

Current situation of Chagas disease vectors (Hemiptera, Reduviidae) in Southern Rio Grande do Sul State, Brazil

Tanise Freitas Bianchi¹, Sabrina Jeske¹, Ana Paula da Paz Grala¹, Italo Ferreira de Leon¹, Cleonara Bedin², Fernanda de Mello², Guilherme Carlos Castilhos da Silva², Marcos Marreiro Villela¹

ABSTRACT

Chagas disease (CD) has been considered endemic in the South of Rio Grande do Sul (RS) State, Brazil. This study aimed at evaluating the occurrence of *Trypanosoma cruzi* vectors based on the main species captured in Southern Rio Grande do Sul State from 2008 to 2019. The study area comprised counties that belong to the 3rd Regional Health Coordination (RHC) and to the 7th RHC, whose headquarters are in Pelotas and Bage, respectively. The study was based on secondary data provided by the partnership between the Federal University of Pelotas, Rio Grande do Sul State (UFPeL-RS) and the State Health Surveillance Center in RS (SCHS-RS). One thousand and four hundred triatomines were captured in the area supervised by the 3rd RHC, mainly in Canguçu (37.7%), Piratini (22.4%) and Pinheiro Machado (15.1%), while, in the area supervised by the 7th RHC, the largest number of triatomines was captured in Lavras do Sul (64.15%). In both areas, *Triatoma rubrovaria* (90.6%) and *Panstrongylus tupynambai* (7.4%) were the most common species. Most were captured inside households but *T. cruzi*-positive insects were not found in the period under study. The results of this study show that, in Southern Rio Grande do Sul State, there is still a high rate of triatomine household invasion and dispersal, mainly by *T. rubrovaria*. Thus, the entomological surveillance should be maintained with the participation of the population and further studies should be deepened in the area.

KEYWORDS: *Trypanosoma cruzi*. *Triatoma rubrovaria*. *Panstrongylus tupynambai*. Vector control. Rio Grande do Sul. Chagas disease.

INTRODUCTION

Chagas disease (CD), which is caused by the protozoan *Trypanosoma cruzi*, has been considered one of the world's most neglected tropical disease by the World Health Organization (WHO). It is a result of the vectors geographical dispersal since the disease affects mainly low-income countries in Latin America, where there are high morbi-mortality rates, considerable socioeconomic impact, limited resources and low political priority to combat the disease^{1,2}.

T. cruzi is mostly transmitted by vectors (hemipterans that belong to the subfamily Triatominae) whose infectant forms are eliminated in triatomine feces and urine, either throughout or right after the blood meal. Protozoans penetrate the human skin through puncture holes resulting from insect stings or their scarification caused by scratching, and through mucous and conjunctiva membranes, when individuals rub their noses, mouths or eyes with infected hands. The main means of infection with *T. cruzi* is by vector transmission, equivalent to 80% of Chagas disease cases³, however, other

¹Universidade Federal de Pelotas, Instituto de Biologia, Programa de Pós-Graduação em Parasitologia, Pelotas, Rio Grande do Sul, Brazil

²Centro Estadual de Vigilância em Saúde, Porto Alegre, Rio Grande do Sul, Brazil

Correspondence to: Tanise Freitas Bianchi
Universidade Federal de Pelotas, Instituto de Biologia, Programa de Pós-Graduação em Parasitologia, Campus Universitário Capão do Leão, S/N, CEP 96010-900, Pelotas, Rio Grande do Sul, Brazil
Tel: +55 53 2341-7686
+55 53 99966 7811

E-mail: tanisebianchi@hotmail.com

Received: 5 March 2021

Accepted: 11 May 2021

modes of infection include blood transfusion; congenital transmission; organ transplantation; laboratory accidents and, mainly, oral transmission, through contaminated foods such as sugar cane juice and acai, with the majority of reported cases found in the Amazon region. The Ministry of Health of Brazil counted 112 outbreaks in the national territory between the years 2005 to 2013, most of them occurred in the Para, 75.9% (85 outbreaks) and Amapa, 12.5% states^{1,2,4}.

The main environment in which *T. cruzi* transmission takes place is poorly constructed and preserved houses that characterize of low social and economic conditions. Vector domiciliation is related to the opportunity for triatomines to find shelters and burrows, food availability and different degrees of anthrophilia of each species⁵.

Camargo *et al.*⁶ carried out a serological survey showing that Rio Grande do Sul (RS) and Minas Gerais (MG) were the Brazilian States that had the highest human seroprevalence index of *T. cruzi* (8.8%).

Chagas disease has been considered endemic in Southern RS⁷. It is noteworthy that these municipalities, geographically remarkably close, had high rates of home infestation by *T. infestans* in previous decades⁸. Therefore, prevalence in patients from this area is still considered one of the highest in the state and sero-reactivity to *T. cruzi* was found in individuals under the age of 30^{9,10}. Even though it is now less common than in the past, implying that vectors may still be one of the modes of transmission in this area.

Although *T. infestans* has been eliminated in RS State, there are other triatomine species that persist in rural households¹¹. Therefore, this study aimed at evaluating the occurrence of CD vectors based on the main species captured in Southern RS, from 2008 to 2019.

MATERIALS AND METHODS

Characterization and period of study

This is a retrospective descriptive study of CD vectors based on secondary data provided by the Chagas Disease Control Program (CDCP), carried out by the partnership between the Universidade Federal de Pelotas (UFPEl), located in Pelotas, RS, and the State Health Surveillance Center in RS that belongs to the State Health Department (SCHS-SHD-RS).

The investigation comprised the latest results provided by the SCHS, i.e., data collected in the last 12 years, from 2008 to 2019. Both data and entomological information from 2008 to 2016 were found in the CDCP-DATASUS system, while the ones between 2017 and 2019 were collected from the Formulary for Entomological Surveillance of CD (FORMSUS-DATASUS).

Choice of the area and its description (study area)

Considering the high prevalence of CD and the number of vectors in Southern RS, shown by previously mentioned evaluations, – the area chosen for this study comprised counties that belong to the 3rd RHC and the 7th RHC in RS, Brazil.

The 3rd RHC, whose headquarters are in Pelotas, RS, comprises 22 counties (Amaral Ferrador, Arroio do Padre, Arroio Grande, Capao do Leao, Canguçu, Cerrito, Chui, Cristal, Herval, Jaguarao, Morro Redondo, Pedras Altas, Pedro Osorio, Pelotas, Pinheiro Machado, Piratini, Rio Grande, Santa Vitoria do Palmar, Santana da Boa Vista, Sao Jose do Norte, Sao Lourenço do Sul and Turucu), 845,135 inhabitants¹² and has a degree of urbanization of 83.64%¹³ (Figure 1). The 7th RHC has its headquarters in Bage, RS, and comprises 6 counties (Acegua, Bage, Candiota, Dom Pedrito, Hulha Negra and Lavras do Sul), 182,579 inhabitants¹² and has a degree of urbanization of 78.49%¹³ (Figure 2).

Regarding environmental aspects, this region is part of the Pampa Biome, characterized by field vegetation and lowland relief, formed by dense, tree and shrub vegetation, on the slopes and along water courses, with the occurrence of wetlands and also of natural pastures¹⁴.

Description of entomological surveillance in the area

In counties that belong to both, 3rd and 7th RHCs, as well as in other Brazilian regions, passive surveillance is carried out by the population when individuals notify the occurrence of vectors to the Triatomine Information Center (TIC). TICs, which have been implemented in tactical spots, such as schools and health centers, are the main strategies of vector control. City health agents go to the TICs monthly and, if there is any confirmed notification, i.e., the captured insect is actually a triatomine, a visit to the household is scheduled within a month (counting from the day the insect was collected). Since the process of registering notifications/confirmations is mandatory, they are available in the SCHS.

According to the rules proposed by the Ministry of Health, the search is carried out in peridomicile and in intradomicile. It includes the analysis of people's households and the search for shelter and animals that may serve as food sources for triatomines¹⁵.

Statistical analysis of data

The following variables were characterized by this study: vector species found in the area, level of triatome infestation per county; index of *T. cruzi* infection of triatomines captured per county (number of captured kissing



Figure 1 - Map of cities that belong to the 3rd RHC; headquarters in Pelotas, RS State, Brazil.

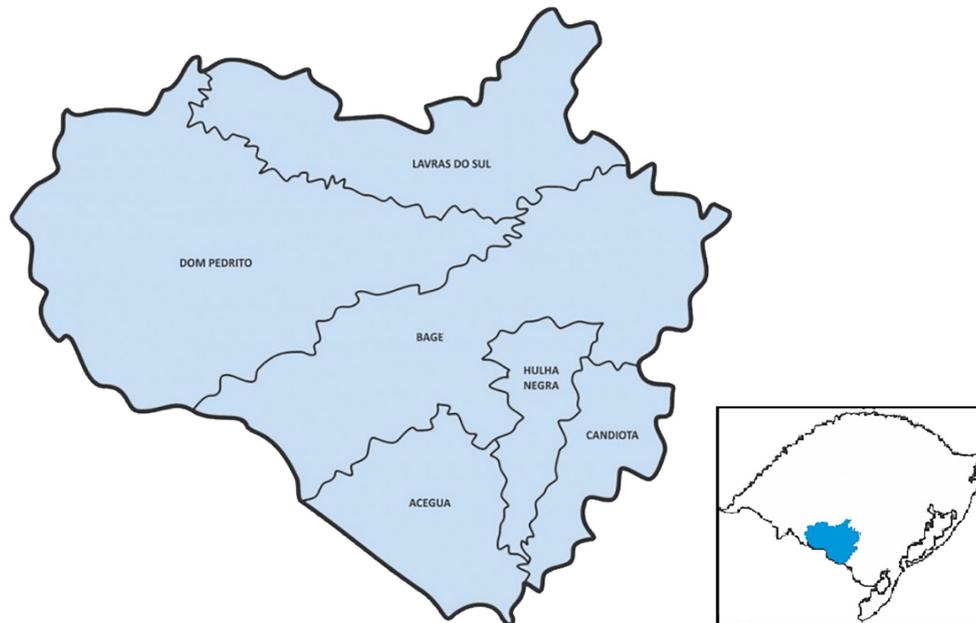


Figure 2 - Map of cities that belong to the 7th RHC; headquarters in Bage, RS State, Brazil.

bugs/number of examined kissing bugs/percentage of *T. cruzi*-positive bugs); infestation frequency of households and the peri-domicile; and geographical dispersal of species captured in both RHC.

Data tabulation was carried out by the Microsoft Excel[®] program and a database was built up. Values were expressed as frequencies (observed value n) and percentages. Variables were statistically compared by the Chi-Square Test (χ^2); values of $p \leq 0.05$ were considered significant. The period

under evaluation (2008-2019) was divided into six biennia. The statistical analysis was conducted by the MINITAB[®] program (version 18, Minitab LLC, Pennsylvania, EUA). The Odds Ratio Test was applied to statistically significant values.

RESULTS

Reports showed that 1,400 triatomines were captured in the counties that belong to the 3rd RHC (Pelotas) from 2008

to 2019 (Table 1). A decrease in the number of captures was observed in this period. The biennium with the largest number of captures was 2008-2009, when 579 insects were registered, corresponding to 41.7% of the total. The comparison between the first three biennia and the last three ones showed a statistically significant difference ($p=0.04$; $OR=3.26$, $IC_{95}=1.04 - 10.16$).

Regarding the counties that comprise the 7th RHC (Bage) (Table 2), 159 triatomines were captured between 2008 and 2019. A decrease in the number of captured insects was also observed in the period, and 2008-2009 was again the biennium with the largest number of captures (63), corresponding to 39.60% of the total. However, the comparison between the first three biennia and the last three ones did not show any statistically significant difference ($p=0.72$).

Canguçu (37.71%), Piratini (22.43%) and Pinheiro Machado (15.07%) were the counties in the 3rd RHC where more triatomines were captured, while in the 7th RHC, Lavras do Sul was the county where more triatomines were captured (64.15%) (Table 4).

In both RHCs, the largest number of specimens belonged to the species *Triatoma rubrovaria* (90.63%), followed by *Panstrongylus tupynambai* (7.37%).

No *T. cruzi*-positive insects were detected in the study period and most triatomines were captured intradomicile (Tables 3 and 4).

Concerning the species dispersal, in the 3rd RHC, *T. rubrovaria* was found in 16 (72.20%) out of 22 counties, while *P. tupynambai* was found in seven counties and *T. carcavalloii* was only found in Canguçu (1 capture) (Table 3). In the 7th RHC, *T. rubrovaria* was identified in Bage, Candiota, Dom Pedrito, Hulha Negra and Lavras (83.30% of counties), *P. tupynambai* was detected in Bage, Dom Pedrito and Lavras and *P. megistus* was only found in Acegua (Table 3).

DISCUSSION

Chagas disease has been investigated in RS State since the beginning of the 20th century. Arthur Neiva, who registered the first triatomines between 1911 and 1914, identified *Triatoma infestans* and *T. rubrovaria* and this was the first record of *T. rubrovaria* in Brazil^{16,17}.

It should be highlighted that *T. rubrovaria* requires attention, since Silva and Silva¹⁸ found that it is a competent agent of *T. cruzi* transmission due to its bionomic characteristics. Besides, its importance may be compared to the one of other species, such as *T. infestans*¹⁹.

The study carried out by Almeida *et al.*²⁰ showed the increasing frequency of *T. rubrovaria* in rural households in Southeastern RS State and stated these authors claimed that this species may be invading areas that had been previously occupied by *T. infestans*, the main domestic CD vector

Table 1 - Number and ratio of triatomines captured in the area that belongs to the 3rd RHC in Pelotas, RS State, Brazil, in different biennia.

Time corse (biennium)	Number of triatomines	% Total	Average per year
2008-2009 ^a	579	41.36	289.50
2010-2011 ^a	246	17.57	123.00
2012-2013 ^a	246	10.36	123.00
2014-2015 ^b	145	10.36	72.50
2016-2017 ^b	107	7.64	53.50
2018-2019 ^b	77	5.50	38.50
Total	1400	100	116.70

^{a x b}* $p=0,04$

Table 2 - Number and ratio of triatomines captured in the area that belongs to the 7th RHC in Bage, RS State, Brazil, in different biennia.

Time corse (biennium)	Number of triatomines	%/ Total	Average per year
2008-2009 ^a	63	39.60	31.50
2010-2011 ^a	09	5.70	4.50
2012-2013 ^a	16	10.10	8.00
2014-2015 ^b	31	19.50	15.50
2016-2017 ^b	16	10.10	8.00
2018-2019 ^b	24	15.10	12.00
Total	159	100	13.25

^{a x b}* $p=0,72$

Table 3 - Triatomine species captured between 2008 and 2019 in the counties that belong to the 3rd RHC in Pelotas, RS State, Brazil, places of capture and analysis of *Trypanosoma cruzi*.

Counties	Species	Intra	Peri	Not assigned	Analyzes	Positive	Total n (%)
Canguçu	<i>P. megistus</i>	01	-	-	-	-	528 (37.71%)
	<i>T. rubrovaria</i>	494	12	-	74	0	
	<i>P. tupynambai</i>	02	01	13	-	-	
	<i>T. carcavaloi</i>	-	-	01	-	-	
	<i>T. circummaculata</i>	-	-	04	-	-	
Cerrito	<i>T. rubrovaria</i>	51	0	-	0	-	51 (3.65%)
Jaguarao	<i>T. rubrovaria</i>	22	16	-	15	-	43
	<i>T. circummaculata</i>	-	-	05	-	-	(3.07%)
Pedras Altas	<i>T. rubrovaria</i>	36	04	-	02	0	52
	<i>P. tupynambai</i>	-	-	09	-	-	(3.71%)
	<i>T. circummaculata</i>	-	-	03	-	-	
Pinheiro Machado	<i>T. rubrovaria</i>	155	16	-	43	0	211
	<i>T. tupynambai</i>	02	01	34	02	-	(15.07%)
	<i>T. circummaculata</i>	-	-	03	-	-	
Piratini	<i>T. rubrovaria</i>	277	10	-	54	0	314
	<i>T. tupynambai</i>	02	-	23	-	-	(22.43%)
	<i>T. circummaculata</i>	-	-	02	-	-	
Santana da Boa Vista	<i>T. rubrovaria</i>	85	01	-	14	0	98
	<i>P. tupynambai</i>	-	-	11	-	-	(7.00%)
	<i>P. megistus</i>	01	-	-	-	-	
Others	<i>P. megistus</i>	06	-	-	-	-	103
	<i>T. rubrovaria</i>	85	06	-	-	-	(7.36%)
	<i>P. tupynambai</i>	-	-	05	-	-	
	<i>T. circummaculata</i>	-	-	01	-	-	
Total		1219	67	114	204	0	1,400 (100%)

Table 4 - Triatomine species captured between 2008 and 2019 in the counties that belong to the 7th RHC in Bage, RS, Brazil. Places of capture and analysis of *Trypanosoma cruzi*.

Counties	Species	Intra	Peri	Not assigned	Analyzes	Positive	Total n (%)
Acegua	<i>P. megistus</i>	01	-	-	01	0	01 (0.63%)
Bage	<i>T. rubrovaria</i>	14	04	-	0	-	23
	<i>P. tupynambai</i>	02	-	03	0	-	(14.46%)
Candiota	<i>T. rubrovaria</i>	06	-	-	0	-	06 (3.78%)
D. Pedrito	<i>T. rubrovaria</i>	19	03	-	0	-	26
	<i>T. circummaculata</i>	01	-	01	0	-	(16.35%)
	<i>P. tupynambai</i>	-	-	02	-	-	
Hulha Negra	<i>T. rubrovaria</i>	01	-	-	0	-	01 (0.63%)
Lavras do Sul	<i>T. rubrovaria</i>	75	-	06	-	-	102
	<i>P. tupynambai</i>	03	15	02	06	-	(64.15%)
	<i>T. circummaculata</i>	-	-	01	-	-	
Total		122	22	15	07	0	159 (100%)

which was eliminated in the State by chemical control¹¹, corroborating the findings of this study.

Triatomine invasion has decreased lately. In both, 3rd RHC and 7th RHC, the largest number of captures took place in the first biennium under investigation (2008-2009),

when about 40% of the total number of bugs were captured. Reduction in the number of captures would be a result of several processes and induction agents, such as the use of residual pesticides, environmental management and improvement of rural households, besides educational

projects carried out in the counties^{7,21}. According to Priotto *et al.*²², the decrease in the number of insects in the households is apparently not associated with the population's knowledge of CD vectors, since some studies have shown how insufficient is the knowledge of people about vectors in endemic areas, preventing the adoption of actions that prevent different endemics from advancing^{23,24}. This supposition is corroborated by the fact that there has been a decrease in people's knowledge on CD vectors and of the disease notification lately, as shown by a study conducted by Bianchi *et al.*²⁵, who observed that older adults are more aware of the topic than youngsters.

Dutra *et al.*²⁶ carried out a study in patients with heart diseases who used the public assistance service provided by the Cardiology Outpatient Clinic that belongs to the UFPel, in Pelotas, RS State. These patients, who came from Canguçu, Herval, Pinheiro Machado and Piratini, counties in which CD was considered endemic in the 1980's. In these localities, vectors were properly identified, a fact that partially corroborates the findings of this study, since these counties are among those that notified large numbers of triatomines in the 3rd RHC. Such factor may make the population in Canguçu more experienced in the identification of kissing bugs. As a result, this investigation shows that many vectors were captured in the county. Another fact that collaborates this finding is the high index of infection caused by *T. cruzi* (10%) found by Rosenthal *et al.*²⁷ in patients from Canguçu, the county that has the highest populational prevalence of antibodies anti – *T. cruzi*.

Therefore, Canguçu, Piratini and Pinheiro Machado exhibited the largest number of captured triatomines, a fact that agrees with findings published by Priotto *et al.*²², who showed that Canguçu, Piratini, Santana da Boa Vista and Pinheiro Machado had the highest indexes of infestation by triatomines, that were also *T. rubrovaria* (93.9%) and *P. tupynambai* (5.1%). It should be emphasized that these counties have a historical importance regarding CD, since they are geographically close to each other and, in the past, used to have low-quality households in their rural areas. As a result, they had high indexes of household infestation with *T. infestans*⁸. Besides, Canguçu is one of five counties in RS State with the largest numbers of TICs (30), a fact that contributes to increase vector notifications. In other words, the population is more sensitive towards vector identification because of historical issues and there are more TICs distributed around the county favoring the notifications about vectors detections¹⁷.

No *T. cruzi*-positive insects were detected in the period, mainly because of the material bad preservation, since most insects were dry when analyses were carried

out, corroborating data by Priotto *et al.*²² in the same and nearby region. Ribeiro *et al.*²⁸ isolated *T. cruzi* strains from triatomines that belong to the species *T. rubrovaria*, captured in households, peridomicile and in the wild in RS State. Considering all examined specimens, the index of natural infection caused by the protozoan was 3.65%, which was similar to the percentage found by Martins *et al.*²⁹ in six areas in Quaraí, RS State, in a study carried out in the municipality of Quaraí, showing that 4.2% of *T. rubrovaria* triatomines were infected. It should be mentioned that, in Quaraí, *P. tupynambai* was also found co-inhabiting stone fissures with a lizard that belongs to the species *Tupinambis merianae* (giant tegu). This lizard has a very varied diet that includes triatomines and it can acquire natural infection by *T. cruzi*, as has already been described.

Regarding the infections, it is important to consider that captured triatomines must be delivered to the laboratory as soon as possible so that their intestinal contents can be evaluated for positivity to *T. cruzi*³⁰. In addition, the implementation of molecular techniques, such as the PCR (Polymerase Chain Reaction), is very important for the protozoan diagnosis, since such techniques increase sensitivity of *T. cruzi* detection, mainly in the cases of dead and dry insects. However, they have high cost in comparison with microscopic evaluations, a limiting factor in the Brazilian public health system^{31,32}.

The small number of triatomines found in the peridomicile, in both RHCs in the period under investigation should be mentioned. However, if the program is working well, promoting the population's and health agents' cooperation, this finding should not be considered normal, since the largest spots and frequency of insects usually occur in the peridomicile because triatomines often find low-quality facilities in this area, such as chicken and pig pens, barns and rupestrian walls, which are typical in RS State^{20,21,33}. They have cracks that enable triatomines to hide and represent a rich source of nutrients, mainly in chicken pens and spots where rodents and wild birds build their nests.

After *T. infestans* was controlled in RS State, the most captured triatomine species became *T. rubrovaria*³⁴, whose food sources are several invertebrate and vertebrate hosts, such as humans. These vectors are mainly found amid rocks and stones³⁵. Silveira and Rezende³⁶ showed that *T. rubrovaria* can colonize human households and other studies highlighted that there has been an increment in the number of *T. rubrovaria* in Southern Brazil in the last 20 years²⁰, a result that was also found by this investigation, since this species represented 90.6% of captures in Southern Brazil from 2008 to 2019.

CONCLUSION

Despite some limitations of this study, such as the fact that it is based on secondary data, it shows unequivocal data that confirm that, in Southern Brazil, even with a decrease in the capture of *T. cruzi* vectors in the period under investigation, there is still significant household invasion and dispersal of triatomines, mainly *T. rubrovaria* and *P. tupynambai*. The population's awareness on CD and its vectors must improve and emphasis has to be given to the search for suspicious insects in the peridomicile, especially in chicken pens, barns and dumpsites. Besides, health agents of the program could conduct an active survey in Southern RS State, mainly in the peridomicile, and deliver the vectors to the CPHL-RS immediately after for *T. cruzi* detection, aiming at corroborating data shown in this study. Thus, even though the program was successful in the biennia under investigation, when there was a significant decrease in the numbers of captured vectors, activities of entomological surveillance are still essential and the community's active participation should be stimulated so as to reach an effective and sustainable control of CD vectors in Southern RS State.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

REFERENCES

- Rassi Jr A, Rassi A, Marin-Neto JA. Chagas disease. *Lancet*. 2010;375:1388-402.
- World Health Organization. Chagas disease in Latin America: an epidemiological update based on 2010 estimates. *Wkly Epidemiol Rec*. 2015;90:33-43.
- Aras R, Gomes I, Veiga M, Melo A. Transmissão vetorial da doença de Chagas em Mulungu do Morro, Nordeste do Brasil. *Rev Soc Bras Med Trop*. 2003;36:359-63.
- Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Doença de Chagas aguda no Brasil: série histórica de 2000 a 2013. *Bol Epidemiol*. 2015;46: 1-9.
- Silveira AC. Situação do controle da transmissão vetorial da doença de Chagas nas Américas. Current situation with Chagas disease vector control in the Americas. *Cad Saude Publica*. 2000;16 Suppl 2:35-42.
- Camargo ME, Silva GR, Castilho EA, Silveira AC. Inquérito sorológico de prevalência da infecção chagásica no Brasil, 1975/1980. *Rev Inst Med Trop Sao Paulo*. 1984;26:192-204.
- Bedin C, Mello F, Wilhelms TS, Torres MA, Estima C, Ferreira CF, et al. Vigilância ambiental: doença de Chagas no Rio Grande do Sul. *Bol Epidemiol*. 2009;11:1-8.
- Baruffa G, Alcantara Filho A. Inquérito sorológico e entomológico da infecção pelo *T. cruzi* na região Sul do Rio Grande do Sul, Brasil. *Ann Soc Belg Med Trop*. 1985;65 Suppl 1:171-9.
- Rosenthal LA, Petrarca CR, Mesenburg MA, Villela MM. Trypanosoma cruzi seroprevalence and associated risk factors in cancer patients from Southern Brazil. *Rev Soc Bras Med Trop*. 2016;49:768-71.
- Stauffert D, Silveira MF, Mesenburg MA, Manta AB, Dutra AS, Bicca GL, et al. Prevalence of Trypanosoma cruzi/HIV coinfection in southern Brazil. *Braz J Infect Dis*. 2017;21:180-4.
- Bedin C, Wilhelms T, Villela MM, Silva GC, Riffel AP, Sackis P, et al. Residual foci of Triatoma infestans infestation: surveillance and control in Rio Grande do Sul, Brazil, 2001-2018. *Rev Soc Bras Med Trop*. 2021;54:e0530-2020.
- Instituto Brasileiro de Geografia e Estatística. Censo 2010. [cited 2021 May 11]. Available from: <http://www.censo2010.ibge.gov.br>
- Rio Grande do Sul. Secretaria de Planejamento, Governança e Gestão. Atlas socioeconômico Rio Grande do Sul: demografia: taxa de urbanização. [cited 2021 May 11]. Available from: <https://atlassocioeconomico.rs.gov.br/grau-de-urbanizacao>
- Empresa Brasileira de Pesquisa Agropecuária. Pampa. [cited 2021 May 11]. Available from: <https://www.embrapa.br/contando-ciencia/bioma-pampa>
- Silva RA, Rodrigues VL, Carvalho ME, Pauliquévis Jr C. Programa de controle da doença de Chagas no Estado de São Paulo: persistência de alta infestação por triatomíneos em localidades na década de 1990. *Cad Saude Publica*. 2003;19:965-71.
- Oliveira G. Tripanosomiasis cruzi (doença de Carlos Chagas) no Rio Grande do Sul, Brasil. *Mem Inst Oswaldo Cruz*. 1942;37:443-537.
- Rio Grande do Sul. Secretaria de Estado da Saúde. Centro Estadual de Vigilância em Saúde. Divisão de Vigilância Ambiental em Saúde. Informe de situação no estado do Rio Grande do Sul e proposta para a certificação da interrupção da transmissão da doença de chagas por Triatoma infestans. [cited 2021 May 11]. Available from: <https://cevs-admin.rs.gov.br/upload/arquivos/201611/03091534-20120427151516chagas-informe-de-situacao-no-estado-do-rio-grande-do-sul.pdf>
- Silva IG, Silva HH. Suscetibilidade de 11 espécies de Triatomíneos (Hemiptera, Reduviidae) à cepa 'Y' de Trypanosoma cruzi (Kinetoplastida, Trypanosomatidae). *Rev Bras Entomol*. 1993;37:459-63.
- Almeida CE, Francischetti CN, Pacheco RS, Costa J. Triatoma rubrovaria (Blanchard, 1843) (Hemiptera-Reduviidae-Triatominae) III: patterns of feeding, defecation and resistance to starvation. *Mem Inst Oswaldo Cruz*. 2003;98:367-71.
- Almeida CE, Vinhaes MC, Almeida JR, Silveira AC, Costa J. Monitoring the domiciliary and peridomiciliary invasion

- process of *Triatoma rubrovaria* in the State of Rio Grande do Sul, Brazil. *Mem Instituto Oswaldo Cruz*. 2000;95:761-8.
21. Santos CV, Bedin C, Wilhelms, TS, Villela MM. Assessment of the Housing Improvement Program for Chagas Disease Control in the Northwestern municipalities of Rio Grande do Sul, Brazil. *Rev Soc Bras Med Trop*. 2016;49:572-8.
 22. Priotto MC, Santos CV, Mello F, Ferraz ML, Villela MM. Aspectos da vigilância entomológica da doença de Chagas no sul do Rio Grande do Sul, Brasil. *Rev Patol Trop*. 2014;43:228-38.
 23. Villela MM, Souza JM, Melo VP, Dias JC. Vigilância epidemiológica da doença de Chagas em programa descentralizado: avaliação de conhecimentos e práticas de agentes municipais em região endêmica de Minas Gerais, Brasil. *Cad Saude Publica*. 2007;23:2428-38.
 24. Villela MM, Pimenta DN, Lamounier PA, Dias JC. Avaliação de conhecimentos e práticas que adultos e crianças têm acerca da doença de Chagas e seus vetores em região endêmica de Minas Gerais, Brasil. *Cad Saude Publica*. 2009;25:1701-10.
 25. Bianchi TF, Jeske S, Sartori A, Grala AP, Villela MM. Validation of a documentar on Chagas disease by a population living in an endemic area. *Braz J Biol*. 2021;81:665-73.
 26. Dutra AS, Stauffert D, Bianchi TF, Ribeiro DR, Villela MM. Seroprevalence of Chagas disease in Southern Brazilian cardiac patients and their knowledge about the parasitosis and vectors. *Braz J Biol*. 2021;81:867-71.
 27. Rosenthal LD, Vieira JN, Villela MM, Jeske S, Bianchi TF. Conhecimentos sobre a doença de Chagas e seus vetores em habitantes de área endêmica do Rio Grande do Sul, Brasil. *Cad Saude Coletiva*. 2020;28:345-52.
 28. Ribeiro AR, Mendonça VJ, Alves RT, Martinez I, Araújo RF, Mello F, et al. *Trypanosoma cruzi* strains from triatomine collected in Bahia and Rio Grande do Sul, Brazil. *Rev Saude Publica*. 2014;48:295-302.
 29. Martins LP, Castanho RE, Casanova C, Caravelas DT, Frias GT, Ruas-Neto AL, et al. Rupestrian triatomines infected by *Trypanosomatidae*, collected in Quaraí, Rio Grande do Sul, 2003. *Rev Soc Bras Med Trop*. 2006;39:198-202.
 30. Almeida PS, Ceretti Júnior W, Obara MT, Santos HR, Barata JM, Faccenda O. Levantamento da fauna de Triatominae (Hemiptera: Reduviidae) em ambiente domiciliar e infecção natural por *Trypanosomatidae* no Estado de Mato Grosso do Sul. *Rev Soc Bras Med Trop*. 2008;41:374-80.
 31. Hamano S, Horio M, Miura S, Higo H, Iihoshi N, Noda K, Takeuchi T. Detection of kinetoplast DNA of *Trypanosoma cruzi* from dried feces of triatomine bugs by PCR. *Parasitol Int*. 2001;50:135-8.
 32. Marcet PL, Duffy T, Cardinal MV, Burgos JM, Lauricella MA, Levin MJ, et al. PCR-based screening and lineage identification of *Trypanosoma cruzi* directly from faecal samples of triatomine bugs from northwestern Argentina. *Parasitology*. 2006;132:57-65.
 33. Almeida CE, Duarte R, Nascimento RG, Pacheco RS, Costa J. *Triatoma rubrovaria* (Blanchard, 1843)(Hemiptera, Reduviidae, Triatominae) II: trophic resources and ecological observations of five populations collected in the State of Rio Grande do Sul, Brazil. *Mem Inst Oswaldo Cruz*. 2002;97:1127-31.
 34. Almeida CE, Folly-Ramos E, Agapito-Souza R, Magno-Esperança G, Pacheco RS, Costa J. *Triatoma rubrovaria* (Blanchard, 1843) (Hemiptera-Reduviidae-Triatominae) IV: bionomic aspects on the vector capacity of nymphs. *Mem Inst Oswaldo Cruz*. 2005;100:231-5.
 35. Salvatella R, Rosa R, Basmadjian Y, Puime A, Calegari L, Guerrero J, et al. Ecology of *Triatoma rubrovaria* (Hemiptera, Triatominae) in wild and peridomestic environments of Uruguay. *Mem Inst Oswaldo Cruz*. 1995;90:325-8.
 36. Silveira AC, Rezende DF. Epidemiologia e controle da transmissão vetorial da doença de Chagas no Brasil. *Rev Soc Bras Med Trop*. 1994;27 Suppl 3:11-22.