

**Seroprevalence of *Trypanosoma cruzi* in a population of pregnant women and evaluation of their knowledge about Chagas Disease and its vectors**

**Soroprevalência de *Trypanosoma cruzi* em uma população de gestantes e pesquisa dos conhecimentos sobre Doença de Chagas e seus vetores**

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**ABSTRACT**

This study aimed at determining prevalence of anti-*Trypanosoma cruzi* antibodies in pregnant women in the extreme south of Brazil and at evaluating their knowledge about Chagas Disease (CD) and its vectors. The study was carried out in Pelotas, Rio Grande do Sul (RS) state, Brazil. The Chemiluminescent Microparticle Immunoassay (CMIA) was used for the triage and, when it was reagent, results were confirmed by the Indirect Immunofluorescence Assay (IFA). A semi-structured questionnaire was applied to evaluate patients' knowledge. The Chi-squared test was used for evaluating correlations among variables under study while an Odds Ratio was conducted when associations were statistically significant. Only 33 (27.7%) out of 119 pregnant women under investigation underwent the serological test to identify *T. cruzi* because the study was carried out in the worst phase of the COVID-19 pandemic. However, prevalence was 6.1% (2 cases). Only 26.9% of pregnant women were able to identify triatominae and 66.4% did not know that the heart could be affected by CD. Associations between variables "lived/have lived in a rural area" and "having found 'kissing bugs' in the household"; "high schooling level" and "knowing that CD causes heart disorders"; and "stating that they know what CD is" and "identifying triatominae" exhibited statistical significance. Results showed that the rate of positive pregnant women is high and needs attention, even though the size of the sample is a limiting factor. Besides, the patients' lack of knowledge about the disease and its vectors shows the importance of educational interventions.

**Keywords:** Chagas Disease, questionnaire, serology, triatominae.

**RESUMO**

O objetivo deste estudo foi determinar a prevalência de anticorpos anti-*Trypanosoma cruzi* em mulheres grávidas no extremo sul do Brasil e avaliar seu conhecimento sobre a doença de Chagas (DC) e seus vetores. O estudo foi realizado em Pelotas, no estado do Rio Grande do Sul (RS), Brasil. O Imunoensaio de Micropartículas Quimioluminescentes (CMIA) foi utilizado para a triagem e, quando reagente, os resultados foram confirmados pelo Ensaio de Imunofluorescência Indireta (IFA). Um questionário semiestruturado foi aplicado para avaliar o conhecimento dos pacientes. O teste do qui-quadrado foi usado para avaliar as correlações entre as variáveis em estudo, enquanto o Odds Ratio foi realizado quando as associações foram estatisticamente significativas. Apenas 33 (27,7%) das 119 gestantes investigadas foram submetidas ao teste sorológico para identificar o *T. cruzi* porque o estudo foi realizado na pior fase da pandemia da COVID-19. No entanto, a prevalência foi de 6,1% (2 casos). Apenas 26,9% das gestantes souberam identificar os triatomíneos e 66,4% não sabiam que o coração poderia ser afetado pela DC. As associações entre as variáveis "mora/já morou em uma área rural" e "já encontrou 'barbeiro' na casa"; "alto nível de escolaridade" e "saber que a DC causa distúrbios

cardíacos"; e "afirmar que sabe o que é DC" e "identificar triatomíneos" apresentaram significância estatística. Os resultados mostraram que a taxa de gestantes positivas é alta e precisa de atenção, embora o tamanho da amostra seja um fator limitante. Além disso, a falta de conhecimento das pacientes sobre a doença e seus vetores mostra a importância de intervenções educacionais.

**Palavras-chave:** Doença de Chagas, questionário, sorologia, triatomíneos.

## 1 INTRODUCTION

Chagas Disease (CD) is endemic to 21 countries in the Americas and belongs to the group of Twenty Neglected Tropical Diseases. About 4,600 people died of CD and its complications per year between 2008 and 2017<sup>1</sup> in Brazil.

In southern Brazil, Rio Grande do Sul (RS) has been the state that exhibits the highest prevalences of CD and its vectors. Brandt *et al.*<sup>2</sup> (1957), Camargo *et al.*<sup>3</sup> (1984) and Baruffa & Alcântara<sup>4</sup> (1985) showed high rates of seroprevalence in their patients between the 1950's and the 1980's.

Control on vector transmission in RS and virtual eradication of *Triatoma infestans*<sup>5,6</sup>, the main vector species in the region, have enabled researchers to pay more attention to other forms of transmission. For instance, there is vertical transmission in RS, since the state has several chronic cases of CD, a fact that enables its transmission during pregnancy. It should be mentioned that the first case of *T. cruzi* mother-to-child transmission (vertical) was described by Mazza *et al.*<sup>7</sup> in Venezuela in 1936. Afterwards, some cases were detected in Brazil<sup>8,9,10</sup>.

The extreme south of Brazil needs updated studies of CD in pregnant women, since the region is considered endemic to the disease. Data were provided by studies that show not only a large number of patients who are HIV+ reactants to anti-*T. cruzi* antibodies (5% coinfection)<sup>11</sup> but also the same prevalence in oncological patients in the region<sup>12</sup>. Regarding blood donor centers, an updated study showed that the index of anti-*T. cruzi* antibodies in blood donors in southern Brazil was 0.27%<sup>13</sup>.

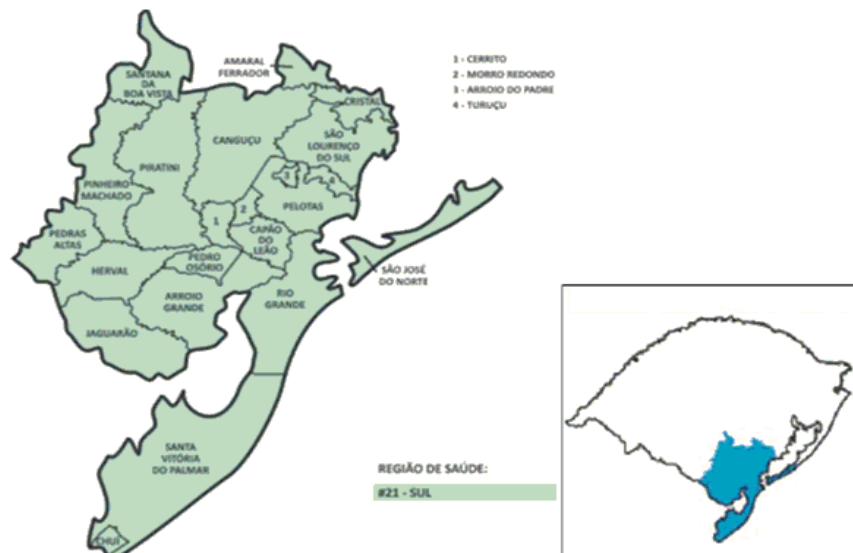
Therefore, this study aimed at investigating prevalence of anti-*T. cruzi* antibodies in a population of pregnant women in the extreme south of Brazil and at evaluating their knowledge about CD and its vectors.

## 2 METHODS

### 2.1 STUDY AREA, TARGET AUDIENCE AND ETHICAL ASPECTS

This study was carried out in the Ambulatory of Gynecology and Obstetrics at the Universidade Federal de Pelotas (UFPel) in Pelotas, RS, Brazil. It involved pregnant women who live in 22 cities (figure 01) that belong to the 3rd Regional Health Coordination (RHC) and use services provided by the Sistema Único de Saúde (SUS) to assist patients throughout pregnancy and puerperium in the extreme south of Brazil. Participants were volunteers and signed the Informed Consent Form (ICF). The study was approved by the Ethics Committee (no. 3,935,759). Data were collected from May to November 2020, when all pregnant women who were assisted in the Ambulatory were invited to take part in the study.

Figure 01: Map of the 22 municipalities belonging to the 3rd Regional Health Coordination, located in the extreme south of the state of Rio Grande do Sul, Brazil.



Source: Secretary of Health of the State of Rio Grande do Sul

### 2.2 SEROLOGICAL TEST

After getting their test requisition forms, pregnant women underwent free serological tests subsidized by the SUS in laboratories. To investigate anti-*T. cruzi* antibodies, the Chemiluminescent Microparticle Immunoassay (CMIA) (ARCHITEC Chagas®, Abott) was used for the triage. When it was reagent, the Indirect Immunofluorescence Assay (IFA) (WAMA® Diagnóstica) was applied to confirm it, as recommended by the Ministry of Health in Brazil.

Participants handed in their results to their physician and, when they were positive, they were followed so as to find the most adequate treatment to the disease. Regarding pregnant

women who did not take the test, even though they had agreed to, phone calls were made to get to know their difficulties in taking the serological tests.

### 2.3 KNOWLEDGE ABOUT CD AND ITS VECTORS

To evaluate pregnant women's knowledge about CD and its vectors, they answered a semi-structured questionnaire – with open and closed questions – adapted from two previously validated questionnaires<sup>14,15</sup>. The following socioeconomic variables were collected: age, schooling, family income, address, type of housing and whether they either lived or have lived in rural areas. When the pregnant women said that they had/have lived in a rural area, further questions were asked. They aimed at knowing how long the patients lived in the rural area, whether they raised pets and, mainly, structural conditions of their households (the main house and its annexes).

The questionnaire addressed the main aspects of CD and its vectors in the following questions:

- a) Do you know what Chagas Disease (CD) is? (1) Yes (2) No
- a) Do you know how it is transmitted? (1) Yes (2) No
- b.1) Can you get CD in the following ways? (1) Contact of skin or eyes with feces of the insect “kissing bug”; (2) Blood transfusion from infected people; (3) From mother to child during pregnancy; (4) Intake of food contaminated with feces of “kissing bugs”; (5) Mosquito bite; (6) Tick bite
- b) Can you identify any of the insects shown to you as “kissing bugs”? (1) Yes (2) No
- c) Have you already found “kissing bugs” in your household or its surroundings? If so, when? Where? What did you do when you saw the insect?
- d) Do you know what to do when you find a “kissing bug”?
- e) Have you already heard about a Triatomine Control Center (TCC)? (1) Yes (2) No
- f) Is there a cure for CD? (1) Yes (2) No/I don't know
- g) Can CD be severe? (1) Yes (2) No/I don't know
- h) What may be caused by CD? (1) heart disorders; (2) problems in the digestive system; (3) alterations in the nervous system; (4) alterations in other organs/systems
- i) Have you or any family member got CD? (1) Yes (2) No. If so, who?
- j) Can CD be prevented/avoided? (1) Yes (2) No/I don't know. If so, how?
- k) Had you already heard about CD? (1) Yes (2) No. If so, where?

Most questions were closed with pre-defined answers, mainly “yes” and “no”. In question “c”, five arthropods – two triatominae (*Triatoma infestans* and *Triatoma rubrovaria*), a spider *Loxosceles* sp., a phytophagous hemipteran insect of the genus *Nezara* sp. and a mosquito *Aedes aegypti* – were shown to the informants in a showcase. When they identified at least one of the “kissing bugs”, their answers were considered correct. Question “e” was an open one and both questions “k” and “l” could trigger new ones, depending on the informants’ answers.

## 2.4 STATISTICAL ANALYSIS

Firstly, results were expressed by descriptive statistics, i. e., values were expressed as frequency (observed value -  $n$ ) and percentage (%). They were organized and analyzed by the Microsoft Excel® Program. The Chi-squared test ( $\chi^2$ ) was used for evaluating seroprevalence of CD in relation to different socio-epidemiological factors and knowledge about the disease and its vectors. Results with  $p \leq 0.05$  were considered significant. The Odds Ratio (OR) was also applied to statistically significant associations. The software used for the statistical analysis was MedCalc© (version 20.115, Ostend, Belgium).

## 3 RESULTS

The study involved 119 pregnant women who come from 14 cities. Participants have lived in Pelotas (96), Capão do Leão (5), Herval (4), Canguçu (2), Piratini (2), Turuçu (2), Amaral Ferrador (1), Cerrito (1), Cristal (1), Jaguarão (1), Morro Redondo (1), Pinheiro Machado (1), Santana da Boa Vista (1) and São Lourenço do Sul (1). Thirty-three (27.7%) out of 119 pregnant women under investigation underwent the serological test to identify anti-*T. cruzi* IgG antibodies and two cases were positive (6.1%). It should be mentioned that one of the positive pregnant women was HIV+; thus, a case of *T. cruzi*/HIV coinfection was found. In addition, in the group of HIV+ pregnant women (16) more patients took the serological test, i. e., 68.8% (11), by comparison with 21.4% (22) who were HIV- (103) ( $p=0.01$ ; OR=3,2).

Concerning characteristics of both positive patients, they were 20 and 32 years old, had never lived in rural areas (neither in São Lourenço do Sul nor in Pelotas, respectively) and their family incomes were the national minimum wage. The oldest one was HIV+. The patient from São Lourenço do Sul had finished Elementary School while the one from Pelotas had finished High School. When they were asked about their knowledge of CD, both said that they did not know how it could be transmitted. However, both showed the “kissing bug” in the showcase, despite their poor knowledge about the disease, mainly related to the organs it affects.

Regarding clinical aspects, the HIV+ patient had undetectable viral load, took the cocktail of antiretroviral drugs against AIDS and had gestational hypothyroidism. She was asymptomatic to any infection caused by *T. cruzi*. The patient from São Lourenço do Sul had Fanconi Syndrome and sickle cell anemia; when she was younger, she had been treated for leukemia and had undergone both blood and bone marrow transfusion. However, she also had asymptomatic CD; neither heart disorders nor digestive ones were clinically detected.

All pregnant women who had read and signed the ICF were stimulated (phone calls and text messages) to take part in the study and difficulties faced by those who had not undergone the tests were identified. Twenty-six (30.5%) out of 86 patients who had not taken the serological test to identify anti-*T. cruzi* antibodies answered that the main factor had been the risk of exposure to Coronavirus.

Concerning socioeconomic aspects, 48.7% (58) of participants got up to 2 national minimum wages while 35.2% (42) stated that they had attended school for 8 years.

Regarding housing, 30.3% (36) answered that they had already lived in a rural area for a certain period, whose average was 18.4 years. In this group, 69.4% (25) said that they lived in good brick houses, 11.1% (4) in wood houses, 8.3% (3) in brick houses, but with cracks on the walls, 5.6% (2) in brick and wood houses and 5.6% (2) in clay/terracotta/wattle and daub houses. When they were asked whether their houses had annexes, such as chicken pens, sties and corrals, their answers showed that 69.4% (25) had chicken pens, 58.3% (21) had sties and 44.4% (16) had corrals close to their houses. Concerning conditions found in chicken pens, 88% (22) said that theirs was made of wood, 80% (20) added that it was not painted and 72% (18) mentioned that it had cracks.

When the showcase with vectors was shown to the informants, only 26.9% (32) was able to identify at least one insect (either *T. infestans* or *T. rubrovaria*). Eleven (30.6%) out of 36 pregnant women who lived/have lived in a rural area said that they had seen vectors in their households, a statistically significant difference from the group that had never lived in a rural area (Table 1).

Table 01: Statistically significant associations between socioeconomic and knowledge variables of pregnant women assisted in Pelotas, RS, Brazil, regarding Chagas disease and its vectors.

Variables	Found “kissing bugs” at home		p- value	Odds Ratio (IC <sub>95%</sub> )
	yes (%)	No (%)		
live/lived in a rural area				
Yes	11 (30.6)	25 (69.4)	0.001	6.9 (2.2 a 21.7)
No	5 (6.0)	78 (94.0)		

	Does CD cause heart problems?			
Education	Yes (%)	No (%)		
0 to 8 years of study	1 (2.4)	41 (97.6)	0.013	13.4 (1.7 a 104.4)
≥ 9 years of study	19 (24.7)	58 (75.3)		
Recognized the triatomine				
Do you know what DC is?	Yes (%)	No (%)		
Yes	25 (78.1)	7 (21.9)	0.0002	5.8 (2.3 a 15.0)
No	33 (37.9)	54 (62.1)		

Source: Authors

Regarding “kissing bugs”, 75% (12) of informants said they had been found in the surroundings of their houses. When they were found, 50% (8) said that they had killed them while 31.3% (5) admitted they had done nothing when they saw them. When all pregnant women were asked what they would do when they found a “kissing bug”, 70.6% (84) said they would kill it, 14.3% (17) would do nothing, 10.1% (12) would hand it in to a health agent and only 5% (6) would take it to a TCC. In agreement with the results, 91.6% (109) of participants said they had never heard of any TCC.

When informants were asked about the relation between organs and CD, 66.4% (79) did not know that the heart could be affected by the disease, while 65.6% (78) did not know that it could also affect the digestive system, the nervous system and other organs/systems. In answers given to this question, there was statistically significant correlation between schooling levels and knowledge about the fact that the disease may cause heart disorders ( $p=0.013$ ) (Table 2). The group that stated that they knew what CD was (58) exhibited a high rate of knowledge about triatominae in the showcase. Thus, there was statistically significant difference between both groups ( $p=0.0002$ ) (Table 2).

When asked whether they, or any family member, had had the disease, 10.1% (12) stated that there had already been cases in the family.

In terms of prevention, 77.3% (92) of pregnant women believe that CD may be prevented/avoided. Measures are listed in Table 2.



Table 02: Aspects of knowledge of pregnant women in the extreme south of Brazil, regarding the prevention of Chagas disease and its vectors.

How can Chagas disease be prevented/avoided?	N	%
Hygiene/Cleanliness/Avoid accumulation of garbage	34	36.9
Don't know*	29	31.5
Care/home care, patio, food	9	9.8
Fumigation/poison on the spot/insecticide/repellent	8	8.7
Others**	4	4.3
Killing of insect /avoid contact with the kissing bugs	3	3.3
Treatment/drug	2	2.2
To take insect to TIP/BUH	2	2.2
Avoid açai	1	1.0

Source: \*even though I said yes (CD can be prevented/avoided) / \*\*"Prevention", "seek help", "knowledge", "survey"

Regarding places where the informants had already heard about CD, most mentioned schools. However, a relevant part of pregnant women said that they did not know where they got information on the disease, as shown in Table 3.

Table 03: Aspects of knowledge of pregnant women in the extreme south of Brazil, regarding the prevention of Chagas disease and its vectors and the place where this knowledge was acquired.

Have you heard about Chagas disease anywhere?	N	%
Yes*	82	68.9
No	37	31.1
Where?		
School/college/course/tecnic course	28	34.1
Hospital/ Health Center / BUH	13	15.9
Television	11	13.4
Internet	4	4.9
Conversation family/cotidiano	2	2.4
Known with the disease	1	1.2
book	1	1.2
dont,' know	22	26.8

Source: Authors

#### 4 DISCUSSION

Prevalence of 6.1% anti-*T. cruzi* IgG antibodies in pregnant women is high and important by comparison with results of a study carried out by Araújo *et al.*<sup>16</sup> (2009), who found 0.3% when they investigated this public in Pelotas, RS, Brazil. However, Capiotti *et al.*<sup>17</sup> investigated pregnant women in Health Centers in Santa Maria, RS, and did not find any positive cases of *T. cruzi* among them.

When results were compared to the ones of studies conducted with pregnant women in other Brazilian regions, there was relevant difference in prevalence. In the Distrito Federal (DF), after serological analyses, a study showed prevalences of 0.19%, 0.21% and 0.17% in 2014, 2015 and 2016, respectively<sup>18</sup>. Figueiro Filho<sup>19</sup> (2007) found 0.1% positivity in pregnant women in Mato Grosso do Sul (MS) state. In Goiás (GO) state, prevalence was 0.5%, but a

specific city in the state – Aparecida de Goiânia – drew much attention due to its prevalence of 7.45% in seroreagent pregnant women<sup>21</sup>. In Minas Gerais (MG) state, estimate in puerperal women was 0.5%<sup>22</sup>.

It should be highlighted that this evaluation has limitations due to its sample, since the small number of pregnant women (33) who took the test may influence the high prevalence (6.1% or 2 cases). The SARS-COV-2 epidemic, which started in Wuhan (China) in December 2019 and became a pandemic – about 230 million infected people and about 6.4 million deaths worldwide<sup>23</sup> – hindered tests, since samples were not collected during their appointments, i.e., pregnant women had to go to the laboratory, show the requisition form and take the test. As the result of high transmissibility of the virus, authorities recommended social distancing in 2020 and services in all sectors were restricted in terms of reach and availability<sup>24</sup>; therefore, access to laboratories and/or institutions in charge of appointments was hindered. Collecting blood and sending it to the laboratory right after the interview would mitigate difficulties. Such methodology has already been used by other studies conducted in the region<sup>25,26</sup> and should be used in further investigations.

Concerning tests applied to pregnant and puerperal women to check *T. cruzi* in Brazil, it should be mentioned that serological tests tend to be applied more often to HIV+ pregnant women because they are considered a risk group, in agreement with the Protocol of Therapeutic Guidelines on Chagas Disease, issued by the Ministry of Health<sup>27</sup>, which requires prenatal testing and recommends that all HIV+ patients should take anti-*T. cruzi* antibody tests. This study found statistically significant difference in *T. cruzi* testing by HIV+ patients ( $p=0.01$ ). It may have happened because physicians in the Ambulatory are used to requesting results of exams taken by this group. Besides, the patients concern themselves with the topic. In contrast, Stauffert *et al.*<sup>25</sup> found that only 3.2% of HIV+ patients from a reference center in HIV/AIDS treatment in southern Brazil got requisition forms to undergo *T. cruzi* serology, a fact that shows low adherence to the protocol.

Both positive pregnant women identified by this study said that they had never lived in a rural area. It confirms the possibility of the urbanization of the disease and new forms of transmission, which used to be mainly rural and propagated by a vector. Control on vector transmission in the state<sup>28,29</sup> is expected to lead to decrease in the number of infected people but a program of prenatal testing is needed to identify cases so that the disease can be controlled and treated during pregnancy. Serological tests of CD must be effectively requested by physicians, mainly in the first trimester of pregnancy, to avoid the possibility of congenital transmission and after-effects to the conceptus<sup>30</sup>. RS has very few requests for laboratory tests

that aim at diagnosing CD. From 2017 to 2020, there were 1.1%, 1.2%, 0.53% and 0.54% of laboratory requests for *T. cruzi* tests, respectively, in the state<sup>31</sup>.

Regarding pregnant women's knowledge, 26.9% identified the "kissing bug" in the showcase. The percentage is much lower than the one (89.1%) found by a study carried out with adults who live in a rural area in Bambuí, MG<sup>32</sup>. Dos Santos *et al.*<sup>5</sup> (2016) conducted a study with residents who had got their houses in the Program of Housing Improvement to Control Chagas Disease in RS and 86.3% was able to identify the insect. Maeda & Gurgel Gonçalves<sup>33</sup> found percentages of 89% and 79% in Águas Claras and Planaltina, DF, respectively, among rural residents who identified and named the insect. In order to face the current challenge, it is clear that CD and its vectors must be more widely publicized among pregnant women in the area under investigation.

The group of pregnant women who said that they knew about CD was more successful in identifying the triatominae that the group that did not know anything about the disease ( $p=0.0002$ ). This result agrees with the one found by Connors *et al.*<sup>34</sup> (2017), since informants who had already heard about the disease identified CD vectors correctly. Statistical significance between variables "lived/have lived in a rural area" and "having found 'kissing bugs' in the household" (Table 1) confirms predominance of triatominae in the rural area<sup>35,36</sup>. Besides, considering participants who said that they had already seen the insect, 75% reported they had seen them in the surroundings of the household. It agrees with studies carried out in different Brazilian regions where the surroundings of the household, mainly chicken pens, constitute the artificial ecotope which vectors choose to invade and colonize due to shelter and food availability<sup>35, 37,38</sup>.

When pregnant women were asked about organs that are affected by CD, few gave right answers; the heart was only mentioned by 16.8%. It differs from answers given by informants in Bambuí, MG, where 84.5% of the population mentioned the heart as the organ that is affected by CD, but it is close to the percentage found by Rosenthal *et al.*<sup>39</sup> in Pelotas, RS, where only 18.9% of rural residents said the heart could be affected by the disease. However, there was statistically significant difference between this variable and schooling. Women who had attended school longer exhibited more knowledge, a fact that shows the importance of formal education. Even though there may be relatively distinct methodologies to address the issue, mainly concerning the public and places under investigation, it may be concluded that pregnant women in this region exhibited insufficient knowledge about this aspect of the disease.

In terms of measures that must be taken to avoid contact with *T. cruzi* vectors, hygiene/cleaning was the most common answer. The same was found by the study conducted

in Bambuí<sup>32</sup> but measures are generalist and show that it is not clear which specific actions should be employed to avoid/mitigate contact with the triatomine. It should be highlighted that no pregnant woman answered “test during prenatal” to avoid CD transmission (mainly to the newborn), even though most answered that the disease may be transmitted congenitally.

This study concludes that the rate of positive pregnant women in the population under investigation is high and correlated with other studies of seroprevalence of anti-*T. cruzi* antibodies carried out with different groups in the extreme south of Brazil. Even though the sample may be considered a limiting factor in this study, it is important and suggests that similar searches should be conducted in the region after the pandemic. Pregnant women’s knowledge was partial and insufficient regarding several questions about vectors and general aspects of CD. It shows the need to prepare and distribute educational material aiming at pregnant women. Besides, these results highlight the need to carry out anti-*T. cruzi* antibody serological tests as part of prenatal tests in endemic areas.

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