *T. cruzi* parasitemia than those living without vectors, confirms the need to match for the risk of exposure. However, because of the limited sample size, it was not possible to properly test for the presence of vectors in the final model. Further studies with larger sample size are therefore necessary.

Our findings may have implications for gestational and parasite immunology. Evidence indicates that the maternal

immune system may tolerate fetal antigens by suppressing cell-mediated immunity while retaining normal humoral immunity. The latter type of immunity is most effective against extracellular pathogens, such as helminths. There was no effect of pregnancy on *M. ozzardi* infections in our study, and several other studies also failed to show a relationship between pregnancy and intestinal helminths.^{12,13}

In contrast, our results confirm that the pregnancy-induced depression of cell-mediated immune response is related to an increase in *T. cruzi* parasitemia. Other causes of impaired cell-mediated immune response, such as long-term treatment with corticosteroids or human immunodeficiency virus (HIV) infection, are also accompanied by an exacerbation of *T. cruzi* parasitemia. A Brazilian study based on the use of xenodiagnosis showed a clear increase in *T. cruzi* parasitemia related to the duration of treatment and the dosage of corticosteroids.³⁰ *Trypanosoma cruzi* parasitemia was also detected more frequently in 29 HIV-positive patients than in 81 HIV-negative subjects matched for age, sex, and date of admission (OR 12.3 95% CI = 3.7–41.2).³¹

CONCLUSION

By means of direct parasitology, we showed the effect of pregnancy on chronic Chagas disease in pregnant women, which resulted in an increase in the parasite load in the bloodstream during the third trimester of pregnancy and a significant decline after delivery, compared with non-pregnant women. This is an important concern because we previously showed that detectable maternal parasitemia observed during the second half of pregnancy is responsible for higher risk of congenital infection.⁷

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