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Risk factors associated with Chagas disease in pregnant women in Santander, a highly endemic Colombian area

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Abstract

OBJECTIVE To determine the prevalence and risk factors associated with Chagas disease in pregnant women in an endemic area of Santander, Colombia.

METHODS Cross-sectional study included 23 municipalities of Santander, Colombia. Serological IFAT and ELISA tests were undertaken to detect IgG anti- *Trypanosoma cruzi*. A questionnaire was conducted for assessing the risk factors of each participant. Newborns were evaluated at birth and followed up to 1 year of age to determine congenital infection.

RESULTS An overall prevalence of 3.2% (95% CI 2.4–4.2) among 1518 pregnant women was detected. Prevalences by provinces were as follows: Guanentina: 6.0% (95% CI 4.1–8.5), García Rovira: 2.9% (95% CI: 1.5–4.8) and Comunera: 0.4% (0.4–2.3). The main risk factors identified were age >32 years old (OR: 2.1; 95% CI: 1.1–3.9); currently having a thatched roof (OR: 11.8; CI95% 2.2–63.2) and a thatched roof during childhood (OR: 3.0; 95% CI: 1.4–6.6); having below primary school education level (OR: 4.6; 95% CI: 2.2–9.5); and a history of a close contact with the vector (triatomine bugs) at least once during their lifetime (OR: 6.9; 95% CI: 3.7–12.9). No congenital cases were detected by parasitological or serological techniques.

CONCLUSIONS Prevalence of Chagas disease in pregnant women is a potential source of infection in this Colombian endemic area. The main risk factors associated with seropositivity were related to conditions favouring the contact with the vector. The results show that it is necessary to continue an active surveillance in order to offer diagnosis and treatment to mothers and their newborns in addition to screening to pregnant women from endemic areas.

keywords Chagas disease, serological diagnosis, *Trypanosoma cruzi*, pregnancy, congenital Chagas disease, risk factors

Introduction

Chagas disease is an important public health problem in Latin American countries. In Colombia, vectorborne is the main transmission route and *Rhodnius prolixus* is the predominant species of vector [1]. Risk factors relate to environmental, cultural and social vulnerabilities such as living conditions, housing and poverty in rural areas; and these have allowed national programmes to target the most endemic areas for vector control [2]. However, additional strategies are needed to control other routes of parasite transmission (congenital, oral, transfusion) [3].

Congenital transmission has gained special importance in some zones where vector transmission has been interrupted. Hence, in some endemic countries like Argentina, the rate of congenital transmission can account for up to 10 times the number of acute vectorborne cases [4]. Congenital cases have been widely reported even in countries where the infection is non-endemic due to international migration from endemic countries [5, 6].

Congenital transmission occurs when the parasites cross the placental barrier during pregnancy [7–9]. There are two effective trypanocidal drugs (benznidazole and nifurtimox) for reducing parasitemia, but they are

explicitly contraindicated during pregnancy due to their potential teratogenic adverse effects [8, 10]. However, there is recent evidence of the potential of trypanocidal drugs for preventing congenital transmission when women are treated before pregnancy [11].

In South American countries, the prevalence of *Trypanosoma cruzi* seropositivity in pregnancy ranges from 0.3% to 49.5% [12–16] and the transmission probability from infected mother to child has been estimated at 4.7% (95% CI: 3.9–5.6) in a meta-analysis [17]. Although about 90% of congenital cases are asymptomatic at birth, some cases present varied symptoms such as low weight, hepatosplenomegaly, jaundice and anaemia [18].

Both the prevalence of infection in pregnant women and the transmission pattern of congenital infection in Colombia are unknown [19]. Only two previous studies have determined the prevalence in pregnant women in parts of the country: 3.3% in Boyacá [20] and 4% in Casanare [21].

This study determines the prevalence of *T. cruzi* seropositivity in pregnant women and accordingly the frequency of congenital transmission in an endemic area previously unexplored in this regard.

Methods

Study design

A cross-sectional study was performed between August 2010 and December 2013 in the Department of Santander (first administrative order), specifically in three provinces (intermediate administrative order): García Rovira, Guanentina and Comunera, and 23 municipalities (second administrative order) (Figure 1).

Pregnant women were recruited from August 2011 until September 2011, and their newborns were followed up until the age of 12 months. The sample size was calculated according to an estimate of about 3000 pregnant women per year attending the health facilities in these 23 municipalities. Based on previous work, the expected prevalence was 3.9% [20, 21], therefore with a confidence level of 95%, and a precision of 1%, a required minimum sample size of 1440 pregnant women was estimated to successfully determine the overall prevalence in this area.

Inclusion of participants and follow-up

The inclusion criteria for municipalities in these three provinces were being a Chagas endemic area for domiciliary transmission and having a primary care facility with a regular pregnancy care programme. The inclusion criteria for individual participants were as follows: (i) acceptance of pregnant women to participate in the diagnosis phase; (ii) acceptance of the mother to participate in the follow-up of her newborn once she was identified as seropositive; and (iii) written informed consent.

Several meetings at municipality level were conducted to inform health workers from the 23 hospitals about the study. The local teams who facilitated the inclusion and follow-up were comprised of a physician, a nurse and laboratory technicians. They were trained for helping out with the inclusion procedures, filling-in questionnaires, collecting samples, contacting patients and directing infected pregnant women towards attending a series of specific consultations with the researchers for the follow-up phase of the study.

For diagnosis of pregnant women and children, two techniques were used as follows: ELISA and IFAT to detect anti-*T. cruzi* IgG. These tests were standardised and evaluated at the National Health Institute [22–24]. Discordant results were then processed by the indirect haemagglutination (IHA) commercial test (Winner®, Rosario, Argentina). Microhaematocrit was processed according to the technique previously described by Freilij [25] and modified by Torrico [26]; the IHA was also used for parasitological diagnosis of newborns. The children underwent serological tests and were considered as cases when the tests were reactive after 9 months of age.

Once a pregnant woman was confirmed as positive by two serological tests, she was informed about the need to follow-up her baby after birth. Clinical examination and samples for microhaematocrit and serology were taken at birth and up to 12 months of age. Aetiological (parasiticidal) treatment with nifurtimox (Lampit®) was offered to all confirmed cases.

Ethical aspects

Pregnant women were invited to participate voluntarily during the prenatal consultation at the local hospital, after being informed about the goal, objectives, procedures and benefits of the study. Written informed consent was obtained from all participants, following national and international regulations. The research protocol was approved by the Committee on Research Ethics of Colombia's National Health Institute.

Survey questionnaires and data quality control

The questionnaires contained information on sociodemographic variables (residency, age, marital status, education and occupancy), housing characteristics (construction materials of the roof, walls and floor) for

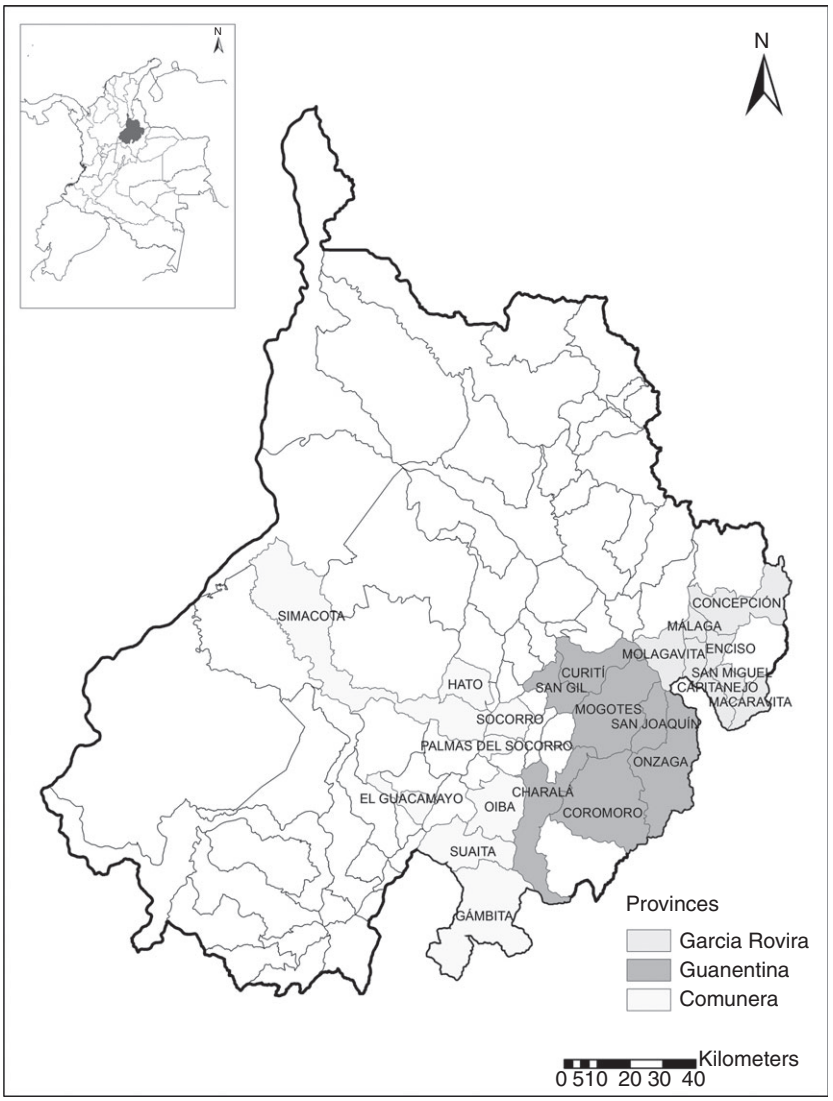

Figure 1 Map of the participant municipalities in the Guanentina, Comunera and García Rovira provinces, Santander, Colombia.

both current and childhood homes, recognition and contact with the vector (past and current), history of transfusions, and information on relatives with previous diagnosis of Chagas disease. During the interview, a box with desiccated insects of the four principal triatomine species (*R. prolixus, Triatoma maculata, Triatoma dimidiata, Panstrongylus geniculatus*) was shown to the pregnant women, who were asked to identify their interaction with the vectors as either 'simple recognition' or 'close contact'. Simple recognition was defined as having seen the vector at least once in their lifetime in their houses or in another place (trees, palms, other houses). This was intended as a proxy indicator of a potential exposure but not remembering any particular contact. Close contact was defined as remembering having been bitten by vectors or seeing them full of blood emerging from the bed where the women slept.

To control for potential information bias, the local teams participating in the study received specific training sessions on the questionnaires and procedures and as a quality control they were regularly reassessed and if necessary received additional training. For data quality control, the fieldwork coordinator revised and evaluated the quality of the questionnaires. In the case of missing data, patients were re-contacted to obtain the specific information. The questionnaires were entered in an MS Excel® database, a random sample of 2% of the questionnaires was reviewed in detail, and patients were called to corroborate the validity and reliability of the information provided.

Data analysis

Frequencies were calculated and displayed as percentages with their 95% confidence intervals (95% CI). Potential risk factors were evaluated through bivariate analysis as odds ratio (OR) and then through stratified and multivariate analysis by logistic regression. For the latter, a stepwise backward removal method, dropping variables with P values <0.2, was used for controlling confounding variables and choosing the final model, for which only variables with P value <0.05 were included. Akaike information criteria (AIC) were also used to choose the best model fit. All the data analysis was performed using Stata 10.0 (Stata Corp. 2007, release 10; StataCorp LP, College Station, TX, USA).

Results

Univariate analysis

A total of 23 health facilities from 23 municipalities in the three endemic provinces participated in the study. All municipalities and all the mothers invited to participate in the study accepted. A total of 1518 pregnant women aged 13–46 years (average: 24.5 years; standard deviation: 6.9 years) were included. The overall prevalence was 3.2%. The Guanentina province had the largest number of cases (30 infected pregnant women). At municipality level, Mogotes presented the highest prevalence (18.3%). The frequency of infected pregnant women by municipalities and provinces is presented in Table 1.

52.2% were from rural areas and 47.8% from urban areas; 67.7% belonged to the lower socio-economic strata (1 or 2) and 80.0% were affiliated to subsidised health care. 55.5% had finished high school and 33.2% had no education at all. 77.9% were either married or had a partner at the time of the survey. 78.5% were housewives and 13.8% were high school students; 43.2% recognised the Chagas disease vector (Triatominae), and only 11.1% reported any contact with it sometime in the past (Table 2).

Bivariate analysis and multivariate analysis

In the bivariate analysis, low socio-economic status, low level of education, age above 30 years, knowledge of the vector, and contact with the vector at least once during lifetime were significantly associated with seropositivity. Having lived in childhood or living currently in rural area and in houses with dirt floor, thatched roof and adobe walls were also statistically significant (Table 2).

To identify potential interactions, a stratified analysis by age groups was conducted with the variables identified as statistically significant in the bivariate analysis, evidencing no modification of the effect with age. In the logistic regression model, the final variables associated with seropositivity were as follows: age >32 years, less than primary schooleducation, previous contact with the vector at least once in the lifetime, and having lived in a house with thatched roof in childhood (Table 3).

Follow-up of newborns and search for congenital infection

The 49 infected women had a successful delivery with no abortions or deaths at birth. After birth, one child died due to respiratory infection at 3 months. One participating mother from the Socorro municipality initially refused to continue in the study after being diagnosed as seropositive, so it was not possible to take samples from the newborn, but it was possible to follow-up the child after 1 year. Follow-up of 48 newborns continued until they reached 12 months of age; no congenital cases were identified through parasitological or serological tests. The transmission rate from mother to children was then estimated at 0% (95% CI: 0.0–7.3%) for this population.

Discussion

The overall prevalence found in this endemic area was 3.2% (95% CI: 2.39–4.24), similar to the prevalence reported for two other Colombian endemic areas in recent studies: 4% in Casanare in 2011 [21] and 3.34% Boyacá in 2007 [20]. Santander has been one of the high endemic departments in Colombia [1]. In previous studies, the prevalence of Chagas disease in general population had reached 52.5% [27] and 6.9% in children [3] in this department. The only previous study in pregnant women in this department was conducted in the capital city, Bucaramanga, in 1993 when a prevalence of 6.9% in women at delivery in a specialised level hospital was found [28]. Thus, the prevalence almost 15 years ago was higher than in the current study, which is the observable trend for other endemic areas [21]. This difference over time could be explained by the scaling-up of vector control measures in this area since the beginning of the 21st century [1]. A similar trend was found in Paraguay in the prevalence in pregnant women in two endemic municipalities in 1995 (15% and 12%) and 2009, when the prevalence dropped to 6% in both sites [29].

One of the most striking results was the high prevalence found in Mogotes, which is one of the most endemic municipalities in Colombia. As part of the Andean

Table 1 Prevalence of *Trypanosoma cruzi* seropositivity in pregnant women in the municipalities of three provinces, Santander, Colombia

Province/Municipality*	Negative	Positive	Total	Proportion (%) of sero[+]	95% CI
Guanentina Province					
Curiti	29	1	30	3.3	0.1–17.2
Charalá	14	0	14	0.0	0.0–23.1
Coromoro	36	2	38	5.3	0.6–17.7
Mogotes	94	21	115	18.3	11.6–26.5
Onzaga	25	0	25	0.0	0.0–13.7
San Gil	222	4	226	1.8	0.5–4.5
San Joaquín	47	2	49	4.1	0.5–14.0
Subtotal	467	30	497	6.0	4.1–8.5
Comunera Province					
Gámbita	13	0	13	0.0	0.0–24.7
Guacamayo	23	0	23	0.0	0.0–14.8
Socorro	324	5	329	1.5	0.5–3.5
Suaita	40	0	40	0.0	0.0–8.8
Oiba	138	1	139	0.7	0.0–3.9
Hato	4	0	4	0.0	0.0–60.2
Palmas del Socorro	3	0	3	0.0	0.0–70.8
Simacota	15	0	15	0.0	0.0–21.8
Subtotal	560	6	566	1.1	0.4–2.3
García Rovira Province					
Capitanejo	91	4	95	4.2	1.2–10.4
Concepción	25	0	25	0.0	0.0–13.7
Enciso	6	2	8	25.0	3.2–65.1
Macaravita	35	2	37	5.4	0.7–18.2
Molagavita	51	2	53	3.8	0.5–13.0
Málaga	186	1	187	0.5	0.0–2.9
San Jose de Miranda	40	1	41	2.4	0.1–12.9
San Miguel	8	1	9	11.1	0.3–48.2
Subtotal	442	13	455	2.9	1.5–4.8
Total	1469	49	1518	3.2	2.4–4.2

*Department is the first administrative order. Municipality is second administrative order. Province is an intermediate level between department and municipality.

countries initiative for the control of Chagas disease in the 1990s, an integrated domiciliary vector infestation control programme was created to guide the vigilance and control actions of high-risk municipalities. This initiative involved the participation of 30 municipalities in Santander but Mogotes was not part of such strategy and no systematic vector control took place there [1]. This result supports the hypothesis that vector control is an important factor in explaining temporal changes to prevalence and accounts for the substantial difference in prevalence between Mogotes and other municipalities.

In our study, age was an important risk factor for *T. cruzi* seropositivity. Specifically, being older than 32 resulted in twice the risk of having positive serology. This confirms similar findings in Argentina [30], Bolivia [31], another endemic area of Colombia [21] and recently in El Salvador [32]. This recurrent finding reflects the typical seroprevalence profile due to accumulated exposure with age in areas of domiciliary transmission.

A history of living in poor housing conditions (specifically in houses with thatched roofs) was a risk factor for seropositivity (OR 3.0; 95% CI: 1.4–6.6), which increases significantly if women continue living in such conditions (OR 11.8; 95% CI: 2.2–63.2). Housing conditions are the most important risk factors associated with triatomine infestation in Colombia and Argentina [2, 33]. Even though some variables such as socio-economic conditions and living in rural areas were statistically significant in the bivariate analysis, they did were not relevant in the final model. This may be due to correlation between those variables and the ones that were finally presented in the model such as thatch roof and level of education. Recollection of at least one 'close contact' (a bite or seeing a vector in bed) also was a clear risk factor, although 'simple recognition' did not represent a risk.

In Colombia, and particularly the department of Santander, several oral outbreaks have been reported in the last years [34–37]. Interestingly, for the majority of

Table 2 Bivariate analysis for potential risk factors associated with *T. cruzi* seropositive pregnant women in Santander, Colombia

Variable	*n*	sero[+]	%	OR	95% CI	P
Socio-demographic factors						
Residence area						
Urban area	725	13	1.79	1		0.003
Rural area	793	36	4.54	2.6	1.3–5.4	
Age						
13–19	382	4	1.05	1		
20–24	447	13	2.91	2.8	0.9–8.4	<0.001
25–29	301	5	1.66	1.6	0.4–5.9	
30–42	388	27	6.96	6.6	2.3–18.8	
Socio-economic strata						
High (3,4,5)	491	9	1.83	1		0.034
Low (0,1,2)	1027	40	3.89	2.2	1.0–4.5	
Healthcare insurance						
Contributive	151	3	1.99	1		0.211
Non-insured	152	2	1.32	1.5	0.3–9.1	
Subsidised	1215	44	3.62	2.8	0.7–11.7	
Education level						
University	172	2	1.16	1		<0.001
High School	842	9	1.07	0.9	0.2–4.3	
Primary School	493	36	7.30	6.7	1.6–28.1	
None	11	2	18.18	18.9	2.4–149.9	
Marital status						
Married	1183	43	3.63	1		0.124
Single	321	5	1.56	0.4	0.2–1.1	
Widow-separated	14	1	7.14	2	0.3–15.9	
Occupation						
Housewife	1192	46	3.86	1		0.068
Student	209	2	0.96	0.2	0.6–1.0	
Worker in urban area	96	1	1.04	0.3	0.0–1.9	
Worker in rural area (agriculture)	21	0	0.00	–	–	
Factors related with triatomine vectors exposure						
Recognition of the vector						
No	809	10	1.24	1		**<0.001**
Yes	656	39	5.95	5.1	2.5–10.2	
Close contact with vectors at least once in lifetime						
No	1186	21	1.77	1		**<0.001**
Yes	169	25	14.79	9.6	5.3–17.7	
No response	163	3	1.84	1	0.3–3.5	
Close contact with vectors during the last year						
No	1336	44	3.29	1		0.283
Yes	26	2	7.69	2.4	0.6–10.7	
Close contact with animals						
No	462	16	3.46	1		0.732
Yes	1056	33	3.13	0.9	0.5–1.7	
Housing conditions						
Current floor						
Cement	1241	32	2.58	1		**<0.001**
Wood	36	2	5.56	2.2	0.5–9.7	
Dirt	190	15	7.89	3.2	1.7–6.1	
Other	51	0	0.00	–	–	
Current roof						
Clay tiles	601	20	3.33	1		
Fibrocement tiles	552	18	3.26	1	0.5–1.9	**<0.001**
Thatched/palm leaves	12	3	25.00	9.7	2.4–38.5	
Other	353	8	2.27	0.7	0.3–1.5	

Table 2 (*Continued*)

Variable	*n*	sero[+]	%	OR	95% CI	P
Current wall						
Brick/cement	1109	28	2.52	1		**0.02**
Wood	19	2	10.53	4.5	1.0–20.6	
Adobe	371	19	5.12	2.1	1.2–3.8	
Other	19	0	0.00	–	–	
Floor of housing during childhood						
Cement	796	16	2.01	1		**0.003**
Wood	68	0	0.00	–	–	
Dirt	639	33	5.16	2.7	1.5–4.9	
Other	15	0	0.00	–	–	
Roof during childhood						
Clay tiles	753	22	2.92	1		**<0.0001**
Fibrocement tiles	408	8	1.96	0.7	0.3–1.5	
Thatched/palm leaves	92	12	13.04	5	2.4–10.5	
Other	265	7	2.64	0.9	0.4–2.1	
Walls of housing during childhood						
Brick/cement	737	12	1.63	1		**0.004**
Wood	70	2	2.86	1.8	0.4–8.1	
Adobe	676	34	5.03	3.2	1.6–6.2	
Other	35	1	2.86	1.8	0.2–14.1	
Other factors						
Relatives diagnosed with Chagas disease						
No	1320	39	2.95	1		**0.022**
Yes	101	9	8.91	3.2	1.5–6.8	
No response	97	1	1.03	0.3	0.1–2.5	
Previous transfusions						
No	1467	48	3.27	1		0.578
Yes	29	0	0.00	–	–	

Bold numbers mean p values statistically signifciant at <0.05.

Table 3 Logistic regression model for *T. cruzi* seropositive pregnant women of Santander

Variable	OR	95% CI	*P*
Current thatch roof	11.8	2.2–63.2	0.004
Close contact with vector at least once on life	6.9	3.7–12.9	<0.001
Low education level	4.6	2.2–9.5	<0.001
Thatch roof during childhood	3.0	1.4–6.6	0.005
Age >32 years	2.1	1.1–3.9	0.024

foodborne outbreaks for which a triatomine infection source has been identified, these have usually been species that are not usually associated with vectorial transmission, and mostly *P. geniculatus* [38]. Given the epidemiological history of vectorial transmission in the areas involved in this study, and also the association with housing conditions for intradomiciliary transmission, oral transmission seems less likely to be a driver of prevalence of infection in this population.

With respect to performance of the diagnostic tests, ELISA and IFI have been evaluated and presented in detailed in previous studies [24]. The reproducibility between these two tests was almost perfect with a kappa coefficient of 0.98 so the HAI test was only necessary to confirm the seropositive status in a few cases.

Because the local doctors and health personal participating in this study were trained for both diagnosis and treatment, the study was a great opportunity to generate awareness among healthcare workers and the community about congenital Chagas disease. Recent studies have highlighted the importance of increasing awareness at local level as a crucial tool to improve diagnosis and disease control [39]. For all pregnant women diagnosed in this study, aetiological treatment was offered and given after delivery through the local hospitals. The decision of treating all mothers (after pregnancy) despite the absence of congenital transmission in this particular study was considered appropriate given that in other endemic departments of the country this type of transmission has been reported [20] and we cannot dismiss the possibility of transmission in future pregnancies. The trypanocidal treatment of women with chronic Chagas infection before

pregnancy prevents congenital transmission of *T. cruzi* to their children [11]. This group of young and mostly healthy women could potentially benefit from aetiological treatment in terms of reducing the possibility of developing heart complications [40].

A potential limitation of this study is that we only included pregnant women who attended antenatal control clinics in hospitals. Despite the fact that the coverage of antenatal care in Colombia has been increasing in the last decades and for 2010 it was estimated at 97% [41], this still could represent a potential bias in omitting the poorest women who only seek hospital care for delivery or who do not seek care at any time and who are the patients at the highest risk of *T. cruzi* seropositivity. Another potential limitation of this study, considering a recent meta-analysis has estimated the transmission rate of congenital Chagas disease at 4.7% (95% CI: 3.9–5.6) [17], is that the sample size of newborns (49 children) was not enough to find congenital cases.

This study updates epidemiological data on Chagas disease prevalence in pregnant women in a Colombian endemic area. The identified risk factors will allow the local health providers to target more precisely the necessary interventions for this specific population. The fact that no congenital cases were found in this series of pregnant women does not mean that this transmission route is not present in this department. There is still a need to guarantee the proper diagnosis at birth and the clinical and laboratory follow-up to all children born from infected women, as recently recommended [42]. Providing care and aetiological treatment to infected women of childbearing age before they become pregnant is also imperative in order to reduce the probability of the disease progression and prevent congenital transmission.

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