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 Cangucu (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 cases, however, other...
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 acac, 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 \textit{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 anthropophilia of each species\(^5\).

Camargo \textit{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 \textit{T. cruzi} (8.8%).

Chagas disease has been considered endemic in Southern RS\(^7\). It is noteworthy that these municipalities, geographically remarkably close, had high rates of home infestation by \textit{T. infestans} in previous decades\(^8\). Therefore, prevalence in patients from this area is still considered one of the highest in the state and sero-reactivity to \textit{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 \textit{T. infestans} has been eliminated in RS State, there are other triatomine species that persist in rural households\(^11\). 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 cases found in Southern RS, shown by previously mentioned evaluations, – the area chosen for this study comprised counties that belong to the 3\(^{rd}\) RHC and the 7\(^{th}\) RHC in RS, Brazil.

The 3\(^{rd}\) RHC, whose headquarters are in Pelotas, RS, comprises 22 counties (Amaral Ferrador, Arroio do Padre, Arroio Grande, Capao do Leao, Cangucu, Cerrito, Chui, Cristal, Herval, Jaguarao, Morro Redondo, Pedras Altas, Pedro Osoiro, Pelotas, Pinheiro Machado, Piratini, Rio Grande, Santa Vitoria do Palmar, Santana da Boa Vista, Sao Jose do Norte, Sao Lourenco do Sul and Turucu), 845,135 inhabitants\(^12\) and has a degree of urbanization of 83.64\(^%\)\(^13\) (Figure 1). The 7\(^{th}\) RHC has its headquarters in Bage, RS, and comprises 6 counties (Aceguia, Bage, Candidota, Dom Pedroito, Hulha Negra and Lavras do Sul), 182,579 inhabitants\(^12\) 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 pastures\(^14\).

**Description of entomological surveillance in the area**

In counties that belong to both, 3\(^{rd}\) and 7\(^{th}\) 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 triatomines\(^15\).

**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 \textit{T. cruzi} infection of triatomines captured per county (number of captured kissing
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 build up. Values were expressed as frequencies (observed value n) and percentages. Variables were statistically compared by the Chi-Square Test ($\chi^2$); 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

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.
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).

Cangucu (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).

**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 Silva\(^{18}\) 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.

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. carcavalloi* was only found in Cangucu (1 capture) (Table 3). In the 7th RHC, *T. rubrovaria* was identified in Bage, Candidota, 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).

### 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 course (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 course (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 (%) |
|----------------|------------------|-------|------|--------------|----------|----------|-------------|
| Cangucu        | P. megistus      | 01    | -    | -            | -        | -        | -           |
|                | T. rubrovaria    | 494   | 12   | 74           | 0        | -        | 528 (37.71%) |
|                | P. tupynambai    | 02    | 01   | 03           | -        | -        | -           |
|                | P. caracalloi    | -     | -    | 04           | -        | -        | -           |
| Cerrito        | T. rubrovaria    | 01    | 03   | 04           | -        | -        | -           |
| Jaguarao       | T. rubrovaria    | 22    | 16   | 15           | -        | -        | 43 (3.07%)   |
| Pedras Altas   | T. rubrovaria    | 36    | 04   | 02           | 0        | -        | 52 (3.71%)   |
|                | P. tupynambai    | -     | -    | 09           | -        | -        | -           |
|                | T. circummaculata| -     | -    | 03           | -        | -        | -           |
| Pinheiro Machado| T. rubrovaria  | 155   | 16   | 43           | 0        | -        | 211 (15.07%) |
|                | P. tupynambai    | 01    | 01   | 02           | 0        | -        | 03 (2.07%)   |
|                | T. circummaculata| -     | -    | 03           | -        | -        | -           |
| Piratini       | T. rubrovaria    | 277   | 10   | 54           | 0        | -        | 314 (22.43%) |
|                | P. tupynambai    | 02    | -    | 23           | -        | -        | -           |
|                | T. circummaculata| -     | -    | 02           | -        | -        | -           |
| Santana da Boa Vista| T. rubrovaria | 85    | 01   | 14           | 0        | -        | 98 (7.00%)   |
|                | P. tupynambai    | -     | -    | 11           | -        | -        | -           |
|                | P. megistus      | 01    | -    | -            | -        | -        | -           |
| Others         | P. megistus      | 06    | -    | -            | -        | -        | -           |
|                | T. rubrovaria    | 85    | 01   | 14           | 0        | -        | 103 (7.36%)  |
|                | P. tupynambai    | -     | -    | 03           | -        | -        | -           |
|                | T. circummaculata| -     | -    | 01           | -        | -        | -           |
| Total          | 1219 | 67   | 114  | 204          | 0        | 1400 (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         | P. megistus      | 01    | -    | 01           | 0        | -        | 01 (0.63%)   |
| Bage           | T. rubrovaria    | 14    | 04   | 03           | 0        | -        | 23 (14.46%)  |
|                | P. tupynambai    | 02    | -    | 03           | 0        | -        | -           |
| Candido        | T. rubrovaria    | 06    | -    | -            | 0        | -        | 06 (3.78%)   |
| D. Pedro       | T. rubrovaria    | 01    | -    | -            | 0        | -        | 01 (0.63%)   |
|                | T. circummaculata| 01    | -    | 02           | 0        | -        | -           |
|                | P. tupynambai    | 01    | -    | 02           | 0        | -        | -           |
| Hulha Negra    | T. rubrovaria    | 01    | -    | -            | 0        | -        | 01 (0.63%)   |
| Lavras do Sul  | T. rubrovaria    | 75    | 03   | 06           | 0        | -        | 102 (64.15%) |
|                | P. tupynambai    | 03    | 15   | 02           | 0        | -        | -           |
|                | T. circummaculata| 01    | -    | 01           | 0        | -        | -           |
| Total          | 122  | 22   | 15   | 07           | 0        | 159 (100%) |

which was eliminated in the State by chemical control\textsuperscript{11}, corroborating the findings of this study.

Triatomine invasion has decreased lately. In both, 3\textsuperscript{rd} RHC and 7\textsuperscript{th} 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. According to Priotto et al., 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.

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., who observed that older adults are more aware of the topic than youngsters.

Dutra et al. 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 Cangucu, 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 Cangucu 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. in patients from Cangucu, the county that has the highest populational prevalence of antibodies anti-T. cruzi.

Therefore, Cangucu, Piratini and Pinheiro Machado exhibited the largest number of captured triatomines, a fact that agrees with findings published by Priotto et al., who showed that Cangucu, Piratini, Santana da Boa Vista and Pinheiro Machado had the highest indexes of infection 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. Besides, Cangucu 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 detections.

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. in the same and nearby region. Ribeiro et al. 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. in six areas in Quarai, RS State, in a study carried out in the municipality of Quarai, showing that 4.2% of T. rubrovaria triatomines were infected. It should be mentioned that, in Quarai, 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. 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.

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 rupetrian walls, which are typical in RS State. 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, whose food sources are several invertebrate and vertebrate hosts, such as humans. These vectors are mainly found amid rocks and stones. Silveira and Rezende 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 years, 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.

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