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Risk factors and consequences of congenital Chagas disease in Yacuiba, south Bolivia

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Summary

OBJECTIVE To determine the risk factors of congenital Chagas disease and the consequences of the disease in newborns.

METHODS Study of 2712 pregnant women and 2742 newborns in Yacuiba, south Bolivia. Chagas infection was determined serologically in mothers and parasitologically in newborns. Consequences of congenital Chagas disease were assessed clinically.

RESULTS The prevalence of Chagas disease in pregnant women was 42.2%. Congenital transmission was estimated at 6% of infected mothers leading to an incidence rate of 2.6% among newborns. Main risk factors of congenital transmission were mothers' seropositivity and maternal *Trypanosoma cruzi* parasitaemia. Parity was higher in infected than in non-infected mothers, but it was not associated with the risk of congenital transmission. The rate of congenital infection was significantly higher in newborns from multiple pregnancies than in singletons. However, we did not observe statistically significant consequences of Chagas disease in newborns from single pregnancies or among twins.

CONCLUSIONS The main risk factors for congenital transmission were infection and parasitaemia of mothers. Consequences of the disease seemed mild in newborns from single pregnancies and perhaps more important in multiple births.

keywords congenital Chagas disease, vertical transmission, pregnant women, newborns, Bolivia

Introduction

Chagas disease is a major parasitic endemic disease in Latin America. The population infected by *Trypanosoma cruzi* is estimated between 9 and 10 million people (Schofield *et al.* 2006), a high proportion of which being women susceptible to transmit the disease congenitally. The disease is strongly associated with poor socio-economic conditions that frequently prevail in these areas (WHO 1991).

Chagas disease represents a major public health problem in Bolivia, as 55% of the territory is considered as endemic, because of the presence of the vector between 300 and 3500 m of altitude. Eight species of triatomes occur in Bolivia, but *Triatoma infestans* is responsible for 80% of vectorial transmission. The seroprevalence of Chagas disease in the Bolivian population ranges from 32% to 93% in adults and is commonly associated with poor rural dwellings (Azogue 1993; Noireau 1999).

According to the National Programme for Chagas disease Control, levels of vectorial infestation were significantly reduced during the last decade, thanks to insecticide treatment of houses and improvement of housing conditions. The rate of vectorial infestation fell down from 66% in the years 1999–2000 to below 2% in the year 2004 everywhere the intervention was performed and regularly maintained (Ministerio de Salud y Previsión Social 1998; Noireau 1999). Thus, the relative importance of congenital transmission has increased and in certain areas has become the major mode of disease spread. We followed up a population of pregnant women for 19 months in a hospital located in the southern part of Bolivia to measure the rate of congenital transmission and to evaluate the risk factors influencing this way of transmission.

Materials and methods

Study population and protocol

The study was conducted in the public maternity of the city of Yacuiba, County town of Gran Chaco, Department of

Tarija, in south Bolivia, at the border with Argentina. At the time of the study, the city's population was 83 500, of whom 21 500 were women of childbearing age. Most deliveries (92%) took place in the public maternity ward (INE-Bolivia). The city is situated in a highly endemic area and Chagas disease prevalence was estimated at 50% from previous surveys in the maternity ward (Brutus L & Schneider D, unpublished data).

Every woman admitted to the hospital of Yacuiba for delivery was asked to participate in the study. Between May 2003 and November 2004, all mother–infant pairs were enrolled and neonates were followedup until December 2005.

'Incidence of congenital Chagas disease' was defined as the number of newborns showing circulating *T. cruzi* divided by the total number of living births; 'congenital transmission rate' denotes the number of newborns with circulating parasites divided by the number of seropositive mothers (Carlier & Torrico 2003).

Written informed consent was obtained from each mother prior to entering the study. Information on obstetrical (gravidity, abortions, caesarean, stillborns) and maternal characteristics (age, village of residence and duration, village of birth, medical history, residual insecticide house sprayings) were collected through interviews by medical staff at enrolment. The protocol received ethical approval by the Bolivian Ministry of Health (INLASA).

Biological investigations

Blood samples were collected in 600 μl Microtainer® tubes with lithium heparinate and separate gel (Becton Dickinson, Franklin Lakes, NJ USA) for all biological tests. Before delivery, pregnant women's blood was collected from finger puncture; at delivery, newborns' blood was collected from the umbilical cord. In the case of caesarean, newborns' blood was collected from finger or heel puncture.

Maternal infection was assessed by *T. cruzi*-specific serologic tests: indirect haemagglutination (HAI Chagas; Polychaco S.A.I.C., Santiago del Estero, Argentina) and ELISA (Wiener, 1st generation, Buenos Aires, Argentina) for confirmation. In the case of discrepancy between the tests, a second ELISA with recombinant antigens (Wiener, 3rd generation, Rosario, Argentina) was performed.

Haemoglobin in newborns was measured with a Hemocue® (Hemocue AB, Ängelholm, Sweden) dosing cyanmethaemoglobin concentration, and the presence of anaemia was defined as <13.5 g/dl haemoglobin.

Parasitaemia in mothers and newborns was diagnosed by microscopic examination of the buffycoat from four heparinized microhaematocrit tubes centrifuged for 5 min at 12 000 ***g***. The interface of buffycoat was observed at 100× magnification.

Because vectorial transmission may exist even in the very first months of life, we used the presence of circulating parasites in cord blood at delivery or in peripheral blood at 1 month of age to define a congenital infection. Whenever possible, congenitally infected newborns were treated for 60 days with benznidazole (2×5 mg/kg/day). Parasitological and serological controls were carried out during and after treatment.

Newborns' weights, lengths and head circumferences were measured at birth. A physical examination was performed following Farr's procedure for the estimation of gestational age (Farr *et al.* 1966); APGAR score at 1 and 5 min and the occurrence of neonatal anaemia (haemoglobin rate under 13.5 g/dl) were recorded.

Results of these studies were communicated to each mother who had also been informed that her newborn would be reexamined for further parasitological and serological controls. Blood samples for the control of infants' parasite status were collected from finger or heel puncture.

Statistical analysis

Results were expressed as the mean (±SD) or percentages of variables measured in mothers or newborns. Student's *t*-test, ANOVA and Mann–Whitney or Kruskal–Wallis nonparametric tests were used to compare means or medians. Chi-squared or Fisher's exact tests, with the correction of Bonferroni for multiple comparisons, were used to compare proportions. Univariate analysis was performed to identify possible risk factors of congenital Chagas disease in newborns from serologically positive mothers. Finally, all variables with $P \leq 0.20$ were introduced in a multiple logistic regression to determine the factors associated with congenital transmission. A separate stratified analysis was performed to identify the consequences of congenital transmission in the first 1220 newborns, in single and multiple births.

Results

Study population

Our study involved 2712 pregnant women and 2742 newborns. Mothers' mean age was 24.4 years. The rate of multiple gestations (twins and triplets) was 2.9% and of caesareans was 13.6%. The mean number of antenatal visits was 3.8 and 40% of the mothers came for their first antenatal visit during the third trimester of gestation.

Table 1 Characteristics of studied population

Characteristics	Mean or %	SD	95% CI	*n*
Mother's age (years)	24.4	6.6	11–48	2680
<20	27.4%			735
20–29	51.2%			1373
30–39	18.6%			499
≥40	2.7%			73
House				
Rural zone	18.3%		16.8–19.7%	2665
Absence of insecticide house-spraying programme	41.9%		40.1–43.8%	2585
Duration of residence in Yacuiba and place of birth of the mothers				
Mean residence duration (years)	12.42	10.0	0–48	2605
Residence duration ≥25 years	18.7%		16.9–20.6%	2605
Mother born in Yacuiba	37.9%		36.2–39.9	2638
Obstetrical history				
Primigravid	35.6%		33.8–37.4%	2702
Mean number of previous pregnancies	2.7	2	1–12	1716
History of previous abortions	23.9%		21.9–25.9%	1716
History of previous stillbirths	3.3%		2.4–4.1%	1714

Almost half of the habitations benefited from antivectorial control and most of them were in urban area. A majority of mothers did not originate from the city and were frequently multigravid (Table 1).

Chagas disease in mothers and newborns

The serological prevalence of Chagas disease among mothers was 42.2% (1144/2711). We observed circulating parasites at delivery in 54 mothers (2%), of whom 51 were seropositive (4.5%) and three were seronegative (0.2%). In addition, a single child born from a seronegative mother without circulating parasites presented parasites at delivery, which implies that the mother harboured circulating parasites although we did not find them. As four seronegative women were observed, either with circulating parasites or with an infected child, it is likely these women were in acute phase of Chagas disease at the time of delivery.

Of 2674 newborns examined at delivery, 52 presented circulating parasites (1.9%). By the age of 1 month, we also found circulating parasites in nine of 849 children testing negative at delivery (1.1%). Among seropositive women, the transmission rate of congenital Chagas disease was 5.1% and the incidence rate was 228 for 10 000 living births. Among seronegative women in acute phase of the disease, the transmission rate of congenital Chagas disease was 50% (two of four women).

We observed 29 multiple births: 28 mothers with twins and one with a triplet; we did not consider the latter for analysis. Serological prevalence of Chagas disease was identical in mothers with singleton and multiple pregnancies ($\chi^2 = 0.37$; $P = 0.53$; Table 2). The prevalence of congenital infection was not significantly different between twins (3.6%) and singletons (2.1%) (Fisher's Exact test $P = 0.36$; Table 2). By evaluating the rate of congenital transmission between single births (56/2615; 2.1%) and twins (2/28; 7.1%), the difference did not reach statistical significance (Fisher's test $P = 0.12$). Notwithstanding, when congenital transmission occurred during multiple pregnancies, all foetuses were infected.

Risk factors of congenital transmission

Univariate analysis was made with newborns from all mothers with singleton, regardless of their parity (Table 3). Only maternal seropositivity and parasitaemia were significantly associated with congenital transmission of the

Table 2 Comparison of Chagas disease prevalence in single and twin newborns

	Number of mothers	Seropositive mothers	Number of newborns	Infected newborns
Single newborns	2683	1132/2657 (42.6%)	2683	56 (2.1%)
Twins	28	12/28 (42.9%)	56	2 (3.6%)
Total	2711	1144/2685 (42.6%)	2739	58 (2.1%)

Table 3 Univariate analysis of risk factors of congenital Chagas disease in mothers (single births only)

Variables	OR	95% CI	*P*	*n*
Rural area	1.20	0.65–2.39	0.5	2623
Without insecticide house-spraying programme	1.51	0.65–1.95	0.66	2547
Mother's age >30 years	1.51	0.84–2.71	0.17	2638
≥1 pregnancy	1.27	0.71–2.26	0.80	2631
Time of residence ≥25 years	1.22	0.57–2.61	0.61	2563
Mother born in Yacuiba	1.02	0.58–1.76	0.97	2596
Without antenatal visit	0.51	0.12–2.13	0.36	2586
Multigestity (≥3 pregnancies)	1.47	0,84–2.58	0.8	2631
Cesarean delivery	1.01	0.50–2.28	0.87	2619
Male newborn	0.97	0.56–1.68	0.9	2624
Hot season (July–February)	1.63	0.96–2.78	0.07	2641
Previous stillbirth	0.88	0.43–1.81	0.73	2622
Mother's parasitaemia positive	27.5	14.1–53.5	<0.001	2638
Mother's serology positive	25.1	7.8–80.5	<0.001	2615

disease ($P < 10^{-4}$ for both factors). Hot season (from July to February) appeared to be a possible risk factor ($P = 0.07$). The same results were found after logistic regression (Table 4). These results remained unchanged when using only seropositive mothers for whom parasitaemia appeared as the only risk factor.

The average number of previous pregnancies was significantly different depending on mothers' serology and newborns' parasitaemia (Table 5). Mann–Whitney test was highly significant showing a tendency to a higher number of previous pregnancies in serologically positive compared with seronegative mothers ($P < 10^{-3}$). However, there was no difference in the mean number of previous pregnancies between mothers giving birth to *T. cruzi* congenitally infected babies and mothers with uninfected babies (Mann–Whitney $P = 0.77$). Similarly, seropositive mothers (whether the newborn was infected or not) were significantly older than seronegative mothers ($P < 10^{-4}$). In addition, the group of seropositive mothers with infected newborns was significantly different, from both seropositive mothers having uninfected children and seronegative mothers (Bonferroni test).

Table 4 Logistic regression models of risk factors of congenital Chagas disease in mothers (single births only, $n = 2634$) using variables showing $P < 0.2$ in the univariate analysis

Variable	OR	*P*	95% CI
Mother's age >30 years	1.03	0.92	0.56–1.88
Hot season (July–February)	1.65	0.07	0.95–2.85
Mother's parasitaemia positive	11.62	10^{-4}	5.92–22.82
Mother's serology positive	19.39	10^{-4}	5.95–62.71

Effects of congenital Chagas transmission on newborns

Singletons and twins highly differ in physical and developmental conditions. Congenital Chagas disease was more frequent ($\chi^2 = 11.51$; $P = 10^{-3}$) among newborns from multiple pregnancies (five cases in 37 births or 13.5%) than in singletons (32 cases in 1232 births or 2.6%). Consequently, separate analyses were performed on these two groups to determine the consequences of congenital Chagas disease (Table 6).

Even if they were systematically lower, none of the quantitative parameters (weight, length or cephalic perimeter) differed significantly between infected and non-infected singletons. There were two infant deaths (6.5%) in infected, and 22 (1.9%) in uninfected children (Fisher's exact test $P = 0.12$). Prevalence of anaemia was more frequent in congenitally infected newborns (21.9%) than in non-infected newborns (10.2%), with a nearly significant difference (Fisher's exact test $P = 0.07$).

Because of limited sample sizes, we did not test the differences in quantitative variables among multiple newborns. However, mean birth weights, lengths and cephalic perimeters were lower in infected than in uninfected babies.

Treatment

Congenitally infected newborns were treated. Appropriate information was given at delivery, 83.8% of 37 congenital Chagas cases started treatment and 67.7% (21/31) completed it adequately. All showed good tolerance to

Table 5 Average number of previous pregnancies and mother's age according to serological and infection status of mothers and newborns

	Previous pregnancies			Mother's age		
Group	Mean	SD	*n*	Mean	SD	*n*
Positive mother and infected newborn	2.6	2.57	52	25.3	7.46	53
Positive mother and non-infected newborn	2.18	2.33	1054	25.8	6.94	1056
Negative mother and non-infected newborn	1.43	1.71	1499	23.44	6.19	1500
Total	1.76	2.07	2661	24.38	6.61	2609

Table 6 Mean characteristics of congenital *T. cruzi*-infected newborns

	Mean weight ± SD (*n*)	Mean length ± SD (*n*)	Mean cephalic perimeter ± SD (*n*)	Mean haemoglobin ± SD (*n*)	Mean gestational age ± SD (*n*)
Single newborns					
Non-infected newborns	3391.6 ± 532.9 (1181)	50.3 ± 2.56 (1173)	33.7 ± 1.75 (1171)	15.8 ± 2.11 (1173)	39.2 ± 1.59 (1166)
Infected newborns	3285.8 ± 406.8 (31)	50.0 ± 2.28 (30)	33.6 ± 1.33 (30)	15.1 ± 2.03 (32)	38.8 ± 1.23 (30)
Total	3388.4 ± 530.3 (1213)	50.2 ± 2.56 (1204)	33.7 ± 1.74 (1202)	15.8 ± 2.11 (1205)	39.2 ± 1.59 (1196)
P, Non-infected *vs.* Infected newborns*	0.27	0.54	0.84	0.06	0.13
Multiple newborns					
Non-infected twins	2510.3 ± 566.9 (32)	46.6 ± 2.99 (30)	32.4 ± 1.84 (27)	16.1 ± 2.31 (30)	37.2 ± 1.96 (26)
Infected twins	2100.0 ± 325.2 (2)	44.0 ± 2.82 (2)	32.0 ± 1.41 (2)	17.6 ± 1.06 (2)	37.4 ± 1.77 (2)
Infected triplet	943.3 ± 98.15 (3)	34.7 ± 0.58 (3)	24.7 ± 0.58 (3)	12.8 ± 0.85 (3)	32.8 ± 0.58 (3)
Total	2361.0 ± 686.5 (37)	45.4 ± 4.41 (35)	31.6 ± 2.85 (32)	15.9 ± 2.38 (35)	36.8 ± 2.26 (31)

*t-test

benznidazole and no adverse reaction was observed. All parasitological controls were negative.

Discussion

This study in the maternity ward of Yacuiba Hospital revealed an overall seroprevalence of Chagas disease of 42.2% in pregnant mothers, with a 1.9% incidence of congenital transmission. The transmission rate was 5.1% among seropositive mothers. Only mothers' seropositivity and parasitaemia were identified as risk factors for congenital infection. We did not observe important adverse consequences of Chagas disease in single newborns.

Congenital Chagas infection was assessed by repeated microscopic examinations of four capillary tubes both at delivery and at first month of life, to prevent possible false negative tests as a result of a low parasitaemia at birth (Azogue & Darras 1995). However, the double examination is not always possible in rural conditions. Microhaematocrit concentration method is very easy to perform and reliable for the diagnosis of congenital Chagas disease diagnosis (Freilij *et al.* 1983; Freilij & Alchteh 1995). Other highly sensitive techniques, such as xenodiagnosis or haemoculture, are difficult to perform, expensive and require higher volumes of blood not suitable for newborns.

When we prepared this survey, we did not have the adequate material for molecular biology analyses (PCR) or recombinant antigens for serology (SAPA – shed acute phase antigen). At that time, these techniques were still under development and not yet standardized (Carlier & Torrico 2003). However, the rapid evolution of the PCR should facilitate its generalization in the near future (Picka *et al.* 2007; Rozas *et al.* 2007). The advantage of the microhaematocrit, although less sensitive, actually remains its facility of use in the field and the immediate result. The PCR could not be used in rural facilities and samples must be sent to a reference laboratory resulting in delayed response. It is indeed, often difficult to find the mothers and their child again after their discharge from the hospital to start the treatment.

Examining the babies at 1 month of age permitted to recover nearly 15% more congenitally infected children. Blanco *et al.* (2000) also used a microhaematocrit technique to detect infected infants in Argentina. In their study, 81% of the congenital cases were diagnosed by this technique (used once or repeatedly) and only 19% by serology. Hence we think, with Azogue and Darras (1995), that a double examination by microhaematocrit may be an interesting alternative to more sophisticated techniques.

We found in Yacuiba a congenital incidence of Chagas disease of 2.3% and a congenital transmission rate of 5.1%, considering diagnosis both at birth and at 1 month of age. Nevertheless, an overall underestimation of congenital Chagas disease cases is possible because of infants lost at the first month control. Based on the hypothesis that among the 763 infants without circulating parasites at birth and lost at the first month control, a similar proportion would have presented circulating parasites (approximately 1.1%), the incidence of congenital Chagas disease would rise to 2.6% and the congenital transmission rate to 6%. Using direct parasitological methods, this transmission rate is comparable with those reported in the city of Cochabamba in Bolivia (4.9–5.9%; Torrico *et al.* 2004) or in Argentina (8.8%; Contreras *et al.* 1999; 7.1%; Blanco *et al.* 2000) but higher than those reported in Brazil (1.6%; Bittencourt *et al.* 1985; 0.7%; Rassi *et al.* 2004) or Uruguay (1.6%; Sarasua *et al.* 1986). Only teams using molecular methods of detection have reported higher

transmission rates (10.4%; Russomando *et al.* 1998; 13.7–28.2%; Garcia *et al.* 2001).

In our study, the presence of circulating forms of *T. cruzi* parasites in the mother's peripheral blood at the time of delivery appeared to be a major risk factor for congenital transmission. We observed circulating *T. cruzi* in 54 pregnant women using the microhaematocrit concentration method. Interestingly, we diagnosed only four cases of acute phase of Chagas disease, i.e. mothers with circulating parasites and negative serology. Among these cases, three women transmitted the disease to their babies. Some authors had previously described an increased risk of congenital transmission during the acute phase of the disease (Bittencourt 1992; Freilij *et al.* 1995). According to Moretti *et al.* (2005), the period of transmission could play a role, as the risk of congenital transmission is higher when infection occurs early in pregnancy. In the remaining 51 mothers, we observed circulating parasites and a simultaneous presence of anti-*T. cruzi* antibodies demonstrating a chronic phase of the disease. Although we cannot completely exclude the possibility of recent re-infection of a long time infected mother, the high number of seropositive subjects displaying circulating parasites diagnosed in our study is striking. It may be better explained by a transient increase in parasitaemia during pregnancy, which had been previously evidenced by indirect concentration methods such as haemoculture or xenodiagnosis (Szarfman *et al.* 1975; Storni & Bolsi 1979; Menezes *et al.* 1992). In this hypothesis, congenital transmission of Chagas disease could be enhanced by the temporary increase of parasite load during pregnancy as a result of immunological modulation (Bittencourt *et al.* 1985; Hermann *et al.* 2004).

Torrico *et al.* (2004) showed a significant difference between the average number of pregnancies in mothers having transmitted *T. cruzi* to their children (1.8 ± 0.2) and that of the seropositive mothers, who did not transmit the parasite to their newborns (2.6 ± 0.1). By analysing seropositive women with single births, we did not find any effect of the average number of pregnancies on the rate of congenital transmission. However, although non-significant, we observed a tendency of increasing mean of previous pregnancies in seropositive women and in women with infected newborns (Table 5).

Anaemia has been described as a consequence of congenital Chagas disease (Zaidenberg 1999; Blanco *et al.* 2000; Barbieri *et al.* 2003). In our study, we found a borderline decrease in the rate of haemoglobinaemia in infected compared with non-infected newborns.

We also observed an unexpectedly high proportion of twins and triplets congenitally infected by *T. cruzi* (five infected twins among 37 infected cases). Examples of congenital infections in twins have been mentioned in other studies in Bolivia (Torrico *et al.* 2004) and Argentina (Freilij & Alchteh 1995; Blanco *et al.* 2000).

Restricting comparisons to the group of singletons (1212 births), we found a lower mean birth weight and a shorter duration of pregnancy in infected newborns, even if, contrary to Torrico *et al.* (2004), the difference did not reach significance. It is noteworthy that these authors did not take into account multiple pregnancies as a confounding factor for the occurrence of clinical signs in newborns. In addition, in our study, a variety of parameters (mean birth weight, cephalic perimeter and size) appeared to be lower in the groups of infected *vs.* non-infected twins. Hoff *et al.* (1978) had mentioned the possibility of a transmission of *T. cruzi* through the placenta and the severity of the infection in twins.

All these findings support the view that infection may increase the risk of prematurity (Bittencourt *et al.* 1972; Freilij & Alchteh 1995; Zaidenberg 1999; Blanco *et al.* 2000). Furthermore, Azogue *et al.* (1985) had observed a significantly higher number of *T. cruzi* infections in premature newborns of 26–37 weeks of gestation than in those aged more than 37 weeks.

Low exposure to re-infection of mothers by *T. cruzi* can reduce the severity of congenital Chagas disease (Dias *et al.* 2002; Torrico *et al.* 2004, 2006). Our results showing only mild effects of congenital Chagas disease on newborns could result from a reduced rate of re-infection in pregnant mothers as a consequence of an efficient vectorial control in the study zone, especially in the urban area.

Aetiological treatment of Chagas disease was completed in only 57% of the infected infants. Cost of benznidazole, theoretically paid by the municipality, drug shortages, high doses, possible adverse reactions or duration of treatment could explain this result, which could be notably improved with an adequate management by the national health system.

Conclusion

We found 42.2% *T. cruzi* infection rate in pregnant women and a congenital Chagas transmission rate of up to 6%. However, the only significant risk factors for congenital transmission were previous mother's infection and detected maternal parasitaemia at birth. Consequences of the disease seemed mild in newborns from single pregnancies. In infected twins or triplets, impairment of foetal development may be more important but we cannot conclude because of our limited sample size. Repeated microhaematocrits are useful for the rapid and reliable diagnosis of *T. cruzi* infection, both in mothers and in newborns, and may contribute to better management of congenital Chagas disease in rural areas of Latin America.

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