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MAJOR ARTICLE

Treatment of Infected Women of Childbearing Age Prevents Congenital *Trypanosoma cruzi* Infection by Eliminating the Parasitemia Detected by PCR

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Background. We evaluated the effectiveness of treating women of childbearing age with benznidazole to prevent congenital Chagas disease (CCD), as well as the usefulness of polymerase chain reaction (PCR) as a tool to predict the risk of transmission.

Methods. Prospective study involving 144 *T. cruzi* seropositive pregnant women. The parasitological status was studied by PCR in 159 pregnancies, 38 of which involved a cohort of previously treated mothers. One hundred sixty children were examined by PCR and serologically studied at 0–6, 9 and 12 months and annually after treatment.

Results. PCR was seen to be useful for predicting the risk of congenital transmission: 18.8% of mothers with a positive PCR result transmitted the infection (16 infected children out of 85 pregnancies). No infected infants were detected among 74 pregnancies when PCR was negative. Of the treated mothers, 92.1% had negative PCR results, compared with 32.2% of untreated mothers. No infected infants were detected from previously treated mothers, compared with 13.2% among untreated mothers ($P = .019$; χ^2). All infants treated before the first year of life were cured.

Conclusions. Treating infected women of childbearing age prevents CCD by reducing the parasitemia. PCR is an useful tool in the CCD diagnostic algorithm.

Keywords. Chagas; congenital; treatment; PCR; prevention; *Trypanosoma cruzi*.

Chagas disease, caused by the parasite *Trypanosoma cruzi,* is endemic in 21 Latin American countries, where is mainly transmitted by the feces of infected triatomine insects. The disease affects about 6–8 million people around the world [1]. With the establishment of strategies focused on reducing vectorial transmission in endemic areas and the introduction of a universal screening in blood and organ donors, the epidemiological importance of the vertical transmission by *T. cruzi* is increasing [2]. It is estimated that more than 8000 infants are born infected every year, which, added to the migration of women infected with *T. cruzi* (mainly to North America and Europe), makes Chagas congenital transmission a worldwide public health problem [3].

It is estimated that the congenital transmission risk in newborns from mothers with Chagas disease is around 5%, but can vary from 0.7% to 18.2%, depending on the different geographic areas and other factors such as parasitemia [4, 5, 6, 7]. It has been demonstrated that a parasite load in blood detected by polymerase chain reaction (PCR) in pregnant women increases the risk of vertical transmission [8, 9].

Today, trypanocide treatment during pregnancy is not recommended due to the possibility of teratogenic effects. However, the treatment of infected women of childbearing age has been proposed as a strategy for congenital transmission prevention [9–11]. In congenital cases, treatment with benznidazole or nifurtimox in the first year of life is successful in nearly 100% of the infected newborns [12, 13].

Because congenital infection is mostly asymptomatic, the screening of pregnant Latin American women and early diagnosis in newborns is essential to avoid disease progression. At present, in newborns, the diagnosis is performed by parasitological techniques before the sixth month of life or by serology from 8 to 12 months (when maternal antibodies disappear). Nonetheless, the low sensitivity of parasitological techniques [14, 15] means that follow up is necessary until 12 months of age to confirm the diagnosis in most newborns. Many infants do not complete this monitoring, which contributes to the underdiagnoses of congenital Chagas disease (CCD) [16, 17].

The aim of this study was to evaluate the usefulness of PCR in the prevention and management of CCD, and the effectiveness of treatment to prevent vertical transmission of *T. cruzi*.

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PATIENTS AND METHODS

Patients

The study was carried out in 144 seropositive pregnant women and their 160 offspring who attended at the Unit of Tropical Medicine of the Hospital Virgen de la Arrixaca in Murcia (Spain), from January 2007 through May 2016.

Among the 144 seropositive-infected women, 136 (95.8%) were from Bolivia, 4 (2.8%) from Paraguay, 2 (1.4%) from Ecuador, and 2 from unknown Latin American countries. They ranged in age from 15 to 45 (mean age ± SD, 31.1 ± 5.9 years). A control group of 150 seropositive nonpregnant women was included. Of these, 145 (97.3%) were from Bolivia, 2 from Paraguay, 1 from Argentina, 1 from Ecuador, and 1 from an unknown Latin American country. They ranged in age from 17 to 43 years (mean age ± SD, 32.2 ± 5.6 years). Women were considered infected when 2 serological tests (see below) were positive, according to the criteria of the World Health Organization. Seropositive women were interviewed to collect epidemiologic data (age, previous pregnancies, country of origin, and region) and to ascertain whether they had been treated or not before pregnancy. The parasitological status of the infected women was studied by PCR (see below) in peripheral blood samples.

All 160 infants included in the study were born in Spain and had not traveled to any endemic area; the age range when the infants were studied was 0–84 months. CCD was considered confirmed when the parasite was detected by PCR and/or microhematocrite at any age, or when serology remained positive at 12 months of life. Confirmed cases of CCD were examined to evaluate clinical manifestations.

Treatment and Follow Up

Thirty-six women were diagnosed and treated before they become pregnant. All infected infants were also treated. Mothers and infants were orally treated with benznidazole, at 10 mg/kg body weight per day in infected infants and 5–7 mg/kg body weight per day for 60 days in infected women. Due to adverse effects related to benznidazole, treatment was suspended in 2 women, who were treated with nifurtimox, completing the treatment before pregnancy, using a dose of 10 mg/kg body weight per day taken twice daily for 60 days. Treatment response was followed up by PCR and serology at the time of the diagnosis and at 60–90 and 150 days, and annually after the start of therapy, in women and infected infants. Patients were considered cured when 2 consecutive serological tests were negative.

Sample Collection

At each time point, 10 samples (2 mL) of peripheral blood were collected from *T. cruzi*–infected women and infants, for PCR and serology, respectively. Serum samples were stored at –80°C until their use for the serological follow up. For molecular detection of the parasite by PCR, blood was immediately mixed with an equal volume of lysis buffer containing 6 M guanidine hydrochloride and 200 mM ethylenediaminetetraacetic acid (EDTA), pH 8.

Serological Methods

Infection in women and infants was detected using 2 serological tests: the chemiluminescent microparticle immunoassay (Architect $i2000_{SR}$ Immunoassay, Abbott, IL) and the indirect immunofluorescence assay (Inmunofluor Chagas kit, Biocientifica S.A., Argentina) following manufacturers' indications.

DNA Extraction and PCR Detection

The guanidine-EDTA blood mixture was boiled for 15 minutes in order to break the minicircles. DNA extraction was carried out in duplicate from blood using the Maxwell 16 Blood DNA Purification Kit (Promega Biotech Iberica, Spain) in 400 µL of sample, according to the manufacturer's instructions. PCR detection of the 330–base pair variable regions of the *T. cruzi* kinetoplastid minicircle genome (kDNA) was carried out under previously reported conditions [18].

Statistical Analysis

All the results were analyzed using the IBM SPSS Statistics 23.0 software. Categorical variables were compared by χ^2 test. The confidence intervals were calculated, and relationships were considered significant if $P < .05$.

Bioethical Criteria

The study was reviewed and approved by the Ethical Committee of the Hospital Universitario Virgen de la Arrixaca. Informed consent forms were signed by all mothers after detailed interviews.

RESULTS

Congenital T. cruzi Transmission

Of the 144 pregnant Latin American women who were included in this study, 1 mother gave birth to twins, 3 mothers became pregnant 3 times, and 9 mothers became pregnant twice during the study period. Thus, a total of 160 infants from 159 pregnancies were followed up for the diagnosis of CCD. Vertical transmission was detected in 16 newborns, which represents a congenital transmission rate of 10% (16 infected newborns out of 160 live births). All of the infected infants were born from Bolivian mothers.

Of the 16 infants with congenital *T. cruzi* infection, 13 (81.25%) did not show any clinical manifestations of symptomatic CCD, and only 3 infants were symptomatic (Table 1). The main symptoms and signs of infections were low birth weight, hepatosplenomegaly, respiratory distress, myocarditis, anemia, and jaundice.

Table1. Diagnosis and Follow Up Post-treatment by PCR and Serology in Infected Infants

Infant	Age at Diagnosis, Months	Time 0 Before Treatment			Time 60–90 Days After Treatment			Time 150 Days After Treatment			Time 1 Year After Treatment			Time 2 Years After Treatment			Time 3 Years After Treatment		
		IFI	CMIA	PCR	IFI	CMIA	PCR	IFI	CMIA	PCR	IFI	CMIA	PCR	IFI	CMIA	PCR	IFI	CMIA	PCR
1[a]	2	+	+	+	+	+	–	–	–	–	–	–	ND	…	…	…	…	…	…
2	1	+	+	+	+	+	–	ND	ND	ND	–	–	ND	…	…	…	…	…	…
3	0	+	+	+	–	–	–	–	–	–	–	–	–	…	…	…	…	…	…
4[b]	1	+	+	+	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
5	0	+	+	+	ND	+	ND	ND	ND	ND	–	–	–	…	…	…	…	…	…
6	0	+	+	+	ND	+	–	–	–	–	…	…	…	…	…	…	…	…	…
7[c]	0	+	+	+	+	+	–	–	–	–	…	…	…	…	…	…	…	…	…
8[a,d]	6	+	+	+	ND	ND	–	ND	ND	ND	–	–	–	…	…	…	…	…	…
9[a,d]	6	+	+	+	+	+	–	+	+	+	…	…	…	…	…	…	…	…	…
9[a,e]	…	…	…	…	ND	+	–	ND	+	–	–	+	–	–	–	ND	…	…	…
10	6	+	+	+	–	–	–	–	–	–	–	–	ND	…	…	…	…	…	…
11	12	+	+	+	ND	ND	–	+	+	ND	–	–	–	…	…	…	…	…	…
12	48	+	+	+	ND	+	ND	ND	ND	ND	+	+	–	ND	ND	ND	ND	+	+
12[e]	…	…	…	…	ND	ND	–	…	…	…	…	…	…	…	…	…	…	…	…
13	48	+	+	+	ND	+	ND	ND	+	ND	+	+	–	ND	+	ND	ND	+	–
14	84	+	+	+	ND	ND	–	ND	ND	ND	ND	+	ND	ND	ND	–	…	…	…
15[c,f]	72	+	+	+	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
16[c,f]	60	+	+	+	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…

Plus signs indicate positive results; minus signs indicate negative results.

Abbreviations: CMIA, chemiluminescent microparticle immunoassay; IFI, indirect immunofluorescence assay; ND, not done; PCR, polymerase chain reaction.

[a]Infants 1, 8, and 9 were symptomatic.

[b]Lost to follow up: infant returned to Bolivia.

[c]Infants 7, 15, and 16 are brothers.

[d]Infants 8 and 9 are twins.

[e]Infants 9 and 12 were followed up twice after their first treatment. Treatment failure was diagnosed on the basis of a positive PCR result at 150 days in infant 9 and at 3 years in infant 12, and a second treatment and post-treatment follow up were performed. Serology remained positive and a cure was not achieved in infant 12.

[f]Infants have been recently treated.

Treatment and Parasitological Blood Status Detected by PCR in Infected Women During Gestation, and Risk of Vertical Transmission

Figure 1 compares the parasitological status (by PCR) of 121 pregnancies in 113 untreated mothers, and in 150 nonpregnant, untreated *T. cruzi*–infected women, with similar epidemiological characteristics (control group).

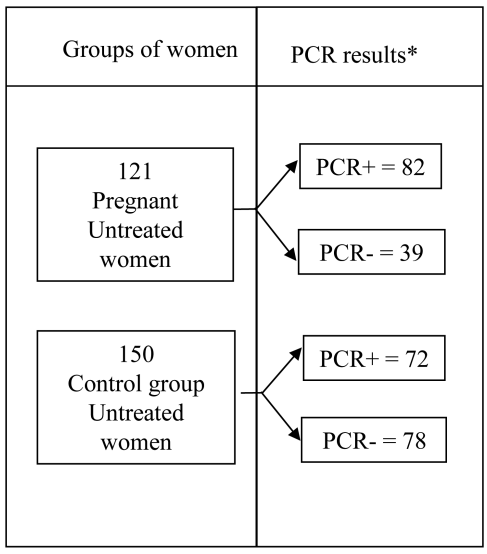

Figure 1. Comparison of parasitological blood status detected by PCR in infected women during gestation.Abbreviation: PCR, polymerase chain reaction.

During the pregnancy, 67.8% of women had a positive PCR result (82 positive PCR results out of 121 pregnancies), and in the control group (nonpregnant women) PCR was only positive in 48% (Figure 1). There was a statistically significant correlation between pregnancy and the increase in parasitemia tested by PCR ($P = .001$; χ^2).

A group of 38 women of childbearing age were diagnosed and treated with benznidazole before they became pregnant. However, due to adverse effects, treatment was suspended in 2 of them and treatment was restarted with nifurtimox before pregnancy. In these women, the evaluation was prospective but the treatment follow-up data and variables were taken back in time. The mean time elapsing between the treatment of the women and when they became pregnant was 3.5 ± 2 years (range, 1–9 years). Before treatment, PCR was positive in 52.8% of the mothers (19 out of 36). All women with positive pretreatment PCR results presented an early conversion of PCR results 90 days post-treatment, and 35 women remained negative postpartum. However, during the follow-up period, the PCR became positive in 3 mothers who had strictly adhered to the treatment (less than 8%) (Figure 2). In 1 case, PCR became positive at 360 days post-treatment (follow up), another 3 years

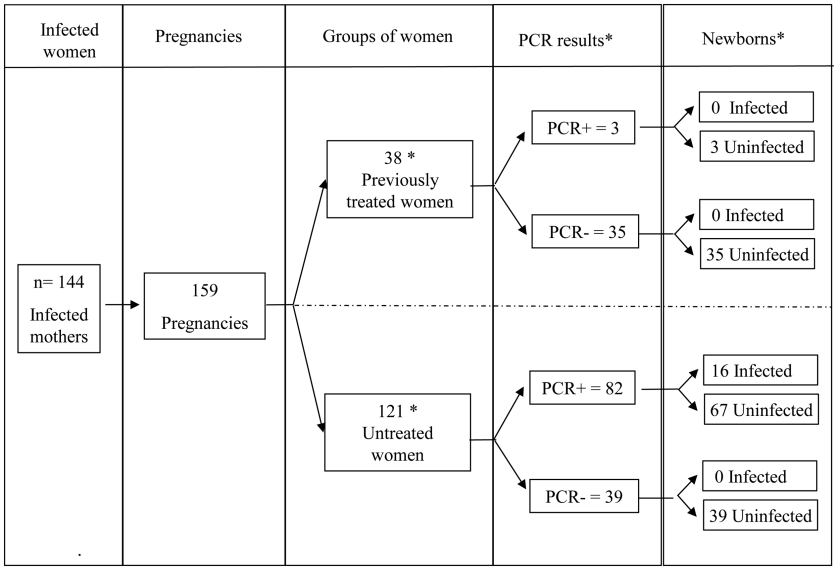

Figure 2. Comparison of vertical transmission and the PCR results of treated and untreated infected mothers.*Two mothers were pregnant twice, before and after treatment. They transmitted the infection prior to being treated.Abbreviation: PCR, polymerase chain reaction.

post-treatment, and 1 case 4 years post-treatment (data not shown).

Nevertheless, none of the treated mothers transmitted the infection to their offspring (Figure 2). In contrast, the congenital transmission rate was 13.2% among untreated mothers (16 infected infants out of 121 pregnancies). Of note, 2 untreated mothers (with a positive PCR result) who gave birth to infected newborns did not transmit the infection in a second pregnancy after they were treated, and their PCR shifted to negative. The results shown in Figure 2 reveal a statistically significant relationship between treatment of infected women and CCD prevention (P = .019; χ^2). The 13 mothers who transmitted the infection to their 16 infants (1 mother gave birth to an infected pair of twins and another mother gave birth to 3 infected infants) (Figure 2) had a positive PCR test during pregnancy. Therefore, the positive predictive value of PCR in vertical transmission was 18.8% (16 infected infants out of 85 pregnancies in which PCR was positive). In contrast, there was no transmission to 74 newborns from 74 mothers with a negative PCR result during pregnancy (Figure 2). Consequently, the negative predictive value of PCR was 100%. A statistically significant relationship was found (P = .0001; χ^2).

Treatment and Follow Up by PCR and Serology in Infected Infants

Infected infants were treated with benznidazole. All showed good tolerance (no side effects were observed). The treatment follow up was performed by serology and PCR in only 13 out of the 16 infected infants, because 1 infant was lost (infant no. 4, Table 1) and 2 infants, who continued to be monitored, were excluded from the analysis due to insufficient follow-up time after the end of treatment (infants no. 15 and 16, Table 1). All 10 infants diagnosed and treated during the first year of life were cured (infants no. 1–11, Table 1). In contrast, of the 3 infants diagnosed and treated after the first year of life, the serology remained positive after 2 (infant no. 14) and 3 (infants no. 12 and 13) years of post-treatment follow up, respectively (Table 1). Also, PCR became negative early (60–90 days after treatment) in all treated infants. Sustained negative PCR results were observed in all infants correctly adhering to the treatment. However, PCR shifted to positive in 2 infants who did not complete the treatment (Table 1)—a 6-month-old who was considered cured after a second round of benznidazole treatment (infant no. 9), and a 4-year-old child who had negative PCR result but with continuous positive serology, despite retreatment (infant no. 12).

DISCUSSION

The overall congenital transmission rate of Chagas disease detected in our study was 10%, which highlights the importance of vertical transmission in maintaining the disease and, particularly, in perpetuating infection in nonendemic areas where Chagas disease is becoming an important challenge. Among infected infants, it is important to highlight the presence of a pair of infected twins and 3 infected infants from the same mother (Table 1). Family clustering of congenital transmission has already been described, as has the observation that twin births represent a risk factor for CCD [19]. Congenital transmission occurs in 5% of pregnancies, and even at higher rates in chronically infected women in some regions of Bolivia, Chile, and Paraguay; but lower (1–2% or less) in most other endemic countries [4]. These differences might be attributable to the strain of the parasite, the immunological status of the infected mothers, placental factors, and the different methodologies used for detecting congenital cases [20].

In the present study, we analyzed the association between pregnancy and *T. cruzi* DNA detected by PCR. A positive PCR result was statistically more frequent among pregnant women (67.8%) compared with nonpregnant women (48%), probably due to the immunosuppression that occurs during gestation. This natural process of immunosuppression in pregnant women infected with *T. cruzi* may lead to an increase in the mother's parasite load, especially during the third trimester of pregnancy, a process that increases the risk of congenital transmission during this time.

As we reported in a previous study [9], women with a positive PCR result have a higher risk of *T. cruzi* transmission to their infants. In the present work, the positive predictive value of PCR in vertical transmission was 18.8%. Conversely, the 100% negative predictive value of PCR should be noted. Therefore, *T. cruzi*–infected pregnant women diagnosed by serology with a negative PCR result did not transmit the infection. This result reinforces the advisability of incorporating PCR as a screening tool for infected pregnant women. Because parasitemia detected by PCR during gestation must be considered a risk factor for CCD, newborns from mothers with a positive PCR result are required to undergo an exhaustive follow up. In contrast, newborns from mothers with a negative PCR result can be considered to have a low risk of acquiring the disease.

Sustained PCR-negative results were also observed in the majority of women who were treated before they became pregnant. Treatment failure, detected by PCR, was identified in only 7.9% of the treated mothers (Figure 2), a percentage similar to our recent findings in a cohort of chronic Chagas disease patients treated with benznidazole [21]. Nevertheless, none of the treated mothers transmitted the infection to their offspring (Figure 2). In the present study, there was a statistically significant relationship between treatment of infected women and vertical Chagas disease prevention. Our data present evidence concerning the impact of treating infected women of childbearing age as a way to avoid congenital transmission of Chagas disease, although treatment of these chronically infected women does not guarantee their cure.

Because maternal antibodies clear at 8–12 months of age, conventional serology does not have sufficient prognostic value for the early diagnosis of congenital infection [6]. The conventional parasitological techniques —culture and microhematocrite—showed low sensitivity [7, 22]. In our study, PCR showed excellent sensitivity (100%) in the 16 infected infants (Table 1) and specificity in the 144 uninfected infants (data not shown), even in infants diagnosed after their first year of life. Thus, the sensitivity of PCR in our study was higher than that previously described for infants in this age range [23].

It has been suggested that PCR performed immediately after birth could amplify the DNA of dead parasites from infected mothers [24], and several studies have shown that a positive PCR result close to the birth correlates with infection [22, 25]. Nevertheless, in our study, congenital *T. cruzi* infection in newborns was confirmed in all cases by a second sample that was taken 2–3 weeks after birth, thus discarding the possibility of false-positives results.

The importance of early diagnosis of congenital infection has been previously reported [12, 13], and almost all congenital cases have been described as cured when treatment was given before the first year of life. If the treatment of congenital newborns is postponed, the disease can progress to the undetermined chronic phase, with a lower rate of treatment success [13, 22]. Thus, in

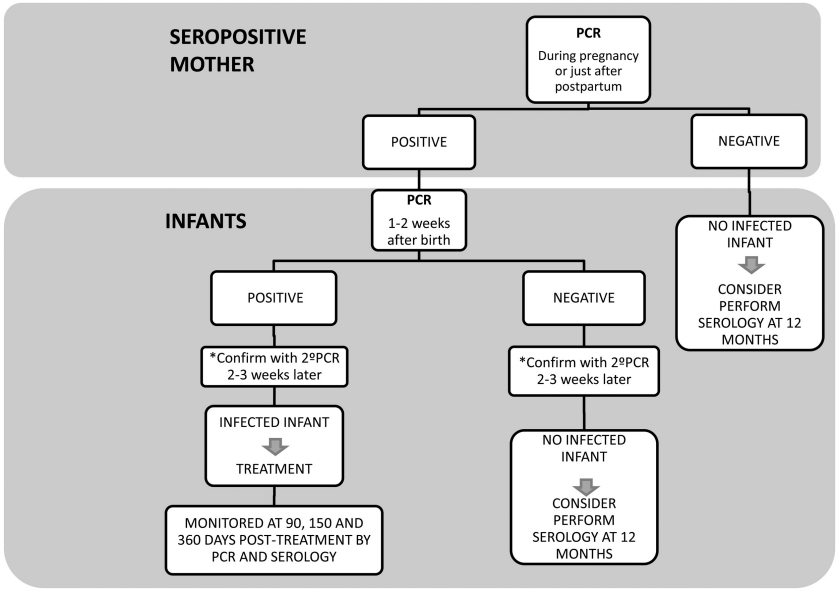

Figure 3. Diagnostic algorithm of congenital Chagas disease.*In the cases of discordance between the first PCR result and the second one, a new PCR must be perform.

this study, in the 3 cases that were diagnosed and treated after the first year of life, serology remained positive and a cure cannot be guaranteed. Conversely, those cases that were diagnosed and treated during the first year of life were cured (100% of the cases) (Table1). These results reinforce the importance of establishing techniques to allow the early and reliable diagnosis of CCD. An increasing number of studies describe PCR as a gold standard technique in the diagnosis of CCD [14, 22, 25]. Recently, nonconventional serology techniques based on the detection-specific *T. cruzi* antibodies against recombinant protein of the parasite have been proposed for the diagnosis of CCD [26, 27]. However, these techniques can only be considered an alternative to PCR in laboratories in which DNA amplification cannot be performed. As previously described, there is a further advantage of PCR for monitoring the success of treating infected infants because it detects therapeutic failures and provides near real-time knowledge on the effectiveness of the treatment [28, 29].

Taking into account that no congenital cases were detected among mothers with a negative PCR, and that no newborns with a negative PCR result became infected, we propose a change in the diagnostic algorithm of CCD (Figure 3).

Infants born from mothers with a negative PCR result during pregnancy can be considered as uninfected. Moreover, in the case of mothers with a positive PCR result during gestation, PCR of the newborns should be performed immediately after birth and 2–3 weeks later. Congenital disease can be ruled out in those babies with 2 consecutive negative PCR results. Additional serology at 9–12 months of age must be performed to ensure the absence of infection. Conversely, infants with 2 positive PCR results should be consider infected, and they should be treated immediately (Figure 3). The proposed diagnostic algorithm is better suited for health centers of nonendemic urban areas where follow up is exhaustive with good patient adherence. In contrast, in endemic areas it is ideal to have an accurate diagnosis closer to delivery before the mother abandons maternity services so that the newborn can be directly referred for treatment.

Therefore, we propose PCR screening of *T. cruzi*–infected pregnant women as a useful tool for identifying a priori those mothers who have a probability of transmitting the infection to their offspring. Additionally, taking into account that no congenital cases were detected in our study among mothers treated prior to their pregnancies, we strongly recommend the treatment of seropositive women of childbearing age as a measure to prevent the transmission of CCD.

Notes

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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