

## Congenital Transmission of *Trypanosoma cruzi* in Argentina, Honduras, and Mexico: An Observational Prospective Study

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**Abstract.** Compared with South America, there is a lack of epidemiologic studies about the risk of congenital transmission of *Trypanosoma cruzi* in Central America and Mexico. It has been suggested that *T. cruzi* genotypes might differ by region and that congenital transmission might vary according to the parasite's genotype. Our objective was to compare *T. cruzi* congenital transmission rates in three countries. We performed an observational prospective study in 2011–2014 enrolling women at delivery in one hospital in Argentina, two hospitals in Honduras, and two hospitals in Mexico. Congenital *T. cruzi* infection was defined as the presence of one or more of the following criteria: presence of parasites in cord blood (direct parasitological microscopic examination) with positive polymerase chain reaction (PCR) in cord blood, presence of parasites in infant's blood at 4–8 weeks (direct parasitological microscopic examination), and persistence of *T. cruzi*-specific antibodies at 10 months, as measured by at least two tests. Among 28,145 enrolled women, 347 had at least one antibody rapid test positive in cord blood and a positive enzyme-linked immunosorbent assay in maternal blood. PCR in maternal blood was positive in 73.2% of the cases, and genotyping identified a majority of non-TcI in the three countries. We found no (0.0%; 95% confidence interval [CI]: 0.0, 2.0) confirmed congenital case in Honduras. Congenital transmission was 6.6% (95% CI: 3.1, 12.2) in Argentina and 6.3% (95% CI: 0.8, 20.8) in Mexico. *Trypanosoma cruzi* non-TcI predominated and risks of congenital transmission were similar in Argentina and Mexico.

### INTRODUCTION

Chagas disease, or American trypanosomiasis, is caused by the protozoan parasite *Trypanosoma cruzi*. It is a major cause of cardiac disease in the Americas. An estimated six million persons are currently infected, including one million women of reproductive age.<sup>1</sup> Mothers can transmit *T. cruzi* to their infants during pregnancy, but infected newborns can be effectively treated if detected early.<sup>2,3</sup> A meta-analysis of available data showed a 5.0% congenital transmission rate in endemic countries, based mostly on studies from South America.<sup>4</sup> Before the current study, there were only limited data about transmission rates in Mexico and Central America. A single confirmed case of congenital transmission has been previously reported in Mexico.<sup>5</sup> Other Mexican studies relied mostly on polymerase chain reaction (PCR) on cord blood to diagnose congenital transmission of *T. cruzi*.<sup>6–8</sup> A recent study identified one case of congenital Chagas disease among 32 infected mothers in El Salvador.<sup>9</sup>

It has been suggested that the congenital transmission rate might vary according to the parasite's genotype, but available data are inconclusive.<sup>10–13</sup> *Trypanosoma cruzi* genotypes have been grouped into discrete typing units (DTUs), with TcI

being assumed to be more frequent in Mexico and Central America and non-TcI (TcII–VI) being dominant in the Southern Cone region of South America.<sup>14,15</sup> However, recent studies suggest that TcI might not be predominant in all regions of Mexico.<sup>16</sup>

A better knowledge of the risk of congenital transmission is a prerequisite to the establishment of universal screening programs for *T. cruzi* infection among pregnant women and infants.<sup>17,18</sup> Institutional delivery is almost universal in Latin America, allowing for hospital recruitment of study populations.<sup>19</sup> The objective of this study was to compare *T. cruzi* congenital transmission rates in Argentina, Honduras, and Mexico, using a common methodology.

### MATERIALS AND METHODS

The protocol was previously published,<sup>20</sup> and the study was registered in ClinicalTrials.gov: Identifier NCT01787968.

**Study design and participants.** This was an observational prospective study conducted in one hospital in Argentina (Instituto de Maternidad y Ginecología Nuestra Señora de las Mercedes, Tucumán), two hospitals in Honduras (Hospital Enrique Aguilar Cerrato, La Esperanza, Intibucá and Hospital Santa Bárbara Integrado, Santa Bárbara), and two hospitals in Mexico (Hospital Materno Infantil, Mérida, Yucatán and Hospital General de Valladolid, Valladolid, Yucatán).

During 2011–2013, we enrolled women at delivery and collected umbilical cord blood to measure antibodies of

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maternal origin. We collected blood with needle and syringe from the placental side after clamping and sectioning the cord and after cleaning it with a sterile gauze pad. We obtained written informed consent immediately after delivery and before testing the cord blood. The eligible women were 18 years old or more, had a live birth, and resided inside of the follow-up areas (Province of Tucumán, Argentina; Departments of Intibucá and Santa Bárbara, Honduras; States of Campeche, Quintana Roo, and Yucatán, Mexico). If one of the cord blood rapid antibody tests was positive, maternal infection was confirmed by serology in maternal blood collected immediately after delivery. Seropositive mothers had at least one positive antibody rapid test on cord blood and a positive enzyme-linked immunosorbent assay (ELISA) on maternal blood. Those newborns with at least one positive antibody rapid test for *T. cruzi* in cord blood were tested with a direct parasitological examination on cord blood and were followed up at their homes at 4–8 weeks and 10 months postpartum by a dedicated and trained team in each country. At the 4–8 weeks postpartum home visit, we repeated the parasitological examination on those infants with a negative examination on cord blood. At the 10-month postpartum visit, we collected venous blood for serology on all followed up infants. During the visits, infants' siblings who assented or consented to participate were tested for *T. cruzi* infection. Infected newborns and siblings were referred for treatment according to local standards, which included a course of 30–60 days of benznidazole or nifurtimox.

**Laboratory procedures.** We performed two antibody rapid tests in cord blood and at 10 months: Stat-Pak (Chembio, Medford, NY) and *Trypanosoma* Detect (T-Detect) (InBios, Seattle, WA). We also used Stat-Pak among siblings. We performed serological confirmation by ELISA (Chagatest ELISA recombinant, version 3.0; Wiener, Rosario, Argentina) in cord and maternal blood and at 10 months of age. A second confirmatory ELISA (Chagas Kit; Hemagen, Columbia, MD) was performed for 10 months samples that were positive for one test or more. We did direct microscopic examination of blood buffy coat in six centrifuged microhematocrit heparinized tubes per sample, after training all laboratory technicians and within a maximum of 6 hours after collecting the samples.<sup>21</sup> The samples to be stored for further molecular testing were immediately mixed with the same volume of guanidine-HCl 6 M, ethylenediaminetetraacetic acid (EDTA) 0.1 M (pH 8). We performed PCR followed by detection of the amplicons by gel electrophoresis on maternal and cord blood using two specific primer sets: Tcz1–Tcz2 and 121–122.<sup>22</sup> The amount of blood collected at 4–8 weeks was not sufficient for PCR. A positive PCR was positive for at least one of the primer sets. Maternal parasitic load was determined by real-time quantitative PCR (qPCR) with Tcz1–Tcz2 primers.<sup>23</sup> A multiplex PCR amplifying the miniexon genes intermediate region allowed us to discriminate the *T. cruzi* DTUs present in maternal blood samples as TcI and non-TcI.<sup>11</sup> A limited number of amplicons were sequenced at Beckman Coulter Genomics (Takeley, United Kingdom) using the Sanger method for confirmation of genotyping. All molecular analyses, except sequencing, were performed in Brussels, Belgium, and were conducted under strict quality control. The molecular methods that were not detailed in the published protocol<sup>20</sup> are included in the Supplemental Material (appendix).

**Outcomes.** The primary outcome was the congenital *T. cruzi* infection, defined as the presence of one or more of the following criteria: presence of parasites in cord blood (direct parasitological microscopic examination) with positive PCR in cord blood; presence of parasites in infant's blood at 4–8 weeks (direct parasitological microscopic examination); and persistence of *T. cruzi*-specific antibodies at 10 months, as measured by at least two tests. The primary outcome analyses were performed among newborns with at least one positive antibody rapid test on cord blood and a positive Wiener ELISA on maternal blood.

**Statistical analysis.** We described the maternal and newborn characteristics and the results of serological tests, PCR, qPCR, and sequencing. Sequences were analyzed by BLAST,<sup>24</sup> edited manually and aligned with *T. cruzi* reference sequences for DTU assignment using MEGA version 5.2.<sup>25</sup> For the primary outcome, we reported the proportions with the exact binomial confidence interval.  $\chi^2$  and Fisher's exact tests were used to compare proportions. We used the Bonferroni correction to compare the proportions between the three countries. We used Kruskal–Wallis tests to compare median equivalent parasites per millilitre (eP/mL). Statistical significance was defined as  $P < 0.05$ . Tests are two-sided. We used SAS (Cary, NC) version 9.3 for data analysis.

**Ethics.** All participating women and siblings provided informed consent or assent to participate in the study. The study was approved by the Tulane University Institutional Review Board, and the Ethics Committees of the Centro de Educación Médica e Investigaciones Clínicas “Norberto Quirno” (CEMIC), Argentina, the Facultad de Ciencias Médicas, Universidad Nacional Autónoma de Honduras, and the Universidad Autónoma de Yucatán, Mexico.

## RESULTS

The study took place between April 2011 and December 2013. Figure 1 shows that 46,421 (78.7%) of the 59,000 women who delivered during the study period were screened for eligibility and that 28,145 were enrolled (9,390 in Argentina, 6,595 in Honduras, and 12,160 in Mexico). Table 1 shows that maternal and neonatal characteristics differed between countries ( $P < 0.01$ ). Enrolled women in Mexico were less likely to be 35 years or older, more likely to be primiparae, and more likely to have a high school or higher education. Honduras had the lowest cesarean section rate. The low birthweight (<2,500 g) rate and the rate of preterm (< 37 weeks) births were low in Mexico.

We identified 495 (1.8%) women with at least one antibody rapid test positive in the cord blood of at least one of their 503 (1.8%) newborns, and we followed-up more than 95% of them (Figure 1). Mothers of newborns with at least one antibody rapid test positive in cord blood also had a positive Wiener ELISA in 87.0% (134/154) of the cases in Argentina and in 78.7% (181/230) of the cases in Honduras, but in only 29.4% (32/109) of the cases in Mexico ( $P < 0.01$ ) (Table 2). The primary outcome analyses were performed among the 350 newborns of the 347 seropositive mothers.

Table 2 shows that PCR in maternal blood was positive in more than 70% of the cases in Argentina (105/134) and Honduras (132/181), but in only 53.1% (17/32) of the cases in Mexico ( $P = 0.015$ ), and that genotyping identified a predominance of non-TcI in the three countries. We obtained

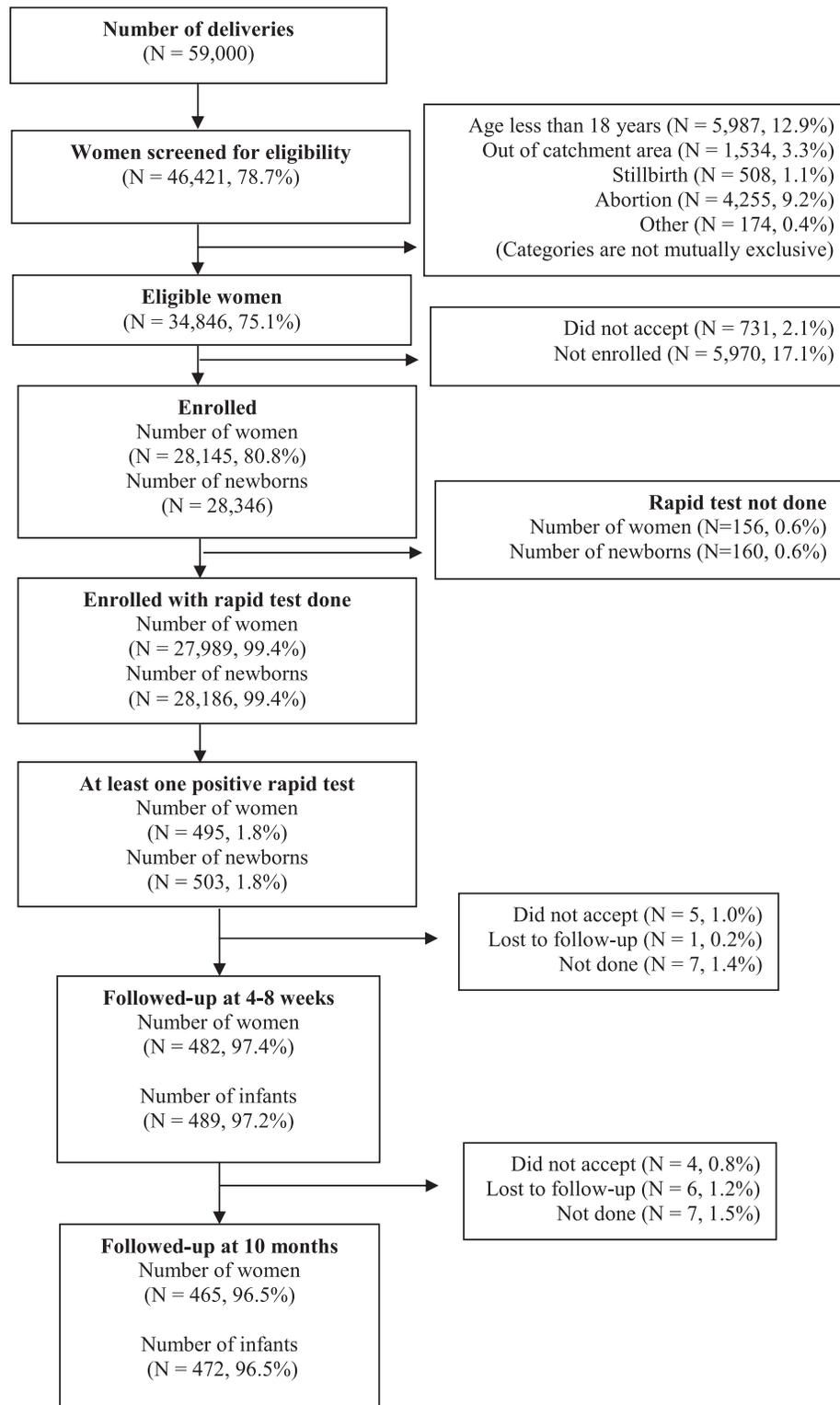


FIGURE 1. Recruitment flow chart.

sequencing of the PCR products in 31 of 107 genotyped maternal samples (13 from Argentina, 17 from Honduras, and one from Mexico). Sequence analyses confirmed PCR genotyping in all cases of mothers that were infected with TcI or non-TcI only, whereas in the case of maternal mixed infection, we obtained the sequences for both TcI and non-TcI in two

mothers and the sequence for one genotype for others. In total, we detected by sequencing: TcI only in 16.1% (5/31), non-TcI in 77.4% (24/31), and mixed infection TcI and non-TcI in 6.5% (2/31) of maternal samples. Parasitic load measured by qPCR was not statistically significantly different in the three countries ( $P = 0.089$ ) (Figure 2), with a median of 3.4 eP/mL in

TABLE 1  
Maternal and neonatal characteristics by country

	Argentina (number of women = 9,390) (number of infants = 9,497)		Honduras (number of women = 6,595) (number of infants = 6,654)		Mexico (number of women = 12,160) (number of infants = 12,195)	
	n/N	%	n/N	%	n/N	%
Maternal age (years)*						
18–19	1,291/9,390	13.8	969/6,595	14.7	1,914/12,160	15.7
20–34	7,080/9,390	75.4	4,883/6,595	74.0	9,322/12,160	76.7
≥ 35	1,019/9,390	10.9	743/6,595	11.3	924/12,160	7.6
Previous deliveries*						
0	4,536/9,384	48.3	2,724/6,595	41.3	6,677/12,137	55.0
≥ 1	4,848/9,384	51.7	3,871/6,595	58.7	5,460/12,137	45.0
Education*						
Elementary school or lower	5,766/9,122	63.2	4,820/6,593	73.1	3,932/12,085	32.5
High school or higher	3,356/9,122	36.8	1,773/6,593	26.9	8,153/12,085	67.5
Mode of delivery*						
Vaginal	5,697/9,388	60.7	5,217/6,595	79.1	6,501/12,150	53.5
Cesarean section	3,691/9,388	39.3	1,378/6,595	20.9	5,649/12,150	46.5
Birthweight (g)*						
< 2,500	761/9,491	8.0	572/6,654	8.6	697/12,194	5.7
≥ 2,500	8,730/9,491	92.0	6,082/6,654	91.4	11,497/12,194	64.3
Gestational age (weeks)*						
< 37	1,126/9,452	11.9	342/6,610	5.2	580/12,156	4.8
≥ 37	8,326/9,452	88.1	6,268/6,610	94.8	11,576/12,156	95.2

\*  $P < 0.01$ .

Argentina, 3.3 eP/mL in Honduras, and 2.5 eP/mL in Mexico. PCR tests on cord blood of seropositive mothers were positive in 45.6% (62/136) of the cases in Argentina, 69.8% (127/182) in Honduras, and 24.1% (7/29) in Mexico.

Congenital transmission as defined previously was 6.6% (9/136) in Argentina, including two cases identified by parasitological examination (filmed) and PCR at birth, one case by parasitological examination (filmed) at 4–8 weeks, and six cases with at least two positive serological tests at 10 months (Table 3, Supplemental Table 1). A similar transmission rate of 6.3% (2/32) was found in Mexico, with one case identified by parasitological examination (not filmed) and PCR at birth and another one identified at 10 months. We found no confirmed case in Honduras, which had a statistically significant lower

transmission rate than the one observed in Argentina ( $P = 0.001$ ) but not in Mexico ( $P = 0.065$ ). All Argentine cases received treatment, and the Mexican cases were referred but not treated. The Mexican case identified at birth by positive parasitological examination and positive PCR had negative serological tests at 10 months (neither positive antibody rapid test nor positive ELISA), despite the fact that it was not treated.

PCR on maternal blood was positive in eight of the nine Argentine congenital cases and in both Mexican cases. Genotyping on maternal blood was successfully performed in four Argentine cases and in one Mexican case, and all cases in Argentina and the one in Mexico showed non-Tcl DTUs. The genotyping was confirmed by sequencing in three Argentine cases. There was a nonstatistically significant trend for higher

TABLE 2

Maternal *Trypanosoma cruzi* serology at delivery and polymerase chain reaction (PCR) and genotyping in maternal blood from seropositive mothers (with at least one positive antibody rapid test in cord blood and a positive enzyme-linked immunosorbent assay [ELISA] Wiener in maternal blood) by country

	Argentina (N = 9,390)		Honduras (N = 6,595)		Mexico (N = 12,160)	
	n/N	%	n/N	%	n/N	%
Rapid tests in cord blood						
Both rapid tests positive	129/9,339	1.4	183/6,583	2.8	38/12,067	0.3
Only Stat-Pak positive	22/9,339	0.2	39/6,583	0.6	36/12,067	0.3
Only T-Detect positive	4/9,339	0.1	9/6,583	0.1	35/12,067	0.3
ELISA Wiener in maternal blood* †						
Positive	134/154	87.0	181/230	78.7	32/109	29.4
Negative	20/154	13.0	49/230	21.3	75/109	68.8
Inconclusive	0/154	0.0	0/230	0.0	2/109	1.8
PCR in maternal blood‡ §						
Positive	105/134	78.4	132/181	72.9	17/32	53.1
Negative	29/134	21.6	49/181	27.1	15/32	46.9
Genotyping in maternal blood‡						
Only non-Tcl	23/105	21.9	54/132	40.9	6/17	35.3
Only Tcl	6/105	5.7	1/132	0.8	1/17	5.9
Both Tcl and non-Tcl	6/105	5.7	10/132	7.6	0/17	0.0
Undetermined	70/105	66.7	67/132	50.8	10/17	58.8

\* Among women with at least one positive rapid test in cord blood.

†  $P < 0.01$ .

‡ Among women with at least one positive rapid test in cord blood and a positive ELISA Wiener in maternal blood.

§  $P = 0.015$ .

||  $P = 0.009$ .

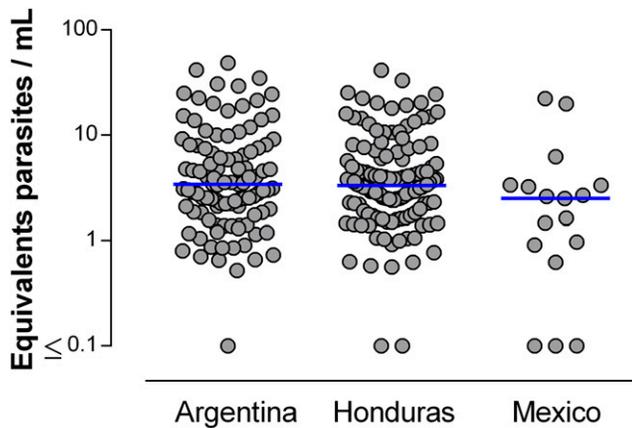


FIGURE 2. Parasitic load (equivalent parasites/mL) measured by real-time polymerase chain reaction (PCR) in maternal blood from seropositive mothers (with at least one positive antibody rapid test in cord blood and a positive enzyme-linked immunosorbent assay Wiener in maternal blood) by country, with medians. This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

parasitic load in Argentine mothers of congenital cases than in nontransmitting *T. cruzi* seropositive women (median parasitic load: 7.5 eP/mL versus 3.1 eP/mL;  $P = 0.088$ ), whereas parasitic loads were similar in Mexican mothers (median parasitic load: 2.1 eP/mL versus 2.5 eP/mL;  $P = 1.00$ ).

In Argentina, three of the nine congenital cases had a premature rupture of the membranes (PROM), and among them one was born preterm (< 37 weeks) and admitted to a special care unit. In one case with PROM delivered at 37 weeks of gestation, the newborn was hospitalized in an intensive care unit at 15 hours of life for multiple symptoms including hepatomegaly, jaundice, and sepsis and was discharged alive after

17 days. The Mexican case identified at birth had an Apgar below 7 at 1 minute, and the other Mexican case had no complications.

We found 12 infected siblings in Argentina, three in Honduras, and none in Mexico (Table 4). One sibling in Argentina was previously treated for *T. cruzi* infection. No sibling was previously treated for *T. cruzi* infection in Honduras. All non-previously treated siblings identified in Argentina were treated; all siblings in Honduras were referred to treatment.

## DISCUSSION

To our knowledge, this is the first study using a common methodology to study congenital Chagas disease in three countries. We assembled a population-based sample by systematically recruiting women at delivery and by successfully following up more than 95% of them. All molecular analyses were performed in a single laboratory, and an independent data center monitored and analyzed the data and ensured that all laboratory analyses were blinded.

The 6.6% congenital transmission rate we observed in Argentina is similar to what was observed in previous studies.<sup>4</sup> There was a trend for parasitic load in maternal blood to be higher among congenital cases than among the general population of *T. cruzi* seropositive women, as suggested by others.<sup>10,26,27</sup> Using the same criteria as the ones used by Torrico et al.,<sup>28</sup> 33.3% (3/9) of cases from Argentina can be classified as “symptomatic newborns,” which is similar to what the same authors observed in their more recent cohort from Bolivia. Torrico et al. also found a frequency of PROM of 36.7%, again very similar to what we found in Argentina. We found and treated 12 infected siblings in Argentina, showing an additional benefit of identifying seropositive mothers.

TABLE 3

Parasitological examination and cord blood polymerase chain reaction (PCR) and *Trypanosoma cruzi* serology at 10 months in infants born from seropositive mothers (with at least one positive antibody rapid test in cord blood and a positive enzyme-linked immunosorbent assay [ELISA] Wiener in maternal blood) by country

	Argentina (N = 136)		Honduras (N = 182)		Mexico (N = 32)	
	n/N	% [CI]*	n/N	% [CI]*	n/N	% [CI]*
<b>At birth</b>						
Positive direct parasitological examination	2† ‡/136	1.5	4‡ §/181	2.2	2‡ §/28	7.1
Positive cord blood PCR among infants with positive parasitological examination	2/2	100.0	0/4	0.0	1/2	50.0
<b>At 4–8 weeks</b>						
Positive direct parasitological examination	1† ‡/129	0.8	0/175	0.0	0/27	0.0
<b>At 10 months</b>						
Positive Stat-Pak	8/114	7.0	8/172	4.7	2/28	7.1
Positive T-Detect	7/113	6.2	3/172	1.7	0/28	0.0
Positive ELISA Wiener	6/115	5.2	0/172	0.0	0/28	0.0
Positive ELISA Hemagen	6/8	75.0	0/11	0.0	1/1	100.0
At 10 months by at least two positive serological tests	6† ‡/113	5.3	0/172	0.0	1§/28	3.6
Congenital transmission¶  #	9† ‡/136	6.6 [3.1, 12.2]	0/182	0.0 [0.0, 2.0]	2§/32	6.3 [0.8–20.8]

\* CI denotes 95% confidence interval.

† Received treatment.

‡ Serology at 10 months was negative.

§ Referred for treatment but not treated.

|| Among infants with at least one positive rapid test and/or a positive ELISA Wiener.

¶ Numerator: positive direct parasitological examination and positive cord blood PCR at birth, positive direct parasitological examination at 4–8 weeks, or two positive serological tests at 10 months. Denominator: at least one direct parasitological examination and cord blood PCR at birth, direct parasitological examination at 4–8 weeks, and/or at least two serological tests at 10 months.

#  $P < 0.01$ . Bonferroni correction applied. Argentina vs. Honduras ( $P = 0.001$ ), Argentina vs. Mexico ( $P = 1.00$ ), Mexico vs. Honduras ( $P = 0.065$ ).

TABLE 4

*Trypanosoma cruzi* serology among siblings born from seropositive mothers (with at least one positive antibody rapid test in cord blood and a positive enzyme-linked immunosorbent assay (ELISA) Wiener in maternal blood) by country

	Argentina (N = 246)		Honduras (N = 408)		Mexico (N = 53)	
	n/N	%	n/N	%	n/N	%
Tested during follow-up	209/246	85.0	362/408	88.7	50/53	94.3
Positive Stat-Pak	21/207	10.1	17/362	4.7	1/50	2.0
Positive ELISA Wiener	13/207	6.3	3/354	0.9	0/47	0.0
Stat-Pak and ELISA Wiener both positive	12/205	5.9	3/354	0.9	0/47	0.0
Referred to treatment	12/12	100.0	3/3	100.0	–	–

The congenital transmission rates observed in Mexico were comparable to Argentina. The case of congenital infection detected at birth in Mexico was referred to treatment but has not been treated yet because of the limited access to treatment in that country.<sup>29</sup> Interestingly, this infant was seronegative at 10 months, suggesting a possible self-cure, as proposed elsewhere.<sup>10</sup> We discarded one cord blood parasitological examination which was classified as positive at first, but had negative PCR on cord blood, was not treated, and was seronegative at 10 months. We cannot exclude that the case identified at 10 months was vector-borne because *Triatoma dimidiata* is present around and inside houses in Yucatán.<sup>30</sup> In a previous household survey in two Mayan villages, we found three children infected by *T. cruzi*, and one of them had a *T. cruzi* seropositive mother.<sup>31</sup>

We did not confirm any case of congenital transmission in Honduras. We had to discard the four parasitological examinations which were classified as positive at first because they were not filmed and were assessed as doubtful by the supervising teams. Moreover, they had a negative PCR on cord blood, were not treated, and were all seronegative at 10 months. The possibility of self-cure, as suggested for the Mexican case previously, seems less probable here since PCR, which is more sensitive than microscopic examination, was negative in cord blood. Our results do not mean that congenital transmission of *T. cruzi* does not occur in Honduras.

Our results pinpoint shortcomings concerning the diagnosis of *T. cruzi* infection, congenital or not. Serological tests need to be improved. Our previous study in four countries showed that Stat-Pak sensitivity was 94.6%, with the Wiener ELISA as the gold standard, but that the sensitivity was lower in Mexico (62.5%) than in Argentina (85.3%) and Honduras (95.4%).<sup>32</sup> We added a second antibody rapid test (T-Detect) in our present study to increase the sensitivity of our initial screening on cord blood. Table 2 shows that in Mexico, 32.1% of positive cord blood antibody rapid tests were positive for T-Detect only. Another challenge is that discrepancies between *T. cruzi* ELISAs were previously reported in Mexico.<sup>33</sup> We also found more discrepancies between serological tests in Mexico than in the other countries (Table 2). The lack of sensitivity of antibody rapid tests and ELISAs might have been a source of underestimation of cases at 10 months.

Our results also strongly highlight the urgent need to improve diagnostic tools to reliably detect congenital infections as soon as possible. Among nine Argentine cases, only three were detected by parasitological examination in cord blood or at 4–8 weeks, confirming previous reports showing that direct parasitological examination sensitivity is limited.<sup>34</sup> We also found that the positive predictive value of direct parasitological examination was low in Honduras and Mexico, despite

extensive training of the laboratory technicians and close supervision. A low positive predictive value is expected when the prevalence is low,<sup>35</sup> and identifying live *T. cruzi* parasites when they have never been detected can be difficult. Parasitological examinations were performed within a maximum of 6 hours after collecting the samples, and a second observer was often not available. We recommend filming parasitological examinations with smartphones to document the findings and to allow an evaluation by a second observer, which we performed in Argentina.

PCR on cord blood was positive in 69.8% of the newborns in Honduras, 45.6% in Argentina, and 24.1% in Mexico, suggesting a highly sensitive PCR (see Supplemental Material). The sensitivity of the PCR might have been increased by high performance DNA extraction, the use of GelRed™ nucleic acid stain, and the classification as positive of very faint amplicon bands, which were more frequently found in cord blood than in maternal blood samples (results not shown). Detection of DNA fragments without live parasites is another potential cause of the high frequency of positive cord blood PCR. Mouse models of congenital transmission showed up to 62% positive PCR among noninfected pups born to infected mothers, suggesting that DNA fragments were detected rather than live parasites.<sup>23</sup> Maternal plasma DNA has been shown to cross the placental barrier into the fetal circulation.<sup>36</sup> An early study found only 3% positive PCR among noninfected infants from infected mothers in Argentina.<sup>37</sup> A study in Bolivia found no positive cord blood PCR among 109 uninfected infants but found transient positive PCR in one 7-day sample from a noninfected infant.<sup>34</sup> A more recent study of 487 infants of *T. cruzi* infected mothers in Bolivia found higher numbers of false positive and borderline qPCR results.<sup>38</sup> Another recent study found 20.0% positive PCR on cord blood among *T. cruzi* seropositive mothers in Guanajuato, Mexico.<sup>7</sup> In Argentina, PCR positive rates as high as 30.8% in Jujuy and 27.2% in Formosa have been recently reported.<sup>39</sup> PCR on cord blood is not universally accepted for diagnosing congenital Chagas disease at birth.<sup>3</sup> We, therefore, only used cord blood PCR negative results to identify false positive direct parasitological examinations because of reading errors.

Our data confirmed the expected predominance of non-Tcl in Argentina but surprisingly indicated a very similar situation in the two other countries, contrary to earlier reports.<sup>14</sup> The high frequency of non-Tcl DTUs in Honduras and Mexico is in agreement with the recent findings of a greater diversity than previously reported in the *T. cruzi* DTUs circulating in Mexico and the US.<sup>16,40–42</sup> It confirms the high frequency of non-Tcl infections in Mexican patients determined by lineage-specific serology.<sup>43</sup> Our genotyping by PCR was limited to discriminating between Tcl and non-Tcl because of the very short

sequence length of the marker used, and differences among non-TcI DTUs should be studied further. PCR on maternal blood was less often positive in Mexico than in the other countries (Table 2), but when the PCR was positive, maternal parasitic load was similar in the three countries. This is somewhat surprising, with vector control programs being at different stages in each country. In Argentina, the interruption of transmission by *Triatoma infestans* in the Province of Tucumán was certified in 2015.<sup>44</sup> Honduras eliminated *Rhodnius prolixus* but is still exposed to other triatomines.<sup>45</sup> Chagas vector control programs are very limited in Mexico.<sup>46</sup> Some have found that repeated vectors exposure increases parasitic load,<sup>47</sup> but others have found the opposite.<sup>26</sup> One limitation of our study is that we do not have information on the country of origin of the mothers, but census data show that less than 0.5% of women residing in our study areas were born abroad.<sup>48–50</sup>

We conclude that mothers are predominantly infected by non-TcI *T. cruzi* parasites across participating hospitals and *T. cruzi* DTU distributions in Mexico and Central America need to be further investigated. Similar risks of congenital transmission exist in Mexico and Argentina.

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