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ORIGINAL PAPER

Seroprevalence of *Trypanosoma cruzi* Infection in Pregnant Women Suggests a High Risk for Congenital Transmission in Central Veracruz, Mexico

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Abstract

Purpose The state of Veracruz, Mexico, is a well-recognized endemic region for Chagas disease, but congenital transmission has not been extensively studied. We estimated here the prevalence and the risk of congenital transmission of *Trypanosoma cruzi* in pregnant women from 27 municipalities of central Veracruz.

Methods 528 sera from pregnant women were analyzed by ELISA and IFA assays for the detection of IgG antibodies against *T. cruzi*.

Results The presence of anti-*T. cruzi* antibodies was identified in women from 17 municipalities, obtaining an overall seroprevalence of 17.0%. A higher seropositivity was observed in the municipalities of Orizaba (25.2%), Nogales (13.6%), and Río Blanco (10.5%). The results suggest that there is a high risk of congenital transmission of *T. cruzi* in the region.

Conclusion There are currently limited actions for the surveillance and control of congenital transmission of Chagas disease in Veracruz.

Keywords *Trypanosoma cruzi* · Seroprevalence · Congenital transmission · Pregnant woman

Introduction

Chagas disease is a zoonosis caused by *Trypanosoma cruzi*, and is a major parasitic infection in the Americas [1], endemic in South, and Central America as well as Mexico and the southern United States [2–4]. It is estimated that there are about 6 million people infected by *T. cruzi* in Latin America [5]. It has been traditionally considered a disease of rural areas where triatomine vectors are the most common source of infection [1]. However, congenital Chagas disease is becoming a growing global health problem which is still often underdiagnosed in many regions. Indeed, vertical transmission from an infected mother to her newborn is an important mode of transmission of *T. cruzi* infection in both endemic and non-endemic areas [6]. Also, large human migrations from rural to urban areas have led to the urbanization of Chagas disease, as well as its globalization [7–9]. According to recent WHO estimates, Mexico is one of the countries with the highest number of people infected by *T. cruzi*, after Argentina and Brazil, and ranked first in Latin America for the number of cases of infection by *T. cruzi* due to congenital transmission, with 1788 annual cases

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[5]. However, very few studies have focused on congenital transmission or on *T. cruzi* infection in pregnant women in Mexico [8]. Recent studies have reported the presence of triatomines and vectorial transmission in localities of the central part of the state of Veracruz, with a very high seroprevalence of *T. cruzi* infection in humans, reaching 16.8% [10, 11]. The objective of this study was to measure the prevalence of *T. cruzi* infection in pregnant women attending prenatal care in a regional hospital in central Veracruz, and estimate the risk of congenital transmission of *T. cruzi*.

Materials and Methods

Study Population and Serum Samples

The study was conducted in a tertiary care hospital in Veracruz, Mexico (Hospital Río Blanco), which provides prenatal care to pregnant women and attends over 4500 deliveries per year. Study participants were women attending routine prenatal care during their second trimester of pregnancy, with no preeclampsia or other complications/risks (vaginal bleeding, placental abruption, abdominal pain, vaginal infections, lack of fetal movement, gestational diabetes among others).

During the period from March 2013 to January 2015, 728 women were screened, and a total of 528 serum samples were collected from pregnant women by venipuncture, after obtaining informed consent (Fig. 1) according to the WHO safety protocols, as well as basic sociodemographic data through a short questionnaire. Blood samples were collected in vacutainer tubes. Serum was separated by centrifugation at 1200 × *g* for 10 min and samples were stored at -70 °C until used. Samples were analyzed for *T. cruzi* infection using two different assays: an in-house enzyme-linked immunosorbent assay (ELISA) based on crude parasite extract and an indirect immunofluorescence (IIF) assay for the detection of IgG antibodies against *T. cruzi*. The sera that showed hemolysis were not included in the study.

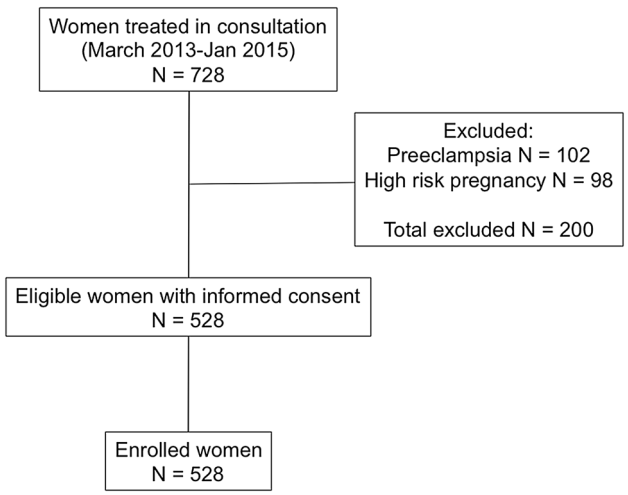

Fig. 1 Sampling design

In-house ELISA

An in-house ELISA test was used [10], based on an epimastigote crude extract of the LJ01 strain (TDIM/MEX/2014/LJ01/T. cruzi, a mixture of TcI and non-TcI DTUs, isolated from *Triatoma dimidiata* collected in the village of Las Josefinas, Veracruz, Mexico) [12]. The strain was cultured in liver infusion tryptose medium supplemented with 10% fetal calf serum. Briefly [10], logarithmic phase parasites were harvested by centrifugation at 1000 × *g* for 10 min at 4 °C. The parasite pellet was suspended in 500 µL of phosphate-buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, and 1.4 mM KH2PO4, pH 7.4) and lysed by cycles of freezing (− 70 °C) and thawing (25 °C). The suspension was centrifuged at 10,000 × *g* for 20 min at 4 °C. The resulting supernatant (extract) was used as crude antigen extract. Protein concentration was determined by the Bradford method. Polystyrene plates (Costar Corporation, Cambridge, MA) were coated with the *T. cruzi* crude antigen extract (10 µg/mL) in carbonate buffer, pH 9.6, and incubated overnight at 4 °C. Unbound antigen was removed and plates were blocked with 200 µL of PBS containing 5% non-fat milk for 2 h at 37 °C. After washing, the plates were incubated with 50 µL of serum samples (1:200 dilution). Each plate also included positive and negative control serum samples. Further, washing steps were performed and a peroxidase-labeled goat anti-human IgG antibody (Pierce, Rockford, IL) was added at a 1:5,000 dilution in PBS/0.05% Tween 20 and incubated for 1 h at room temperature. After eight washes, 100 µL of 2,2,-azinobis (3-ethylbenzthiazoline)-6-sulphonic acid (Zymed, San Francisco, CA) was added as substrate and the reaction was allowed to proceed for 20 min at room temperature. The reaction was stopped with 2% sulfuric acid, and absorbance was read at 415 nm with an ELISA microplate reader (Multiskan MS; Labsystems, Vantaa, Finland). The use of a local strain was previously found to increase the sensitivity of the detection of *T. cruzi* infection [12].

Immunofluorescence Assay

The sera that gave positive results by ELISA were processed by IIF. Briefly [10], a suspension of 10^5 epimastigotes of the Y strain were placed on a slide, fixed and blocked with PBS containing 10% fetal bovine serum (FBS) for 1 h at 37 °C in a humid chamber. Sera of donors and controls were diluted 1:50 in PBS and incubated for 30 min in humid chamber, subsequently, the slides were washed and incubated with the

second anti-human IgG antibody conjugated to FITC at a 1:20 dilution for 30 min at 37 °C. After incubation, the slides were observed in a fluorescence microscope. The presence of a green fluorescence on parasites was considered a positive test using as a benchmark a positive control. In all serological assays, a positive control consisting of a pool of sera from chagasic patients was used, as well as, and a negative control consisting of a pool from healthy volunteers. Only samples reactive to both tests were considered as positive for *T. cruzi* infection.

Data and Statistical Analysis

Seroprevalence data were compared using χ^2 or Fisher's exact tests, and the kappa index was calculated when applicable. Also when relevant, 95% confidence intervals (CIs) were calculated. Sociodemographic data were similarly compared by χ^2 tests, to assess potential associations with the serological data, and a multivariate logistic regression and a multiple regression analyses were performed with the variable that resulted significantly associated with *T. cruzi* seropositivity. A spatial database of serologic results for each municipality was also created in the program QGis (2.6.1-Brighton) to produce seroprevalence maps, and spatial analysis (Kernel density) was conducted using the program JMP (Version 9.0.1).

Ethical Aspects

The project was reviewed and approved by the Ethical Committee of the Faculty of Chemical Sciences (FCQ/067/01/2013), and by the research committee of the regional hospital of Rio Blanco (JSVII/HRRioBlanco/2013/004).

Results

Characteristics of Trainees

A total of 528 pregnant women were enrolled in the study, with an average age of 25 years old, ranging from 13 to 43 years old (Table 1). About one-third were in their first pregnancy, the others had had from one to ten previous pregnancies. Nearly two-thirds were from rural areas. The majority had reached primary or secondary school education. Most had domestic animals (63%), and lived in houses with cement floor (68%), laminated metal roof (55%), and cement walls (62%). The majority were familiar with triatomines, and 20% reported previous contact with these bugs (Table 1).

Table 1 Demographic characteristics of the study population

	25 ± 6 (13–43)
Age	
< 20 years	153/528 (30.0%)
21–30 years	253/528 (47.9%)
31–40 years	111/528 (21.0%)
> 40 years	11/528 (2.1%)
Previous pregnancies	
None	194/528 (36.7%)
1 or more	334/528 (63.2%)
Environment	
Urban	222/528 (42.0%)
Rural	306/528 (57.9%)
Education	
Primary	193/528 (36.5%)
Secondary	201/528 (38.1%)
High	88/528 (16.6%)
University	46/528 (6.8%)
Socioeconomic level[a]	
Low	169/528 (32.0%)
Medium	359/528 (68.0%)
Domestic animals	
Yes	333/528 (63.0%)
No	195/528 (36.9%)
Floor	
Cement	360/528 (68.2%)
Tiles	95/528 (18.0%)
Dirt	73/528 (13.8%)
Roof	
Cement	143/528 (27.1%)
Tiles	92/528 (17.4%)
Laminated metal	293/528 (55.5%)
Walls	
Cement/plaster	328/528 (62.1%)
Blocks	43/528 (8.1%)
Wood	153/528 (29.0%)
Other	4/528 (0.8%)
Know triatomines	
Yes	306/528 (58.0%)
No	209/528 (40.0%)
No answer	13/258(2.0%)
Previous contact	
Yes	115/528 (21.8%)
No	385/528 (72.9%)
No answer	28/528 (5.3%)
Previous transfusion	
Yes	35/528 (6.6%)
No	492/528 (93.4%)

Age is given as mean ± SD and range

[a]Economic and sociological parameter that combines a person's job preparation based on your income, education, and employment

Analysis of Seroprevalence and Sociodemographic Data

All 528 samples were tested for antibodies against *T. cruzi* by ELISA and IIF using a local parasite strain as antigen[12] corresponding to an overall seroprevalence of 17.0% (90/527, 95%CI = 9.91–24.1%). It is worth mentioning that the serum was not included in the serological study for presenting hemolysis. The agreement between the ELISA assay and the IIF was excellent ($\kappa = 0.96 \pm 0.01$). Although seroprevalence of *T. cruzi* infection varied with age, ranging from 4% for women less that 16 years old to 28% in 26–30 years old, there was no significant association of infection with age ($P = 0.86$, Table 2). Previous parity was also not

Table 2 Characteristics of seropositive pregnant women

	Seronegatives ($N = 437$)	Seropositives ($N = 90$)	P
Age			
< 20 years	126 (28.9%)	27 (30.0%)	0.86
21–30 years	207 (47.4%)	46 (51.1%)	
31–40 years	96 (21.9%)	15 (16.7%)	
> 40 years	8 (1.8%)	2 (2.2%)	
Previous pregnancies			
0	278 (63.6%)	55 (61.1%)	
1 or more	159 (36.4%)	35 (38.9%)	
Environment:			
Urban	158 (36.1%)	63 (70.0%)*	**< 0.001**
Rural	279 (63.8%)	27 (30.0%)	
Education			
None	18 (4.1%)	1 (1.1%)*	**0.01**
Primary school	154 (35.2%)	19 (21.1%)	
Secondary school	164 (37.5%)	37 (41.1%)	
High school	68 (15.6%)	20 (22.2%)	
University	33 (7.6%)	13 (14.4%)	
Socioecon. level			
Low	152 (34.8%)	16 (17.8%)*	**0.001**
Medium	285 (65.2%)	74 (82.2%)	
Floor			
Tiles	68 (15.1%)	27 (30.0%)*	**0.002**
Cement	303 (69.3%)	57 (63.3%)	
Dirt	66 (15.1%)	6 (6.7%)	
Roof			
Tiles	70 (16.1%)	21 (23.3%)*	**0.01**
Cement	111 (25.5%)	32 (35.6%)	
Laminated sheets	254 (58.4%)	37 (41.1%)	
Walls			
Cement/plaster	265 (60.6%)	64 (71.1%)	0.13
Block	36 (8.3%)	4 (4.4%)	
Wood	136 (31.1%)	22 (24.4%)	
Know triatomines			
Yes	255 (58.3%)	51 (56.7%)	0.43
No	174 (39.8%)	35 (38.8%)	
No answer	8 (1.8%)	4 (4.4%)	
Previous contact			
Yes	101 (23.1%)	14 (15.6%)	0.24
No	314 (71.8%)	70 (77.8%)	
No answer	22 (5.0%)	6 (6.7%)	

Bold values indicate $p < 0.05$

*statistically significant

associated with *T. cruzi* infection ($P=0.65$). Similarly, having domestic animals ($P=0.68$), knowing triatomine bugs ($P=0.43$) and self-reporting of triatomine bites ($P=0.24$) were not associated with infection. On the other hand, living in urban area ($P<0.0001$), higher socioeconomic level ($P=0.001$) and higher education level ($P=0.01$) were significantly associated with *T. cruzi* seropositivity (Table 2). Floor ($P=0.002$) and roof ($P=0.011$) building materials were also associated with infection (Table 2). Nonetheless, none of these associations remained significant in a multivariate logistic regression model, with the exception of living in urban/rural area, and the resulting model fitted very poorly to the serological data (R2 = 0.08). However, when performing the multiple regression analysis, an association with age was found, mainly in the population of less than 20 years ($P=0.005$), followed by the age group between 21 and 30 years ($P=0.004$). An association was also found with the level of education (high school, $P=0.01$), and with the high socioeconomic level ($P=0.03$), the association of seroprevalence with the housing construction material was maintained, floor and roof: cement ($P=0.01$), walls (cement and wood $P=0.02$), on the other hand, knowledge of the vector and previous contact with it showed an association with the presence of seroprevalence, suggesting that these variables contribute to the distribution of seropositivity of *T. cruzi* in the population studied.

Geographical Distribution of Positive Cases

The presence of anti-*T. cruzi* antibodies was identified in women from 17 municipalities (Fig. 2), and a higher seropositivity was observed in the municipalities of Orizaba with a seropositivity of 25.2% (95% CI = 21.07–27.7), Nogales and Río Blanco with a seropositivity of 13.6% (95% CI = 10.3–16.9), and 10.5% (95% CI = 6.7–14.3), respectively (Fig. 3a). The analysis of the spatial distribution of the women with anti-*T. cruzi* antibodies, by nonparametric Kernel test allowed us to establish the points of greatest

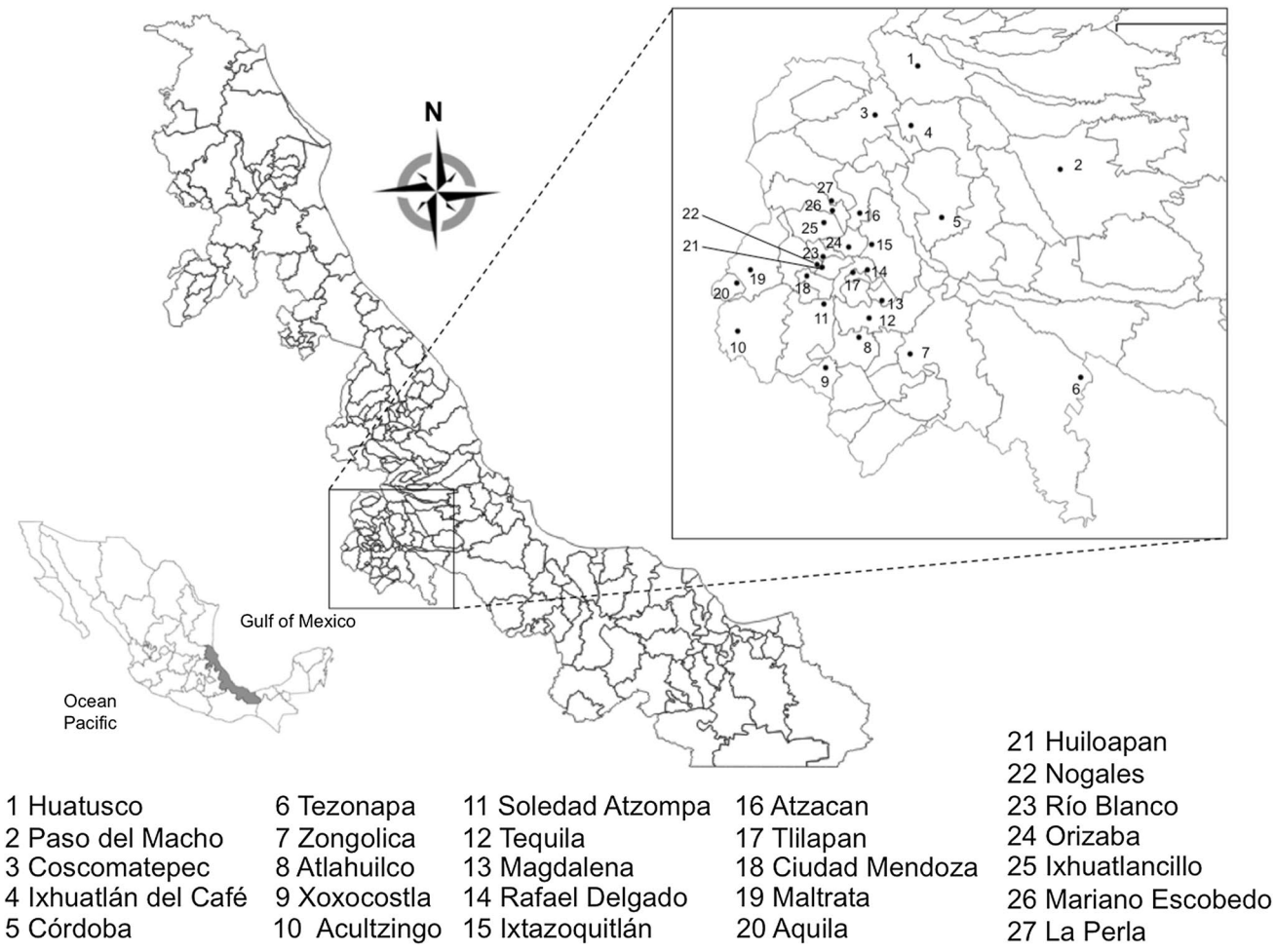

Fig. 2 Study area. Mexico (bottom left), the state of Veracruz (center), and the study area (inset). Black circles indicate the position of the indicated municipalities

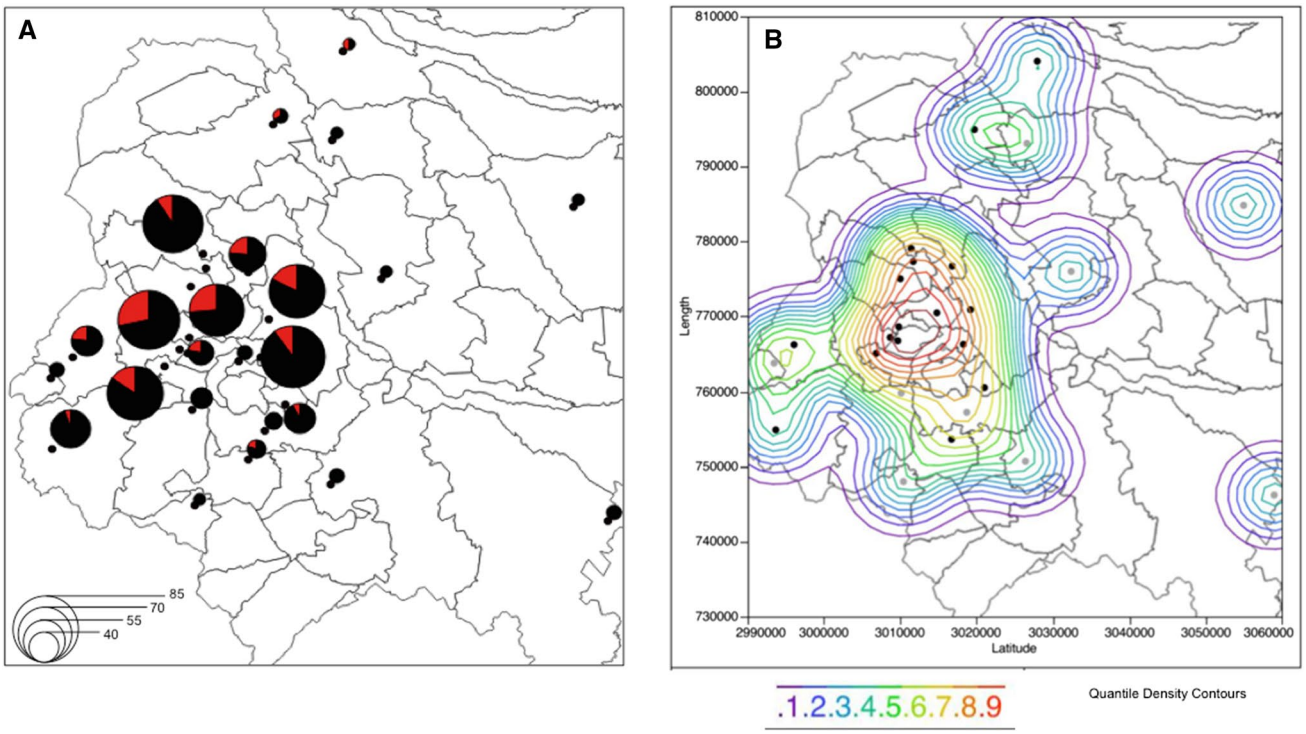

Fig. 3 Cases of positive serology for *T. cruzi* in pregnant women in central Veracruz, Mexico. **a** Geographic distribution of cases. Circles are proportional to the number of serum samples analyzed in each municipality as indicated on the bottom left scale. Red areas in the pie charts represent the proportion of *T. cruzi*-seropositive patients. **b** Nonparametric density estimation (Kernel), fit to ordered by abscissa for Chagas disease indicating the presence of antibodies to *T. cruzi* in the municipalities (black points) (color figure online)

concentration or density, corresponding to areas with the greatest risk. Thus, the density curves confirmed that the municipalities of Orizaba, Nogales, Río Blanco, Ixtazoquitlán, Mariano Escobedo, Ciudad Mendoza, Ixhuatlancillo, and Rafael Delgado concentrated most of the seropositive cases (coordinates: length 771,608, latitude 3,016,084) (Fig. 3b).

Discussion

Mother-to-child transmission of *T. cruzi* is becoming a major public health priority in both endemic and non-endemic regions. Therefore, it is urgent to better understand the epidemiology of mother-to-child transmission and to develop effective prevention programs [13]. In Mexico, accurate information on the prevalence of Chagas disease is scarce and the importance of the disease in public health is still being debated, despite many punctual studies reporting local cases since many years [14]. Here we found a seroprevalence of *T. cruzi* infection of 17.0% in pregnant women in a major hospital in central Veracruz, who's newborns are thus at risk of congenital transmission. This seroprevalence is in complete agreement with our previous seroepidemiological study in the region, reporting a seroprevalence of 16.8% in the general population [10]. The seroprevalence of *T. cruzi* infection in pregnant women varies between different countries, geographical areas, and rural and urban localities from < 1 to 70.5% [15], and in some states of Mexico, it fluctuates between 4.0 [16] and 12.0% [17]. Although it is well known that the state of Veracruz is endemic for Chagas disease, the geographical distribution and extent of the disease are not yet well documented [10]. The very high seroprevalence of *T. cruzi* infection we detected in pregnant women in this population suggests a very high risk of congenital transmission. While no follow-up of the seropositive pregnant women could be performed by the research team, all were referred to the Ministry of Health for confirmation of diagnostic and care. It is, however, unclear how many of them did transmit to their newborns. Based on an average congenital transmission rate of 5% [18], 4–5 cases of congenitally infected newborns can be expected from our cohort of pregnant women. Importantly, current guidelines for prenatal care in Mexico include screening for several infectious diseases, but not for Chagas disease, which should thus be urgently considered in updated public health policies based on our and other's data on congenital transmission [5]. Our short questionnaire failed to clearly identify factors associated with *T. cruzi* infection in pregnant women in this region. The higher proportion of seropositive women

from urban areas may be related to migrations of women and mothers from rural areas with high *T. cruzi* transmission to urban centers, which drives the urbanization of Chagas disease [19–22]. This phenomenon of migration of both endemic and non-endemic areas may be mainly motivated by the extreme poverty in which these families live. However, this condition is influenced by several more specific factors, including: lack of employment, lack of access to social protection, lack of access to infrastructure and services such as health care and schooling, and the demand for domestic workers among others [23].

Finally, it is worth mentioning that this study had some limitations, for example, the presence of infection in pregnant women was not confirmed, so neither an assessment of the clinical status was performed to evaluate the form of the disease. On the other hand, the mothers' umbilical cord blood was not analyzed when giving birth, and the newborn was not followed up.

In conclusion, the spatial cluster of *T. cruzi* infection we detected may reflect this migration to more urbanized municipalities, although the presence of vectorial transmission in many of these areas also seems likely based on the rather widespread knowledge of triatomine bugs reported by the pregnant women. Further studies should focus on a much wider exploration of potential risk factors for *T. cruzi* infection in these pregnant women, which may help in their identification during prenatal care. Understanding the frequency of congenital *T. cruzi* transmission is important for the implementation of appropriate screening strategies for pregnant women and early treatment programmes for infected newborns.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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