Congenital Transmission of *Trypanosoma cruzi* in Non-Endemic Areas: Evaluation of a Screening Program in a Tertiary Care Hospital in Barcelona, Spain

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Abstract.

The impact of Chagas disease is no longer restricted to endemic areas. The aim of this study is to evaluate a 2-year period of a vertical transmission screening program of *Trypanosoma cruzi* infection in a tertiary care hospital in Barcelona (Spain). Two enzyme-linked immunosorbent assays (recombinant and crude antigen) were performed in parallel to pregnant women at risk of *T. cruzi* infection. Discordant results were confirmed by a third diagnostic test. In the case of a positive result, the newborn was tested at birth and after 8 months of life. A total of 1,473 women met the inclusion criteria for the screening program with a resulting seroprevalence for *T. cruzi* of 3.5% (2.2–5.2% confidence interval [CI] 95%). One case of congenital infection was identified. Screening programs for vertically transmitted *T. cruzi* acute infection are beneficial in non-endemic areas for early detection and treatment of acute infection.

INTRODUCTION

Chagas disease is a zoonosis caused by *Trypanosoma cruzi*, an important endemic parasitic infection in Latin America. It has been traditionally considered a disease of rural areas where the triatomine vector is the most common source of infection. Nevertheless, over the past years there has been an important change in the epidemiologic pattern of the disease. The important migration phenomenon from rural to urban areas and from Latin American countries to the rest of the world, mostly the United States and Europe, has caused the disease to be no longer an infection confined to rural Latin America. As a result there is now a preponderance of non-vectorial sources of infection, such as blood transfusions, organ transplants, and vertical transmission both in endemic and non-endemic countries.[1,2]

Vertical transmission is likely to go under-detected as the disease is mostly asymptomatic both in mothers and newborns. In an infected individual, the disease has a long-lasting subclinical phase that can last over 30 years at the end of which 20–30% of the cases develop cardiac or gastrointestinal disease.[3] Therefore, women of childbearing age who acquired the disease in their countries of origin before migration, or even those who have not migrated and were themselves infected congenitally, can transmit the disease. Transmission rates for *T. cruzi* vary from 1% to > 12%[4]; this poses non-endemic countries in a risk situation. In fact, several cases of congenital transmission have been reported in United States, Switzerland, and Spain in the last years.[5–8] Most congenital *T. cruzi* infections are asymptomatic or cause nonspecific signs, requiring laboratory screening for detection. A small proportion cause severe acute morbidity and mortality (hepatosplenomegaly, anemia, meningoencephalitis and/or respiratory deficiency). Infants who survive the acute infection carry the same 20–30% risk of cardiac or gastrointestinal disease. Treatment during infancy is more effective and better tolerated than in adulthood.[9]

In Spain, the Latin American immigration has grown exponentially since the late 90s. There is an estimated 390,000 Latin Americans residing in the Catalonia region, according to the 2008 census.[10] Of these, 7,000 pregnancies could be expected each year (fecundity rate of 40 of 1,000 women). Recent studies have shown seroprevalence rates of *T. cruzi* infection in Latin American pregnant women in our milieu of 3.4%.[5] Thus, we might be facing around 240 at-risk deliveries each year. In this context, we considered the need to setup a screening program in our hospital addressed to pregnant women of Latin American origin for early diagnosis and treatment of congenitally infected newborns, which began in April 2008. The aim of this study is to evaluate a 2-year period (April 2008–May 2010) of the vertical transmission screening program in terms of coverage, seroprevalence of *T. cruzi* infection in the selected population, and vertical transmission rates.

MATERIALS AND METHODS

A screening program was conducted in a tertiary care hospital in Barcelona (Hospital Vall d'Hebron) from April 2008 to May 2010. The target population consisted of pregnant women at risk for *T. cruzi* infection, presenting in our hospital for delivery. The inclusion criteria for screening were being born in areas where Chagas disease is endemic, having received a blood transfusion or an organ transplant in an endemic area or from a donor born in an endemic area or having a mother born in areas where Chagas disease is endemic. Individuals that had more than one delivery during the study period were considered independent opportunities for screening. The physicians and nurses working in the delivery ward explained the screening process, and a blood sample was collected.

Serologic diagnosis in the mothers was performed using in parallel two enzyme-linked immunosorbent assays (ELISA), one of them with recombinant antigens (Novagnost Chagas, Siemens, Germany over the period April 2008–November 2009, and Bioelisa Chagas, Biokit, Barcelona, España from November 2009 until the end of the study period) and the other with a crude antigen (Ortho *T. cruzi* ELISA, Jonhson & Jonhson, EUA), according to the World Health Organization's (WHO) diagnostic criteria. Both ELISA were performed according to manufacturer's instructions. We considered positive or negative results if both tests were concordant. All discordant sera were tested by an in-house Western blot method using a lysate from *T. cruzi* epimastigotes. Sera were considered positive when they recognized at least five antigenic bands of the standard pattern (28, 32, 38, 39, 40, and 48 kDa), indeterminate when they recognized one to four bands, and negative when no bands were present.

In the situation of a positive result, the microbiologist contacted the pediatrician for a follow-up of the newborn. The attending pediatrician recorded major examination findings and microbiological tests. A parasitological diagnosis using the microhematocrit concentration technique[11] and a real-time polymerase chain reaction assay (RT-PCR) were performed once within the first month of life. The RT-PCR was performed using peripheral blood samples pre-treated with 6 M guanidine hydrochloride during 24 hours. The DNA extraction was carried out with automatic silica–membrane technology (NucliSENS EasyMag, BioMerieux). Primers and probe from satellite sequence were selected and used in a TaqMan-based assay as previously described by Piron and others.[12] In the situation of a positive PCR result the test was repeated to exclude a false positive result caused by the high sensitivity of PCR tests.

To exclude possible mother transference or parasitological DNA strains, in positive PCR newborns a second PCR was performed to confirm the positivity of the test. All newborns were followed up and underwent a serologic test at 8 months of age to confirm or refute the diagnosis of congenital transmission. According to international criteria, a vertical

transmission was confirmed in the presence of a positive microhematocrit or a positive serology after 8 months of age.

After delivery, women that resulted positive in the screening process underwent a complete physical examination, chest x-ray, electrocardiogram, echocardiogram, and barium esophageal, and colonic examinations. Indeterminate forms of the disease were defined in women with a positive reaction for *T. cruzi* with no gastrointestinal or cardiac alterations in the evaluation. All infected mothers were offered treatment with Benznidazole after completion of breastfeeding and other at-risk family members were screened.

RESULTS

A total of 5,044 women attended our hospital for delivery in the study period. Of these, 1,473 met the inclusion criteria for the screening program. The mean age was 29.5 years (SD 6) and the most frequent countries of origin were Equator (34%), Bolivia (18%), and Peru (13%). The serological test was performed in a total of 633 women, providing a global coverage of 43% (Figure 1). When considering coverage rates by country of origin the results vary from 57% in Colombians or 50% in Bolivians down to 11% in Uruguayan women.

Twenty-two women (21 from Bolivia and 1 from Equator) were seroreactive for *T. cruzi*, yielding a prevalence of 3.5% (2.2–5.2% confidence interval [CI] 95%). Seroprevalence for *T. cruzi* infection in our sample of Bolivian women was 14.5% (10.4–19.4%, CI 95%). None had a miscarriage and only one had a preterm delivery. Regarding types of delivery, 20 were vaginal delivery and 2 were caesarean delivery. The median infant weight was 3,229 g (SD 516). One of the seroreactive mothers had cardiac involvement (left ventricular dilatation with nodal dysfunction), four had digestive involvement (3 women with dolichosigma and 1 woman with dolichocolon). The rest were considered as indeterminate chronic infection.

Of the 22 seropositive women, we had a follow-up at birth and/or after 8 months of 20 newborns. One case of congenital infection was identified, yielding a vertical transmission rate of 5% (Table 1).

The infected neonate was a male born through vaginal delivery admitted to the hospital because of prematurity (35 weeks) and low birth weight (1,870 g). The physical examination at birth showed hepatosplenomegaly, and blood tests over the first days of life were compatible with increasing cholestasis and cytolysis. A study for neonatal hepatitis ruled out TORCH and other viral infections as well as metabolic diseases. By the time the baby was 20 days old, the mother's seroreactivity to *T. cruzi* was known and thus, a specific study for *T. cruzi* infection in the newborn was performed. The microhematocrit was negative but RT-PCR performed at Day 21 of life was positive and confirmed at Days 25 and 30.

At this point, taking into account the progressive clinical deterioration with jaundice and hepatic damage, after ruling out the alternative diagnoses of the patient's situation and based on the consecutive positive RT-PCR, we finally decided to start an empiric treatment with Benznidazole. Normalization of clinical and laboratory parameters was observed. Follow-up of RT-PCR was negative after 25 days of treatment and ELISA at 7 months of age was also negative. Cardiac assessment was also normal.

DISCUSSION

The increase of immigrants coming from countries where Chagas disease is endemic, has led to the establishment of initiatives for early detection of congenital *T. cruzi* infection in our country. Our study evaluates the results of a screening program in clinical practice over a 2-year period conducted in a university hospital in Barcelona. The 3.5% seroprevalence obtained globally and 14.5% for Bolivian women is consistent with recent data reported in

our area. Two studies conducted in Valencia and one in Barcelona yielded a global seroprevalence among Latino American pregnant woman that ranges between 3.4% and 9.7%, and 17.5% and 26% in the Bolivian population.[5,13,14] Moreover, the rate of vertical transmission is similar to the one obtained in those studies (2.7–7.3%).

In the period evaluated, we detected 22 cases of *T. cruzi* infection in mothers and one in a newborn within the screening program. One of the mothers had a discordant result according to the serologic diagnosis using in parallel two ELISA (recombinant and crude antigen). In the absence of a reference gold standard test to confirm the diagnosis, we performed a qualitative immunological method (in-house Western blot) as has been suggested for serological confirmation by other authors.[15,16] In the case of a positive result in this test, the individual was considered infected to offer to the newborn the best diagnostic option.

We considered one of the newborns a case of congenital transmission caused by a combination of three factors: first, repeatedly positive RT-PCR with a high parasite load performed above the third week; second, absence of other causes that could explain this clinical course after an exhaustive diagnosis process and third, RT-PCR negativization and progressive clinical improvement after etiological treatment. In our opinion, because the negative follow-up serology at 8 months was negative, does not exclude the possibility of a congenital transmission. It is well known that if treatment is started promptly (in the first 3 months of life) a negative seroconversion is observed within the following months.[17–19]

Standard diagnosis of congenital Chagas disease still relies on microscopic observation of blood stream trypomastigotes, or a positive serology beyond the sixth to the ninth month of life for those infants who did not receive diagnoses at birth.[20] The PCR is still not considered as diagnostic criteria because of its difficult interpretation and the lack of a standardized technique.[21] A positive PCR in the first days of life could be caused by a maternal transfer of parasite DNA. Nevertheless, the animal model showed that DNA detected by PCR derives from intact, extracellular, or recently lysed parasites. Several previously published studies show that PCR has higher sensitivity compared with direct methods, shows a better concordance with standard diagnosis criteria, and would increase the early detection of the infection mostly when a high parasite load has been detected.[17,18] This has also been shown for patients with reactivated *T. cruzi* infection post-transplant.[25] Moreover, the possibility of delayed processing facilitates its implementation on the laboratory routine compared with a micromethod, which requires a prompt and experimented interpretation, because the sensitivity quickly decreases with time between collection and reading.

Despite the great effort invested in this program we could only achieve a global coverage of 43%. The implementation of the program in the daily routine of the hospital meant a series of difficulties that we believe are responsible for the low coverage rates. Moreover, it was mandatory to setup a strategy for integrated work in several discipline physicians (obstetricians, pediatricians, microbiologists, epidemiologist, midwifes, and infectologists) that were not familiar with the disease. Some of them were physicians in a training period, with a high turnover and thus, the team connection was not always warranted. Those difficulties are also found in the screening programs previously published and only reflect the need to create a multidisciplinary and motivated team who coordinates the program.[17,22] In an effort to increase the coverage, several informative sessions were addressed to the members of the departments over the period. Other possible ways to increase compliance could be to have within the screening program a case manager that can keep track of “at-risk” mothers, and expanding the informative sessions not only to health care personal, but also to Latin American women coming from endemic areas to increase their awareness of the disease.

We consider the screening program to be beneficial for several reasons. Detecting and treating the disease in young women of childbearing age may reduce vertical transmission in subsequent pregnancies and prevent the development of cardiac complications. This fact is relevant considering that a great proportion of Latin American immigrant population in our area is static and if they return to their countries of origin are at low risk of reinfection. On the other hand, early treatment of infected newborns is associated with high cure rates, as we observed in the case of congenital infection that we detected in our program. Moreover, other children born to women with newly diagnosed Chagas disease or other family members can also benefit from the screening and treatment. In our case, we detected one case of *T. cruzi* infection in a 32-month-old girl whose mother had been diagnosed in her following pregnancy.

In addition, we believe that in the context of screening programs for vertically transmitted *T. cruzi* acute infection, molecular biology methods performed at 30 days of life would increase early detection of the infection, ensuring an effective treatment. More prospective studies both in endemic and non-endemic countries are needed to evaluate these techniques.

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*OUTLEGENDS*F1*FIGURE 1. Flow chart of the study.

TABLE 1

Laboratory results in mothers and newborns*

Mother				Newborn				
				At birth			Follow-up at 8 months	
	ELISA			ELISA			ELISA	
Id	Crude antigen	Recombinant antigen		Crude antigen	Recombinant antigen	PCR	Crude antigen	Recombinant antigen
1	Pos	Pos		–	–	Neg	Neg	Neg
2	Pos	Pos		–	–	Neg	Neg	Neg
3	Pos	Neg†		–	–	–	Neg	Neg
4	Pos	Pos		–	–	Neg	Neg	Neg
5	Pos	Pos		–	–	Neg	Neg	Neg
6	Pos	Pos		–	–	Neg	Neg	Neg
7	Pos	Pos		Pos	Pos	Neg	Neg	Neg
8	Pos	Pos		–	–	Neg	Neg	Neg
9	Pos	Pos		–	–	–	Neg	Neg
10	Pos	Pos		Pos	Pos	Neg	Neg	Neg
11	Pos	Pos		Pos	Pos	Neg	–	–
12	Pos	Pos		Pos	Pos	Neg	Neg	Neg
13	Pos	Pos		Pos	Pos	Neg	Neg	Neg
14	Pos	Pos		Pos	Pos	Neg	Neg	Neg
15	Pos	Pos		–	–	Neg	Neg	Neg
16	Pos	Pos		–	–	–	Neg	Pos†
17	Pos	Pos		–	–	–	–	–
18	Pos	Pos		Pos	Pos	Neg	Neg	Pos‡
19	Pos	Pos			–	Neg	Neg	Neg
20	Pos	Pos		Pos	Pos	Pos	Neg	Neg
21	Pos	Pos		Pos	Pos	Neg	Neg	Neg

22	Pos	Pos		–	–	–	Neg	Neg

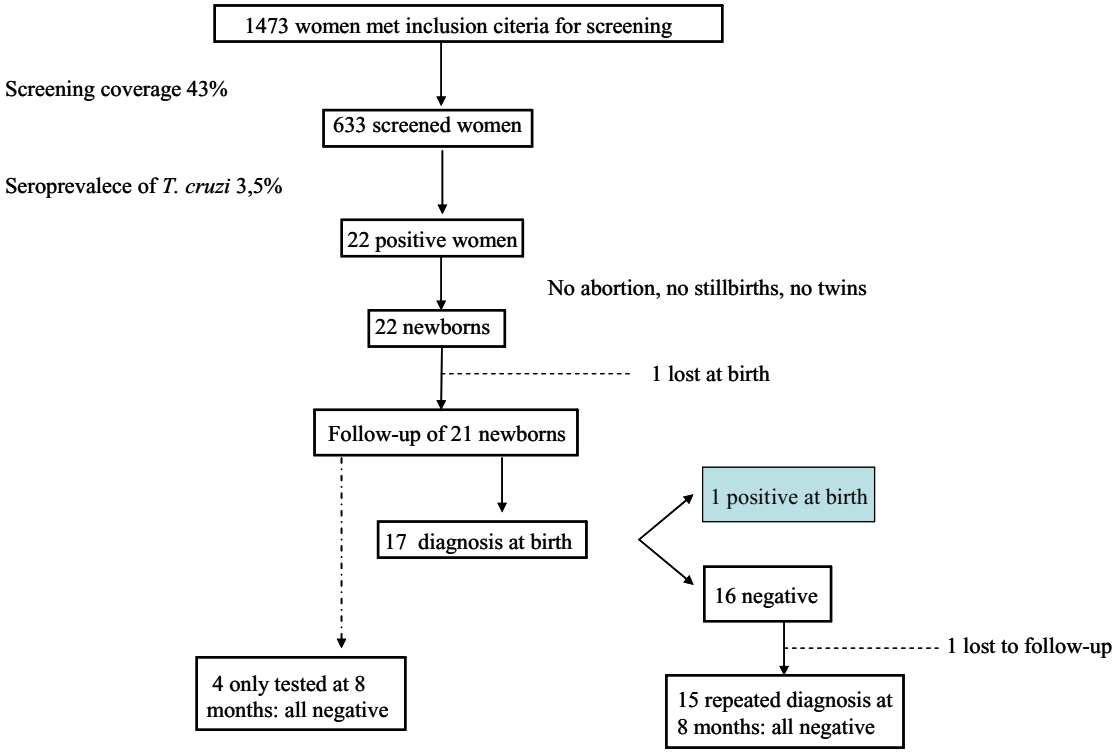
* Two enzyme-linked immunosorbent assays (ELISAs) were used; one used a recombinant antigen (Novagnost Chagas, Siemens, Germany over the period April 2008 until November 2009 and Bioelisa Chagas, Biokit, España from November 2009 until the end of the study period) and the other a crude antigen (Ortho *T. cruzi* ELISA, Jonhson & Jonhson, EUA), according to WHO's diagnostic criteria.

† Western blot test was performed with a positive result.

‡ Western blot test was performed with a negative result.

Id = identification number; Neg = negative; PCR = polymerase chain reaction; Pos = positive; – = not performed.

Figure 1


1473 women met inclusion citeria for screening
Screening coverage 43%
633 screened women
Seroprevalece of T. cruzi 3,5%
22 positive women
No abortion, no stillbirths, no twins
22 newborns
1 lost at birth
Follow-up of 21 newborns
1 positive at birth
17 diagnosis at birth
16 negative
1 lost to follow-up
4 only tested at 8 months: all negative
15 repeated diagnosis at 8 months: all negative