



## Seroprevalence of Chagas disease in Southern Brazilian cardiac patients and their knowledge about the parasitosis and vectors

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### Abstract

Chagas disease (CD) is considered a typical low-income population sickness of the developing countries in Latin America. Given the historical relevance of CD in individuals in southern Rio Grande do Sul (RS) State, Brazil, the aim of this study was to identify the knowledge of the CD and its vectors by cardiac patients, and the prevalence of anti-*T. cruzi* antibodies in these individuals in Pelotas, city located in Rio Grande do Sul (RS) state, Brazil. The subjects with cardiac disease were submitted to a semi-structured questionnaire as well as two serological tests in order to detect anti-*T. cruzi* IgG antibodies. Of the individuals that born in municipalities showing the highest triatomine infestation rates in recent decades, 81.8% were able to recognize the vector insect ( $p = 0.0042$ ; OR = 5.9), and 83.3% reported either themselves or someone in their families to have CD ( $p = 0.043$ , OR = 5.2). Of the 54 patients submitted to serological analysis, only 01 patient (1.9%) was positive for anti-*T. cruzi* antibodies, a 55 year old man from the rural area of Canguçu county. This study provides support for the evaluation to be extended to other cardiology centers, given the importance of Chagas disease in Brazil.

**Keywords:** triatomines, knowledge, cardiopathy.

### Soroprevalência da doença de Chagas em pacientes cardíacos do sul do Brasil e seu conhecimento sobre a parasitose e vetores

#### Resumo

A doença de Chagas (DC) é considerada uma doença típica da população de baixa renda dos países em desenvolvimento da América Latina. Dada a relevância histórica da DC em indivíduos do sul do Estado do Rio Grande do Sul (RS), o objetivo deste estudo foi identificar o conhecimento da doença de Chagas (DC) e seus vetores em pacientes cardíacos, e a prevalência de anticorpos anti-*T. cruzi* nesses indivíduos, em Pelotas, cidade localizada no Rio Grande do Sul (RS), Brasil. Os pacientes cardiopatas foram submetidos a um questionário semiestruturado, e também a dois testes sorológicos para detecção de anticorpos anti-*T. cruzi* IgG. Dos indivíduos que nasceram em municípios com as maiores taxas de infestação por triatomíneos nas últimas décadas, 81,8% foram capazes de reconhecer o inseto vetor ( $p = 0,0042$ ; OR = 5,9), e 83,3% relataram que eles próprios ou alguém em suas famílias tem DC ( $p = 0,043$ , OR = 5,2). Dos 54 pacientes submetidos à análise sorológica, apenas 01 paciente (1,9%) foi positivo para anticorpos anti-*T. cruzi*, um homem de 55 anos da área rural do município de Canguçu. Este estudo fornece subsídios para que a avaliação seja estendida a outros centros de cardiologia, devido à importância da doença de Chagas no Brasil.

**Palavras-chave:** triatomíneos, conhecimento, cardiopatia.

#### 1. Introduction

Chagas disease (CD), also known as American trypanosomiasis, is thought to be typical of low-income populations (Dias, 2000). In Latin America, 5.4 million people are estimated to be *Trypanosoma cruzi* carriers at risk of developing the disease in its chronic cardiac manifestation. In Brazil, there are between 1.5 and 2 million

people infected with the parasite, of whom 20 to 40% will develop the disease in its cardiac form (Nobrega et al., 2014).

However, Martins-Melo et al. (2014), from a systematic review and meta-analysis, evaluated that the prevalence of CD in Brazil, in addition to being increasingly urbanized, can reach 4.6 million people.

There are several ways of disease acquisition, the most important of which is the vector path (Dias, 2000). Although the vector-borne infection has been on the decline on account of control campaigns in Southern Cone countries since the 1990s, this does not allow for non-concern on control measures, inasmuch as other triatomine species can serve as *T. cruzi* transmission vectors (Bacal et al., 2011). In addition, some transmission aspects have changed in the last decades and oral transmission has been pointed out as being the most frequent mode in some areas of Brazil, mainly due to the ingestion of açaí or cane juice contaminated with infectious protozoan forms (Ferreira et al., 2014).

Among Chagas disease (CD) manifestations, Chagas cardiomyopathy is the most important due to its high morbidity and mortality, thus having a relevant medical and social impact, and being referred to as the third cause of pacemaker device implantations and heart transplants (Botoni et al., 2013).

It often affects the working age population, thus inflicting a great socioeconomic burden on the State, once it is a disease that causes physical capacity reduction, leading to early retirements and, consequently, high economic losses (Nunes et al., 2013).

The state of Rio Grande do Sul (RS) has been historically considered to be a CD endemic area (Baruffa and Alcântara Filho, 1985), showing one of the highest *T. cruzi* seroprevalence rates, whose more frequent manifestation is cardiac (Bedin et al., 2009).

The municipality of Pelotas in southern Rio Grande do Sul is one of these areas where CD prevailed in the past, attaining rates of 0.41% to 4% of positive individuals among blood donors (Baruffa and Alcântara Filho, 1985). Furthermore, recent studies on different groups, such as cancer patients (Rosenthal et al., 2016) and individuals coinfecting with HIV (Stauffert et al., 2015), revealed a 5% anti-*T. cruzi* antibody prevalence rate, 3.8 times as high as Ministry of Health estimates (Brasil, 1998).

As a consequence of the severity of the disease in its chronic cardiac phase and the high prevalence estimated for anti-*T. cruzi* antibodies among the southern RS population, this study aimed to identify the knowledge about Chagas disease and its vectors, and to evaluate the anti-*Trypanosoma cruzi* antibody prevalence in patients with a heart disease history in southern Rio Grande do Sul State, Brazil.

## 2. Methodology

A cross-sectional study was carried out with public service users of the Cardiovascular Outpatient Clinic of the Federal University of Pelotas (UFPEL), located in Pelotas, RS, seat (placed on) of the 3rd Regional Health Coordination of the State. The study was performed between November 2016 and April 2017.

Research inclusion criteria were: having a heart condition, being over 18 years old and having agreed to participate in the research by signing an Informed Consent Agreement. A semi-structured questionnaire was used to

determine socioeconomic aspects which included age, color/race, occupation, current activity, family income, schooling and area/municipality of origin, as well as epidemiologic aspects such as awareness of CD and its vectors (including the identification of “kissing bugs” in a showcase), modes of CD transmission, family history and possible risk factors associated with this parasitic disease, as well as clinical aspects: systemic arterial hypertension (SAH) and diabetes mellitus (DM).

Serum from patients was tested for anti-*T. cruzi* antibody detection by means of Chemoluminescent Microparticle Immunoassay (CMIA) (ARCHITECT Chagas®, Abbott) and Indirect Immunofluorescence Reaction (IIFR) (WAMA® Diagnostica), in compliance with the norms established by the Ministry of Health, which advocates the use of two specific techniques for CD diagnosis. The study was approved by the Ethics Committee of the Federal University of Pelotas, under n. 1.555.334.

Data obtained through questionnaires were processed by the Epi-Info software version 6.04 (CDC). The  $\chi^2$  test was performed with a 95% confidence interval to analyze possible epidemiological risk factors, with values of  $p \leq 0.05$  being considered significant; for statistically significant variables, the odds ratio (OR) measure was used.

## 3. Results

As a result, the study population consisted of 75 patients with heart disease who had come to the outpatient clinic for a cardiologist consultation during the analysis period. The subject ages ranged from 34 to 85 years, and the group included 44 men and 31 women. Of the patients interviewed, only two had had serological tests for *T. cruzi* (both negative). Of the total samples collected, 21 hemolyzed due to the use of antiplatelet agents by the patients, resulting in 54 serological findings.

Table 1 shows socioeconomic and awareness aspects of CD and its vectors of the 75 patients who participated in the study. Most patients (64%) were between 60 and 85 years old, with white predominance (80%); 12% were illiterate, 70.7% had a family income of up to one minimum wage and 65.3% lived or had already lived in rural areas.

In this study, 35% (26) patients showed metabolic syndrome, thus having a high risk of cardiovascular complications (13); moreover, 88% (66) of the patients had hypertension and 37.3% (28) had DM.

Taking into account the serological evaluation of 54 individuals, a positive result (1.9%) was obtained for anti-*T. cruzi* IgG antibodies (CMIA reagent and confirmed IIFR). The positive patient, who was not aware of his serological condition for the protozoan, was a hypertensive retired black male who had incomplete elementary schooling and a monthly income of a minimum wage, drank alcoholic drinks seven or more times a week, and who reported having found a kissing bug in his house; he also had a habit of keeping firewood stacks in the house, and had had a chicken coop near the house. It is important to emphasize that the positive patient came from the city of

**Table 1.** Correlation between the socioeconomic and demographic variables of the patients and their capacity to identify the vectors of Chagas disease.

Variables	The patient identify the triatomine/kissing bug?		p (Odds Ratio)
	No	Yes	
<b>Age group (years)</b>			
34 to 59	13(48.1)	14 (51.9)	0.71
60 to 85	21(43.8)	27 (56.3)	
<b>Gender</b>			
Female	16(51.7)	15 (48.4)	0.36
Male	18(40.9)	26 (59.1)	
<b>Literacy</b>			
Illiterate	05(55.6)	04 (44.4)	0.51
Literate	29(43.9)	37 (56.1)	
<b>Family income</b>			
Until 01 minimum wage	23(43.4)	30 (56.6)	0.60
> 01 minimum wage	11(50.0)	11 (50.0)	
<b>Municipalities</b>			
A*	04(18.2)	18 (81.8)	0.0042 (5.9)
B**	30(56.7)	23 (43.4)	
<b>Anyone in your family have/had CD?</b>			
Yes	02(16.7)	10 (83.3)	0.043 (5.2)
No	32(50.8)	31 (49.2)	
<b>Lived in rural areas?</b>			
Yes	21(42.9)	28 (57.1)	0.55
No	13(50.0)	13 (50.0)	

A\* Canguçu, Herval, Pinheiro Machado, Piratini; B\*\* other 15 municipalities in the region.

Canguçu, in addition, considering only the serological tests performed in Canguçu patients (09 cases), the positivity reached 11.1%.

Regarding the knowledge of the patients about the identification of the vectors of the CD, 54.7% of the patients correctly pointed out *Triatoma infestans* and *Triatoma rubrovaria* species. However, if only the interviewed patients from Canguçu, Herval, Pinheiro Machado and Piratini municipalities are considered, the percentage of correct answers rose to 81.8% ( $p = 0.0042$ ; OR = 5.9).

There were several choices for the question “Which are the modes of transmission of CD?” that were read one by one for each interviewee, and the results are shown in Table 2. The oral transmission was significantly more remembered among younger patients (34-59 years) ( $p = 0.05$ , OR = 2.8).

#### 4. Discussion

The patients interviewed presented clinical and sociodemographic similarity to those from other published studies (Sangenis et al., 2017; Oliveira et al., 2006; Brandão et al., 2005). In the research carried out by Sangenis et al. (2017) in a hospital in the “serrana region” of Rio de Janeiro, 209 cardiac patients were analyzed, with the majority of individuals being elderly men, who in the first decades of life lived in houses made of wood. The prevalence of infection by *T. cruzi* was 0.5%, that is,

**Table 2.** Forms of Chagas disease transmission, reported by the population of southern Rio Grande do Sul, Brazil.

Forms of Chagas disease transmission	Affirmative responses from the population	
	Percentage	
Vector/bug	53	70.7%
Oral/contaminated food	45	60.0%
Blood transfusion	40	53.3%
Congenital/mother to fetus	38	50.7%
Mosquito bite	38	50.7
Tick bite	38	50.7

only a positive sample for the parasite, the same amount verified in our research. The common risk factors were the consumption of game meat and the knowledge of the barber, and the main causes of hospitalization were arrhythmias and heart failure.

Regarding the positive patient of the present research, he showed a chagasic patient classic profile (Dias, 2015), that is, had been born in 1961 (55 years) and was a native of the municipality of Canguçu, an endemic triatomine area (Baruffa and Alcântara Filho, 1985); also, the Chagas Disease Control Program was only implemented when he was 14 years of age (1975) (Bedin et al., 2009). Furthermore, the individual was from a rural area and reported having

lived in a stick house until adolescence. The patient was followed up, by his doctor, for possible changes due to CD. The fact of having been born in Canguçu, is in agreement with a study conducted by Rosenthal et al. (2016), since the authors found that patients from this municipality, presented a 6.57 higher chance of having anti-*T. cruzi* antibodies. Regarding aspects attributed to the epidemiology of CD, Gasparim et al. (2018) conducted a study with 270 individuals positives in the state of Paraná, south of the Brazil, and the majority of those infected were over 65 years old, lived in the urban area and were infected in childhood through the CD vectors. Considering the clinical manifestations of the disease, 36% corresponded to the cardiac form, 30% to the indeterminate form, 20% to the cardiodigestive form and 14% to the digestive form.

The fact that patients from the municipalities of Canguçu, Herval, Pinheiro Machado and Piratini, recognize more the vectors of the disease, may be due to that these patients come from areas presenting high triatomine bug infestation in the 1970s and 1980s (Baruffa and Alcântara Filho, 1974, 1985), and although there has been a significant reduction in the number of catches of these vectors in the dwellings of the area, these municipalities are still among the ones that present the highest number of triatomine notification by the local population (Priotto et al., 2014), which may contribute to maintaining greater awareness and participation of individuals in the diagnosis and “kissing bug” reports.

Upon comparing the general 54.7% index of vector identification of the population from southern Rio Grande do Sul with those from a study performed in Bambuí, Minas Gerais State (Villela et al., 2009), the authors of this study are shown to have obtained higher triatomine insect identification indexes by the population (89.1% for adults and 66.7% for school children); in this specific case, it is important to consider the tradition and pioneering actions of this Minas Gerais municipality in the control of vectors and prevention campaigns of CD, which have occurred regularly since 1940 (Dias, 2009). Corroborating with our results, Sousa et al. (2018), making a systematic review of the literature aiming to analyze aspects of the transmission of *T. cruzi*, in relation to the species of triatomines from studies that contemplated the Northeast region of Brazil, also identified lack of information about educational activities, and concludes that education is an important tool to ensure success in programs for prevention and eradication of diseases, once they provides instruments for the maintenance of institutional control policies.

The fact that the highest number of citations for oral transmission occurred among younger patients may be due to a memory of the outbreak acute cases of CD caused by the ingestion of contaminated cane juice in Santa Catarina (a neighboring state of Rio Grande do Sul), which occurred in 2005, and was widely publicized by the media (Andrade et al., 2011). In addition, the coexistence of these patients with insect vectors inside their dwellings was smaller when compared to the older population (Dias, 2000), which may have led to lower awareness, or a lesser appreciation, of the vector path. However, it is

important that the population is aware of the importance of the transmission of CD by its vectors, as the encounter of triatomines can occur, even if occasionally, inside residences present in metropolitan regions (Silva et al., 2020). Additionally, educational interventions may trigger actions that contribute to prevent parasitic diseases such as Chagas disease (Bianchi et al., 2020).

The index of patients serologically positive for *T. cruzi* found in the present study was low when compared to those found by other studies carried out in this region (Rosenthal et al., 2016; Stauffert et al., 2015); nonetheless, it pointed towards a positive reaction in the Cardiology Outpatient Clinic of the Federal University of Pelotas for anti-*T. cruzi* antibodies.

## 5. Conclusion

In conclusion, one patient was positive for anti-*T. cruzi* antibodies, native from the municipality of Canguçu, one of the locations with the highest seropositivity rates in previous studies. Regarding knowledge of the population, it was observed that the patients from the municipalities that presented the highest infestation rates by triatomines in previous decades could identify the insect more easily and reported having had, or having, a family member with CD significantly more often. The oldest interviewees pointed to the vector form as the main mode of transmission of the parasite, while the younger ones pointed to the oral route. Physical improvements in the dwellings, together with vector surveillance and control practices carried out during the last decades, have probably contributed to the low frequency of CD among this population. Furthermore, this study provides support for the evaluation to be extended to other departments of cardiology in this area, given the importance of Chagas disease in Rio Grande do Sul, Brasil.

## References

- ANDRADE, S.G., CAMPOS, R.F., STEINDEL, M., GUERREIRO, M.L., MAGALHÃES, J.B., ALMEIDA, M.C.D., REIS, J.N., SANTOS, V.C., VALADARES, H.M., REIS, M.G. and MACEDO, A.M., 2011. Biological, biochemical and molecular features of *Trypanosoma cruzi* strains isolated from patients infected through oral transmission during a 2005 outbreak in the state of Santa Catarina, Brazil: its correspondence with the new *T. cruzi* Taxonomy Consensus (2009). *Memorias do Instituto Oswaldo Cruz*, vol. 106, no. 8, pp. 948-956. <http://dx.doi.org/10.1590/S0074-02762011000800009>. PMID:22241116.
- BACAL, F., GUIMARÃES, A.C. and NETO, J.M.R., 2011. Epidemiologia e aspectos epidemiológicos no Brasil. I Diretriz Latino-Americana para diagnóstico e tratamento da cardiopatia chagásica. *Arquivos Brasileiros de Cardiologia*, vol. 97, no. 3, pp. 1-48.
- BARUFFA, G. and ALCÂNTARA FILHO, A., 1974. Prevalência sorológica da doença de Chagas em cinco municípios da zona sul do Rio Grande do Sul. *Revista do Instituto Brasileiro de Medicina Tropical*, vol. 16, no. 3, pp. 140-144.
- BARUFFA, G. and ALCÂNTARA FILHO, A., 1985. Inquérito sorológico e entomológico da infecção pelo *T. Cruzi* na Região Sul

- do Rio Grande do Sul. *Annales de la Société Belge de Médecine Tropicale*, vol. 65, no. 1, suppl. 1, pp. 171-179. PMID:3931582.
- BEDIN, C., MELLO, F., WILHELMS, T.S., TORRES, M.A., ESTIMA, C., FERREIRA, C.F. and SEHN, L., 2009. *Vigilância ambiental: doença de Chagas no Rio Grande do Sul*. Porto Alegre: Secretaria da Saúde do Rio Grande do Sul, pp. 1-8. *Boletim Epidemiológico*, vol. 11, no. 3.
- BIANCHI, T.F., JESKE, S., SARTORI, A., GRALA, A.P.P. and VILLELA, M.M., 2020. Validation of a documentary on chagas disease by a population living in an endemic area. *Brazilian Journal of Biology = Revista Brasileira de Biologia*. In press. <http://dx.doi.org/10.1590/1519-6984.228876>.
- BOTONI, F.A., RIBEIRO, A.L.P., MARINHO, C.C., LIMA, M.M.O., NUNES, M.C.P. and ROCHA, M.O.C., 2013. Treatment of Chagas cardiomyopathy. *BioMed Research International*, vol. 2013, pp. 849504. <http://dx.doi.org/10.1155/2013/849504>. PMID:24350293.
- BRANDÃO, A.P., BRANDÃO, A.A., NOGUEIRA, A.R., SUPLYCY, H., GUIMARÃES, J.I. and OLIVEIRA, J.E.P., 2005. I Diretriz Brasileira de Diagnóstico e Tratamento da Síndrome Metabólica. *Arquivos Brasileiros de Cardiologia*, vol. 84, no. 1, pp. 1-28. PMID:15841836.
- BRASIL. Ministério da Saúde, 1998. *Doença de Chagas: triagem e diagnóstico sorológico em unidades hemoterápicas e laboratórios de saúde pública*. Brasília: Coordenação Nacional de Doenças Sexualmente Transmissíveis e Aids, 76. p. Série TELELAB.
- DIAS, J.C.P., 2000. Vigilância epidemiológica em doença de Chagas. *Cadernos de Saúde Pública*, vol. 16, no. 2, suppl. 2, pp. 43-59. <http://dx.doi.org/10.1590/S0102-311X2000000800005>. PMID:11119319.
- DIAS, J.C.P., 2009. Revisão geral e evolução imediata de casos agudos de doença de Chagas estudados no Posto Avançado Emmanuel Dias (Bambu, MG, Brasil) entre 1940 e 1969. *Revista Médica de Minas Gerais*, vol. 19, no. 4, pp. 325-335.
- DIAS, J.C.P., 2015. Chagas disease: still a challenge around the World. *Revista da Sociedade Brasileira de Medicina Tropical*, vol. 48, no. 4, pp. 367-369. <http://dx.doi.org/10.1590/0037-8682-0269-2015>. PMID:26312924.
- FERREIRA, R.T.B., BRANQUINHO, M.R. and LEITE, P.C., 2014. Transmissão oral da doença de Chagas pelo consumo de açaí: um desafio para a vigilância sanitária. *Revista Vigilância Sanitária em Debate*, vol. 2, no. 4, pp. 4-11.
- GASPARIM, A.Z., FONTES, C.E.R., ROSSONI, D.F. and TOLEDO, M.J.D.O., 2018. Epidemiological and clinical profile of patients with Chagas disease in the Central-North area of Paraná, Southern Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, vol. 51, no. 2, pp. 225-230. <http://dx.doi.org/10.1590/0037-8682-0173-2017>. PMID:29768559.
- MARTINS-MELO, F.R., RAMOS JÚNIOR, A.N., ALENCAR, C.H. and HEUKELBACH, J., 2014. Prevalence of Chagas disease in Brazil: a systematic review and meta-analysis. *Acta Tropica*, vol. 130, pp. 167-174. <http://dx.doi.org/10.1016/j.actatropica.2013.10.002>. PMID:24139912.
- NÓBREGA, A.A., ARAÚJO, W.N. and VASCONCELOS, A.M.N., 2014. Mortality due to Chagas Disease in Brazil According to a Specific Cause. *The American Journal of Tropical Medicine and Hygiene*, vol. 91, no. 3, pp. 528-533. <http://dx.doi.org/10.4269/ajtmh.13-0574>. PMID:25002301.
- NUNES, M.C.P., DONES, W., MORILLO, C.A., ENCINA, J.J. and RIBEIRO, A.L., 2013. Chagas disease: an overview of clinical and epidemiological aspects. *Journal of the American College of Cardiology*, vol. 62, no. 9, pp. 767-776. <http://dx.doi.org/10.1016/j.jacc.2013.05.046>. PMID:23770163.
- OLIVEIRA, F.A.S., BICALHO, G.V.C., SOUZA FILHO, L.D., SILVA, M.J. and GOMES FILHO, Z.C., 2006. Características epidemiológicas dos pacientes com Doença de Chagas. *Revista Brasileira de Medicina de Família e Comunidade*, vol. 2, no. 6, pp. 107-113. [http://dx.doi.org/10.5712/rbmf2\(6\)34](http://dx.doi.org/10.5712/rbmf2(6)34).
- PRIOTTO, M.C.M., SANTOS, C.V., MELLO, F., FERRAZ, M.L. and VILLELA, M.M., 2014. Aspectos da vigilância entomológica da doença de Chagas no Sul do Rio Grande do Sul. *Revista de Patologia Tropical*, vol. 43, no. 2, pp. 228-238.
- ROSENTHAL, L.A., PETRARCA, C.R., MESENBURG, M.A. and VILLELA, M.M., 2016. *Trypanosoma cruzi* seroprevalence and associated risk factors in cancer patients from Southern Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, vol. 49, no. 6, pp. 768-771. <http://dx.doi.org/10.1590/0037-8682-0202-2016>. PMID:28001226.
- SANGENIS, L.H.C., NIELEBOCK, M.A.P., REGAZIO, F.M., MENDES, M., ROCHA, R. and FIGUEREDO, L., 2017. Soroprevalência da doença de Chagas em cardiopatas internados em um hospital de referência da região Serrana do estado do Rio de Janeiro, Brasil. *Cadernos Unifoa*, vol. 33, pp. 131-138.
- SILVA, R.A., VIRGÍNIO, F., ESTEVÃO, V.A.O., MARTINS, M.L., DUARTE, A.N., SILVA, G.P., CARVALHO, P.R., SANTOS, A.R., CURADO, I. and SEI, I.A., 2020. First report of colonization by *Panstrongylus megistus* (Burmeister, 1835) (Hemiptera, Reduviidae, Triatominae) in the Metropolitan Region of São Paulo, Brazil. *Brazilian Journal of Biology = Revista Brasileira de Biologia*. <http://dx.doi.org/10.1590/1519-6984.225562>. PMID:32074173.
- SOUSA, D.M., RICARDO-SILVA, A.H., FREITAS, S.P.C., COSTA, F.A.C., KEIKO TOMA, H., CARVALHO, A.A. and SANTOS MALLETT, J.R., 2018. Chagas disease in Northeast of Brazil: findings from a systematic review of literature. *Revista Ciência & Saberes*, vol. 4, pp. 952-964.
- STAUFFERT, D., SILVEIRA, M.F., MESENBURG, M.A., GASPAR, T., MANTA, A.B., BICCA, G.L. and VILLELA, M.M., 2015. Serological diagnosis of Chagas disease in HIV-infected patients. *Revista da Sociedade Brasileira de Medicina Tropical*, vol. 48, no. 3, pp. 331-333. <http://dx.doi.org/10.1590/0037-8682-0268-2014>. PMID:26108013.
- VILLELA, M.M., PIMENTA, D.N., LAMOUNIER, P.A. and DIAS, J.C.P., 2009. 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. *Cadernos de Saúde Pública*, vol. 25, no. 8, pp. 1701-1710. <http://dx.doi.org/10.1590/S0102-311X2009000800006>. PMID:19649411.