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Seroprevalence of *Trypanosoma cruzi* Among Mothers and Children in Rural Mayan Communities and Associated Reproductive Outcomes

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Abstract. Our objective was to determine the seroprevalence of *Trypanosoma cruzi* infection among mothers and children in two rural Mayan communities in Yucatan, Mexico and examine sociodemographic characteristics and adverse reproductive outcomes associated with maternal infection. We performed household surveys in the communities of Sudzal and Teya. Mothers were interviewed, and blood samples were obtained to perform rapid tests and enzyme-linked immunosorbent assays (ELISAs). We surveyed 390 mothers and 685 children. The overall seroprevalence was 2.3% among mothers and 0.4% among children. In Sudzal, we found a seroprevalence of 4.4% among mothers and 0.7% in children. In Teya, we found a seroprevalence of 0.9% among mothers and 0.3% among children. Compared with uninfected mothers, seropositive mothers reported more stillbirths (relative risk = 4.7; 95% confidence interval = 2.1–10.4). *T. cruzi* infection is present in these communities, and infected children indicate active transmission. Seropositivity in mothers is associated with a history of adverse reproductive outcomes.

INTRODUCTION

The protozoan parasite *Trypanosoma cruzi*, which causes Chagas disease (American trypanosomiasis), currently infects about 10 million people in Latin America.[1] Parasites are transmitted primarily by blood-sucking triatomine insects but also, through blood transfusion or from a mother to her fetus. In most cases, infection is acquired early in life and later, causes cardiac arrhythmias, chronic cardiomyopathy, and heart failure.[2] Congenital infections occur in children of chronically infected mothers who were, in most cases, themselves infected through insect vectors during their childhoods in endemic areas of Latin America.[3] Studies about miscarriages and stillbirths among women infected with *T. cruzi* have been inconclusive.[4] Autopsies have shown cases of stillbirths infected by *T. cruzi*,[5] but how often this infection occurs is unclear.

In contrast with South America, information regarding *T. cruzi* infection in Mexico is very limited.[6] Cases of acute human infection as well as chronic cardiomyopathy have been documented in the Yucatán peninsula in southern Mexico, an area considered endemic for Chagas disease.[7–9] Estimates of seroprevalence dating from the 1980s have ranged from less than 1%[10] to more than 10%.[11,12] To our knowledge, population-based seroprevalence data among mothers and children are not available for Yucatán, and no prevention program targeting infants and children is in place.

Entomologic studies have shown that the main insect vector in Yucatán is *Triatoma dimidiata* and that it has good vectorial capacity for *T. cruzi* transmission.[13–15] However, there is limited information about the frequency of transmission of *T. cruzi* by *T. dimidiata* in the field.[16]

We estimated in a previous article that about 37,000 pregnant women and 1,800 newborns are likely to be infected with *T. cruzi* in Mexico.[17] In a recent study, we identified 5 seropositive mothers of 500 women who gave birth at an urban hospital in Mérida, Yucatán.[18] Newborn children of seropositive mothers were tested for presence of *T. cruzi* antibodies 10 months after birth to determine potential congenital infection. None of the children were seropositive. One newborn of an infected mother died of cardiac arrest at 2 weeks of age, but *T. cruzi* infection was not confirmed.[19] The study was part of a multicenter research project on the prevalence of *T. cruzi* infection among women and their infants at delivery in Argentina, Bolivia, Honduras, and Mexico. It also validated the use of a rapid blood test (Chagas Stat-Pak; Chembio, New York, NY) combined with enzyme-linked immunosorbent assay (ELISA) tests. A limitation of the study was that it considered only congenital transmission, whereas infants and children are likely to also be at risk for vectorial transmission.

The purpose of this study was to determine the prevalence of *T. cruzi* infection in mothers and children residing in underprivileged rural Mayan communities of Yucatán, a population considered to be at high risk of vector exposure.[9,14,20] The study also examined sociodemographic characteristics and adverse reproductive outcomes associated with maternal infection.

MATERIALS AND METHODS

Design. We performed a household serological survey of children 0–12 years of age and their mothers in two rural Mayan villages from December 11, 2008 to March 25, 2009 (Sudzal and Teya). The 2005 census of Mexico reported that the total population of Sudzal and Teya was 3,107.[21] The number of children 0–12 years old was estimated to be 301 in Sudzal and 452 in Teya. These villages previously participated in an entomologic survey of Chagas disease vectors.

Community information sessions. With the assistance of public health students and in collaboration with local authorities, the research coordinator informed the community about the study. We held information sessions for parents and students at local kindergartens, elementary schools, and high schools and distributed brochures with information about Chagas disease, the insect vector, the importance of early diagnosis, and the study.

Households georeferencing. Following procedures developed by the Centers for Disease Control and Prevention, we

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used handheld personal digital assistants (PDAs) equipped with global positioning system (GPS) units to collect the latitude and longitude of each household.[22] Interview data were directly entered into the PDAs. Data were stored on secure digital memory cards and transferred to a laptop computer. Unique study identifiers were used, and all data were confidential.

Data collection and management. After georeferencing all households, we conducted household surveys. We used the PDAs to select households to be included in a given day and navigate to them. A medical student and a local research assistant carried out the survey. They were trained to use the PDA, contact the mothers, obtain informed consent, conduct interviews, perform blood collection, and enter the data into the PDAs. The medical student and research assistant stayed in the villages during the week, and the research coordinator supervised the work during the first 2 weeks of data collection in each village and two times per week thereafter.

We visited each household in the villages and asked if children ages 0–12 years old lived there. Repeated visits to households were performed as needed until the presence or absence of children was confirmed. If children were present, the study was explained to the mother. Informed consent was obtained from mothers who agreed to participate in the study. Children 7–12 years old were asked to assent to the study.

We interviewed the mothers and collected data about each individual's age, sex, education level, and history of blood transfusion. We asked the mothers about their knowledge of *T. dimidiata* or the pik (as the insect is called locally), if they knew that it transmitted a disease, and if they had been bitten by triatomines themselves or if someone in the household had been bitten. We also asked the mothers about their reproductive health history using information from their health cards when available. When health cards were not available, we collected data based on the mothers' recall. We asked how many miscarriages, stillbirths, and Cesarean sections each mother had and whether they had children who died within 1 month of birth.

Mothers of confirmed seropositive children and/or confirmed seropositive mothers were informed and advised to contact the local health center for follow-up. Repeated household visits were performed until follow-up at the health center was confirmed. At local health centers, patients with confirmed *T. cruzi* infection are evaluated for clinical signs of Chagas disease and referred to higher-level facilities if necessary. The national standard of care is to treat cases of Chagas disease with nifurtimox or benznidazole if they are confirmed by the National Institute of Epidemiological Reference and Diagnosis in Mexico (Instituto de Diagnóstico y Referencia Epidemiológico [InDRE]).

Laboratory procedures. Seven drops of blood (140 μL) were collected from the finger of the child or the mother by finger prick. Blood was collected in 1.5-mL Eppendorf tubes, and the tubes were stored at 4°C for subsequent testing in the laboratory, where we performed Chagas Stat-Pak (Chembio Diagnostic Systems, Medford, NY) rapid tests and ELISA tests (Chagatest Recombinant ELISA, version 3.0; Wiener Laboratories, Rosario, Argentina). Previous studies have reported sensitivity and specificity higher than 99% for the Wiener ELISA.[23] The Chagas Stat-Pak is a rapid immunochromatographic screening test for detection of antibodies against *T. cruzi* in plasma, serum, or whole blood. Compared with the Wiener ELISA, sensitivities and specificities higher than 99% have been shown for the Chagas Stat-Pak in samples from Nicaragua, El Salvador, and Honduras.[24] Studies from Peru have shown lower sensitivity of 26.6–33.0% and lower specificity of 97.7–100%.[25] We studied 2,495 pregnant women in Argentina, Bolivia, Honduras, and Mexico and found a sensitivity of 94.6% and a specificity of 99.0% for the Chagas Stat-Pak in cord blood compared with the Wiener ELISA in maternal blood.[18] We also compared these commercial tests (Stat-Pak and ELISA Wiener) with non-commercial tests using a local Mexican strain of *T. cruzi* and found similar results for both tests in umbilical cord blood samples.[19]

Blood samples were centrifuged, and serum samples (5 μL) were used for Stat-Pak rapid tests according to the manufacturer's instructions. Stat-Pak results were read 15 minutes after the introduction of serum and recorded by digital photograph.

Serum samples (10 μL) were used to perform Wiener ELISA tests according to the manufacturer's instructions. The criteria for evaluating the results were (1) readings of at least two of three negative controls corrected for the reaction to the blank must be less than or equal to 0.150 optical density (OD) and (2) average reading of positive corrected controls must be greater than or equal to 0.600 OD as instructed by the manufacturer. If one or both conditions were not met, we repeated the tests. The presence or absence of antibodies for *T. cruzi* was determined by comparing the OD of the sample with the cutoff. The cutoff was defined as equal to the average readings of the negative controls plus 0.300 OD. The undetermined area was ±10% of the cutoff.

Seropositive cases were reported to InDRE, and results were confirmed using immunofluorescence antibody (IFA) and ELISA tests. Seropositivity was defined as two reactive test results.

Data analysis. We analyzed the relationship between sociodemographic characteristics and knowledge of the vector in mothers and *T. cruzi* seroprevalence in mothers and children. Next, we took maternal age into account as a potential confounding factor and restricted the analysis to mothers 36 years old and older. We also analyzed the relationship between *T. cruzi* infection and reproductive health history for all ages and mothers 36 years old and older, again considering age as a confounding factor. We assumed that *T. cruzi* infection in mothers occurred during childhood and may have affected women's subsequent reproductive health history.

Fisher's exact tests were used for univariate analyses. Relative risks (RRs) and 95% confidence intervals (95% CIs) were also calculated for reproductive history. Statistical analyses were performed using SPSS Statistics 17.0 (Chicago, IL) and OpenEpi 2.3.1.[26]

Ethical approvals. We obtained approval from the Autonomous University of Yucatan Ethics Committee and the Tulane University Institutional Review Board.

RESULTS

The study team obtained 293 blood samples from children and 160 blood samples from their mothers in Sudzal. In Teya, we obtained 392 samples from children and 230 samples from their mothers. Response rates from eligible households were 94% and 95%, respectively. Table 1 shows the sociodemographic characteristics of mothers: 31.8% were 36 years old or older (range = 15–59 years; only one mother was older than 55 years old), 33.8% had given birth more than three times,

TABLE 1

Sociodemographic characteristics of mothers of children ages 0–12 years old in Sudzal and Teya, Yucatán, México

Characteristic/category	*n* (%)
Age, years	
15–35	266 (68.2)
36–59	124 (31.8)
Number of pregnancies	
1–3	258 (66.2)
4–16	132 (33.8)
Elementary school education or less	
Yes	253 (64.9)
No	137 (35.1)
Mayan speaker	
Yes	305 (78.2)
No	85 (21.8)
Resident of Sudzal/Teya > 1 year	
Yes	381 (97.7)
No	9 (2.3)
Sleeps in hammock	
Yes	371 (95.1)
No	19 (4.9)

and 64.9% had an elementary school education or less. The majority spoke Mayan, and most had lived in Sudzal or Teya for more than 1 year and slept indoors in hammocks. There were no statistically significant differences between the sociodemographic characteristics of mothers in Sudzal and Teya (data not shown).

In Sudzal, we found a *T. cruzi* seroprevalence of 4.4% in mothers and 0.7% in children (Table 2). In Teya, we found a seroprevalence of 0.9% in mothers and 0.5% in children. The seroprevalence in both communities combined was 2.3% in mothers and 0.4% in children, with a total seroprevalence of 1.1%. The difference in seroprevalence among mothers and children combined between Sudzal (2.0%) and Teya (0.5%) was statistically significant ($P < 0.05$). The three children infected with *T. cruzi* were 9, 10, and 12 years of age; one of them also had a *T. cruzi*-seropositive mother. InDRE performed confirmatory tests for eight of the seropositive mothers and all seropositive children. Both IFAs and ELISA tests performed by InDRE were positive in all cases but one case. Our Wiener ELISA and InDRE ELISA results for this mother were positive, whereas IFA and Stat-Pak results were negative. We report the mother here as seropositive. None of the seropositive mothers or children had a history of blood transfusion.

TABLE 2

T. cruzi seroprevalence in children (0–12 years old) and their mothers in Sudzal and Teya, Yucatán, México

Communities/study population	Positive/tested (%)
Sudzal	
Mothers	7/160 (4.4)
Children	2/293 (0.7)
Subtotal (mothers and children)	9/453 (2.0)
Teya	
Mothers	2/230 (0.9)
Children	1/392 (0.3)
Subtotal (mothers and children)	3/622 (0.5)
Sudzal and Teya	
Mothers	9/390 (2.3)
Children	3/685 (0.4)
Total (mothers and children)	12/1,075 (1.1)

TABLE 3

Characteristics and *T. cruzi* seroprevalence of mothers of children ages 0–12 years old in Sudzal and Teya, Yucatán, México

Characteristics/category	Positive/tested	Percent	*P* value
All ages			
Age, years			
15–35	2/256	0.8	
36–59	7/124	5.6	0.01
Number of pregnancies			
1–3	2/258	0.8	
4–16	7/132	5.3	0.01
Low educational level*			
Yes	9/253	3.6	
No	0/137	0.0	0.03
Knows the vector†			
Yes	5/325	1.5	
No	4/65	6.2	0.46
Bitten by the vector‡			
Yes	7/133	5.3	
No	2/257	0.8	0.01
36 years old or older			
Number of pregnancies			
1–3	1/31	3.2	
4–16	6/93	6.5	0.68
Low educational level*			
Yes	7/87	8.0	
No	0/37	0.0	0.10
Knows the vector†			
Yes	4/109	3.7	
No	3/15	20.0	0.04
Bitten by the vector‡			
Yes	6/53	11.3	
No	1/71	1.4	0.04

*Elementary school education or less.
†Knowledge of potential for *T. dimidiata* to transmit disease.
‡Reported that someone in the household has been bitten by *T. dimidiata*.

T. cruzi infection was more frequent among older mothers, mothers who had four or more pregnancies, and mothers who did not complete elementary school (Table 3). The association of infection with the number of pregnancies and maternal education was not statistically significant when we considered only mothers 36 years old and older. *T. cruzi* infection was associated with the lack of knowledge of disease transmission by triatomines and reporting that a pik had bitten someone in the household. These factors were consistently associated with infection, even when we analyzed only data for mothers 36 years old and older.

Table 4 shows the RR of reproductive health outcomes by *T. cruzi* serostatus. We calculated the RR for mothers of all ages and mothers 36 years old and older. Women of all ages infected with *T. cruzi* were 4.7 (95% CI = 2.1–10.4) times more likely to have experienced a stillbirth and 2.5 (95% CI = 1.1–5.4) times more likely to have experienced a miscarriage than uninfected women. Having experienced a Cesarean section or the death of a child less than 1 month of age was not found to be associated with *T. cruzi* infection. After adjusting for age, infected mothers 36 years old and older were 2.8 times more likely to have experienced a stillbirth than uninfected mothers (95% CI = 1.3–5.8).

DISCUSSION

Our results show that *T. cruzi* infection occurs in more than 2% of women of reproductive age in the Mayan communities that we investigated. The observation of *T. cruzi* infection

TABLE 4

RR of reproductive health outcomes in mothers of children ages 0–12 years old by *T. cruzi* serostatus (all ages and 36 years old and older) in Sudzal and Teya, Yucatán, México

	All ages			≥ 36 years old		
Reproductive history*	Seropositive (%)	Seronegative (%)	RR (95% CI)	Seropositive (%)	Seronegative (%)	RR (95% CI)
Miscarriage	4/9 (44.4)	67/381 (17.6)	2.5 (1.1–5.4)	4/7 (57.1)	34/117 (29.0)	2.0 (0.9–3.9)
Stillbirth	4/9 (44.4)	36/381 (9.4)	4.7 (2.1–10.4)	4/7 (57.1)	24/117 (20.5)	2.8 (1.3–5.8)
Death of a child < 1 mo of age	2/9 (22.2)	31/381 (8.1)	2.7 (0.7–9.7)	2/7 (28.6)	18/117 (15.4)	1.9 (0.5–6.4)
Cesarean section	4/9 (44.4)	143/381 (37.5)	1.2 (0.5–2.4)	2/7 (28.6)	38/117 (32.5)	1.0 (0.2–2.9)

*Positive history indicates at least one occurrence.

in three children indicates that there is active transmission. Congenital transmission is a possible source of the infection in one child whose mother is also seropositive, but the two other children were most likely infected by triatomine vectors. We found a difference in *T. cruzi* seroprevalence between Sudzal and Teya. The communities possess similar environmental characteristics (domestic space, peridomestic space, and sylvatic space), but the vector infection rate is higher in Sudzal than in Teya (unpublished data), which may explain this difference.

We recruited 95% of children ages 0–12 years old and their mothers in the study villages. The high participation rate that we obtained might be attributed to the rapport established between the interviewers and the participants, the high motivation of staff members with specialized duties, and the overall in-depth involvement of the entire research team. In addition, the PDA/GPS system simplified navigation between households for follow-up visits.

Other epidemiological studies have used random sampling to select participants.[27] In our study, the total population in Sudzal and Teya was 3,137, with 301 children (0–12 years old) in Sudzal and 452 children in Teya. We included the entire population of children and their mothers, because it was nearly the same as the sample size needed to detect seropositivity of 3% with a 95% CI ± 1.5% and a design effect of two, taking intrahousing correlation into account.

The average *T. cruzi* seroprevalence in Mexico reported in the literature from serological surveys, clinical manifestations, and blood bank reports is up to 5.88% for the entire country and 3.17% for Yucatán.[28] Studies from rural areas of Yucatán reported a seroprevalence of 17.3% in 1972 and 18% in 1985,[11,12] but both studies used only one immunofluorescence test; also, some results might have been false positives. Also, a National Seroepidemiology Survey reported a seroprevalence of 1.5% in Yucatan using an indirect hemagglutination test.[10] This result is very similar to the total seroprevalence of 1.1% among mothers (2.3%) and children (0.4%) that we found 21 years later.

In a recent study, we found 0.6% *T. cruzi* seroprevalence among pregnant women delivering in an urban hospital in Merida, which also receives mothers from a large geographic area around the city.[19] This result is similar to the seroprevalence of 0.9% that we found in Teya but lower than the seroprevalence that we observed in Sudzal. The results of this study are also similar to the recently reported seroprevalence in a rural Mayan village in the nearby state of Campeche (2.3%).[29]

We found that more *T. cruzi*-infected mothers had a history of stillbirths than non-infected mothers. Animal model studies have shown that 80% of mice with acute *T. cruzi* infection either are infertile or experience fetal or neonatal losses associated with placental parasite invasion.[30,31] Another study suggests that intrauterine growth retardation occurs during chronic murine *T. cruzi* infection, but it did not note any difference between other reproductive outcomes of infected and uninfected mice.[32,33] Studies in non-human primates have not observed differences in fertility, menstrual cycle parameters, or numbers of stillbirths or miscarriages between uninfected and infected females.[34]

Congenital Chagas disease in humans has been associated with pre-term labor, low birth weight, and neonatal complications.[35] The association between the presence of maternal *T. cruzi* antibodies and miscarriages and stillbirths is controversial.[4] For example, in Argentina, Hernandez-Matheson and others[36] found an association between *T. cruzi* infection and miscarriages and stillbirths, but Blanco and others[4] did not. In Bolivia, Torrico and others[37] found an increased risk of miscarriages among infected mothers, although this finding was not statistically significant after adjusting for age. We found similar results and concluded that infected mothers did not have a strong increased risk of miscarriage. However, we found that the association between history of stillbirth and *T. cruzi* infection remained statistically significant after taking age into account. The association could be caused by residual confounding, congenital transmission, or increased risk of stillbirth among infected mothers even without congenital transmission.

There is an urgent need to address the problem of Chagas disease among children. Identifying and treating infected children is a priority, because studies indicate that, if children receive proper treatment, there is a high probability that they will not develop clinical disease.[38–40] There is also a need for community initiatives to increase awareness of the risk of Chagas disease. We found that a low level of education and lack of knowledge about the potential for *T. dimidiata* to transmit a disease were risk factors for *T. cruzi* infection. Campaigns for Chagas disease prevention have been shown to generate good community participation.[41,42] Most women in the two communities speak Mayan and have a low education level, indicating the need for tailored health promotion programs administered in their native tongue. Additionally, strategies targeting vector control should be feasible, sustainable, and compatible with their lifestyle.

CONCLUSIONS

Mothers and children in Mayan communities in Yucatan are infected with *T. cruzi*. Two cases of non-congenital childhood infection indicate likely ongoing vectorial transmission in these communities. It is important to emphasize the possible role of Chagas disease as a cause of perinatal mortality as

well as morbidity and mortality later in life in Yucatan and other regions of the Americas. All infected children should receive immediate medical treatment. Additional studies are needed to explore the potential association between maternal *T. cruzi* infection and stillbirth.

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