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Original Article

Risk of *Trypanosoma cruzi* transmission in southern Minas Gerais, Brazil – Data from 2014 to 2020

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ABSTRACT

Trypanosoma cruzi, the etiologic agent of Chagas disease, is widely distributed in the Americas and is transmitted through vectorial, transfusional, and oral routes. This study aimed to evaluate the risk of vectorial transmission of Chagas disease in municipalities located in southern Minas Gerais, Brazil, by analyzing triatomine specimens collected from 2014 to 2020. All 1522 hematophagous triatomines were identified as *Panstrongylus megistus*, and were subjected to parasitological and molecular examinations. From 2014 to 2016, approximately 10% of insects were positive in the microscopic analysis of intestinal content, and 27% were positive as detected by the quantitative polymerase chain reaction (qPCR) of the same sampling. However, in the last investigated years, an increase in infected triatomines was observed in microscopic analysis (22%) and qPCR methods (41%). This corroborates the findings of acute human Chagas disease cases, which have increased in the study area from a maximum of 2 cases in previous years to 20 cases in 2019, and 17 cases in 2020 through June. Additionally, bloodmeal sources of infected triatomines were investigated; human blood was detected in up to 85.7% of the samples. Moreover, canine blood was also detected in triatomine intestinal content in recent years, reaching 91% of analyzed insects in 2018. Data on bloodmeal sources have demonstrated human-vector contact and have suggested the participation of dogs in the parasite transmission cycle. These results indicate the risk of *T. cruzi* vectorial transmission in Southern Minas Gerais and São Paulo owing to the boundary between these states. Thus, enhanced surveillance and vector control of Chagas disease are highly recommended in these areas.

1. Introduction

Chagas disease is caused by the protozoan *Trypanosoma cruzi*, which can be transmitted to humans by insects of the subfamily Triatominae (Hemiptera: Reduviidae) and also by transfusional and organ transplantation routes (Chagas, 1909). Oral transmission due to ingestion of contaminated food has also been responsible for outbreaks of acute Chagas disease (Benchimol-Barbosa, 2010).

Human infection is still endemic throughout Latin America, and 8 million people worldwide are estimated to be infected (Coura, 2013). After acute phase, the immunological events influence disease outcome, helping determine whether the patient will remain asymptomatic, or progress to cardiac or digestive clinical forms (Andrade et al., 2014). The disease is related to losses of approximately 752,000 working days due to premature deaths, and the estimated annual global burden of disease is approximately US $627 million in healthcare costs (Pérez-Molina and Molina, 2018). The highest lifetime economic losses in Latin America occur in Brazil, being related to lost productivity from cardiovascular disease-induced early mortality (Lee et al., 2013).

Primarily an ancient enzootic infection, trypanosomiasis has been

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detected in several mammalian orders (Jansen et al., 2018). An increase in the prevalence of human Chagas disease can occur because of deforestation and other anthropogenic environmental changes (Walsh et al., 1993). In Brazil, especially in the Southern Minas Gerais state, some relevant environmental impacts have occurred owing to the implementation of the Furnas Hydroelectric Plant in the 1960s, which has been associated with an increase in infectious diseases (Silveira and Araújo Neto, 2014; Camasmie Abe and Miraglia, 2018). Agriculture (coffee and citric fruits) plays an important role in the economic activity of this region, which also implies deforestation and migratory events. Additionally, some important university centers are located in Southern Minas Gerais, which attract people from different geographic regions. It means that people harboring different parasites can migrate to these cities, resulting in parasite introduction related to migrations (Gonçalves et al., 2003; Ezenwa, 2004).

Considering the municipalities belonging to the Regional Superintendence of Health of Alfenas, Southern Minas Gerais, Brazil, there have been no reported acute cases of human Chagas disease since 2006 (data from Brazilian Health Ministry, available at www.tabnet.saude.mh.gov.br). However, two cases were confirmed in 2015, and one in 2016. After a year without any cases, one case was confirmed in 2018. However, this number increased significantly in 2019, with 20 confirmed cases. Until June 2020, 17 cases were already confirmed in this Regional Superintendence. This boost is an epidemiological alert to the region, which also includes the São Paulo state because of the boundary between one municipality (Arceburgo) and this state.

Furthermore, the common presence of *Panstrongylus megistus* in the region, an anthropophilic and effective vector, is another critical issue (Vinhaes and Dias, 2000). This hematophagous insect can obtain bloodmeals from a wide range of animals, and analyzing these blood sources can help understand the transmission cycle. In this study, we aimed to evaluate the risk of vectorial transmission of *Trypanosoma cruzi*, investigating the infection rates and feeding sources of triatomines captured between 2014 and June 2020 in Southern Minas Gerais.

2. Materials and methods

2.1. Study area

The study was conducted in the municipalities belonging to the Regional Superintendence of Health of Alfenas, Southern Minas Gerais, Brazil (Fig. 1). This superintendence is linked to the state autarchy, which supervises health issues in all 26 municipalities that compose this regional district. The municipalities are Alfenas, Alterosa, Arceburgo, Areado, Bandeira do Sul, Botelhos, Campos Gerais, Campo do Meio, Cabo Verde, Carvalhópolis, Conceição da Aparecida, Carmo do Rio Claro, Campestre, Divisa Nova, Fama, Guaranésia, Guaxupé, Juruaia, Machado, Monte Belo, Muzambinho, Nova Resende, Paraguaçu, Poço Fundo, São Pedro da União and Serrania. This area is a transition between the Cerrado biome and the Atlantic forest, with a rainy temperate climate and well-defined seasons (Pereira et al., 2002).

2.2. Sampling

Agents from the Minas Gerais state Epidemiologic Surveillance Team conducted active searches and collected insects from January 2014 to June 2020 in both peridomestic and domestic environments. These municipal agents are part of the Brazilian National Chagas Disease Control Program (PCDCh), implemented in 1950 with the main purpose of controlling vectorial transmission (Vinhaes and Dias, 2000). With the decentralization of health actions (Brasil. Portaria n°. 1.399, 1999), shifting responsibilities in endemic diseases control to Municipal governments, collecting methodology can vary between different locations. Even so, in all municipalities triatomine investigations were performed primarily in rural areas, but also in urban ones. Active search comprised the main habitations and also surrounding elements (construction materials, fences, animal shelters, etc). Furthermore, according to PCDCh, the recommended action in houses where infected bugs were found are residual insecticide spraying and a subsequent new active search.

Samples collected in all the investigated houses from the 26 municipalities were included in this study. They were sent to the Laboratory of Clinical Parasitology at Universidade Federal de Alfenas, Minas Gerais, where the samples were classified according to feeding habit, and analyzed for *T. cruzi* infection. Both live and dead specimens were analyzed, as well as engorged and unfed ones. Other insects found not to be hematophagous triatomines, including phytophagous and predatory insects, were excluded from the study; hematophagous triatomines were identified as previously described (Carcavallo et al., 1998).

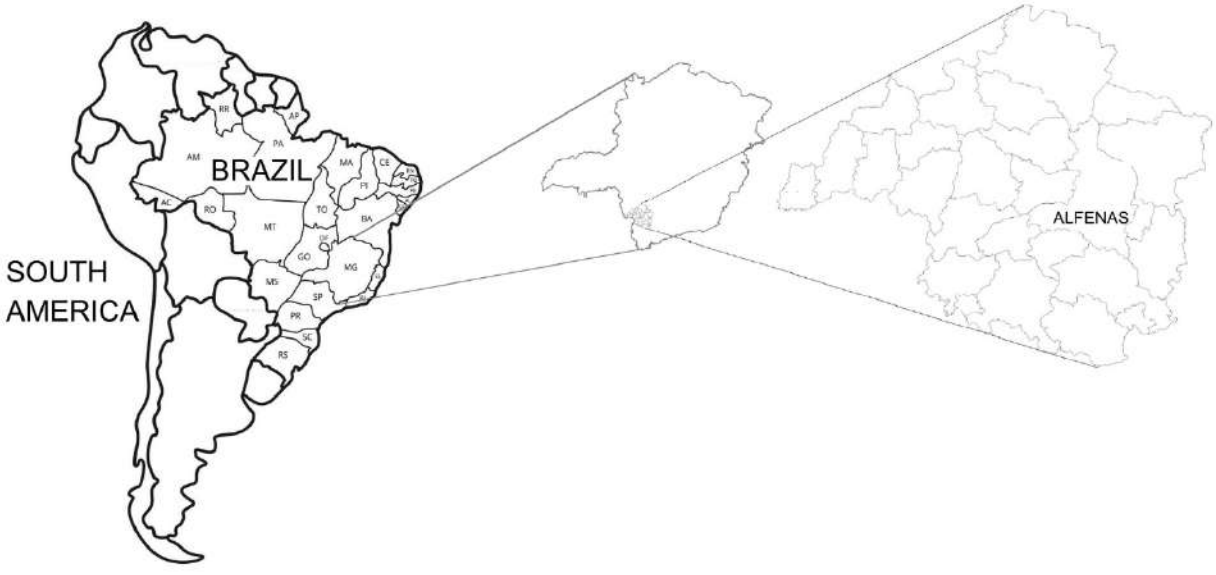


Fig. 1. Study area. Geolocation of Minas Gerais state in Brazil, showing the 26 municipalities of the Regional Superintendence of Health of Alfenas, where triatomines were collected from 2014 to 2020.

2.3. Natural infection of triatomine bugs

2.3.1. Direct microscopic examination

Triatomines were examined individually; material obtained from abdominal compression was placed on a microscope slide with a drop of 0.9% saline, covered with a coverslip, and analyzed under an optical microscope at 400× magnification to identify flagellate forms.

2.3.2. Quantitative polymerase chain reaction (qPCR) with T. cruzi primers

DNA was extracted using the phenol-chloroform protocol, as described elsewhere (Gomes et al., 1998). *T. cruzi* infection status was determined by amplification of a 166 bp region of repetitive nuclear satellite DNA using a TaqMan qPCR reaction with Cruzi 1/Cruzi 2 primers and Cruzi 3 probe, labeled with 5′FAM (6-carboxyfluorescein) and 3′MGB (minor groove binder) (Piron et al., 2007). Reactions were performed using a StepOne Real-Time PCR System (Applied Biosystems, CA, USA), and the reaction mixtures contained 1 μL of DNA, 5 μL of 2× TaqMan Universal PCR Master Mix (Promega, Madison, WI, USA), 1 μL of a mixture of primers and probe, and DNase/RNase-free water to a final volume of 10 μL. Negative and positive controls were used in each step to monitor the reactions. The cycling conditions were as follows: the first step at 50 °C for 2 min, the second step of 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min.

2.3.3. qPCR to detect canine and human bloodmeal

In order to verify whether dogs and/or humans served as a source of bloodmeal for the triatomines, specific oligonucleotides were used. The primer sequences were β1 (5′-ACC ACC AAC TTC ATC CAC GTT CAC C-3′) and β2 (5′-CTT CTG ACA CAA CTG TGT TCA CTA GC-3′) to amplify a 140 bp human β-globulin gene (Lee et al., 2001) and 5′-AGG CTG AGA ACG GGA AAC TT-3′ and 5′-ATT AAG TTG GGG CAG GGA CT-3′ to amplify a 911 bp fragment from canine glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (Kullberg et al., 2006). Reactions were carried out in a final volume of 10 μL containing 0.5 μL of each primer, 1 × SYBR GREEN reaction master mix 1 (Applied Biosystems, CA, USA), 1 μL of DNA and DNase/RNase-free water to the final volume. PCR conditions included denaturation at 95 °C for 5 min, followed by 35 cycles of a 95 °C for 1 min, annealing 62 °C for 1 min, extension at 72 °C for 1 min, and a final extension for 10 min. In each reaction, positive DNA control extracted from human and canine blood was added as well as a negative control (water).

3. Results

During the study period (January 2014 to June 2020), a total of 1522 specimens of triatomines were collected and investigated. The number of collected insects varied yearly, with 422 in 2014, similar amounts in 2015 and 2016 (115 and 114, respectively), 295 in 2017, 257 in 2018, and 268 in 2019. From the beginning of 2020 until June, 51 insects were collected.

After external morphological identification, all specimens were classified as *P. megistus*. However, 93.2% of them could be submitted for parasitological and molecular analysis because some specimens were desiccated or did not present intestinal content, which hindered these analyses.

After intestinal content analysis, some triatomines tested positive on microscopic examination for flagellated parasites. However, as expected, more specimens tested positive when DNA of this intestinal content was extracted to proceed to *T. cruzi* qPCR. In an attempt to perform a chronological analysis of these detections, Fig. 2 shows the positivity rates of triatomine intestinal content in both methods separately, according to the investigated period. From 2018 onwards, an elevation in the percentage of infected triatomines was clearly observed.

The number of confirmed human Chagas disease cases (data from Brazilian Health Ministry, available at www.tabnet.saude.mh.gov.br) has greatly increased in the study region from 2019 onward, as shown in

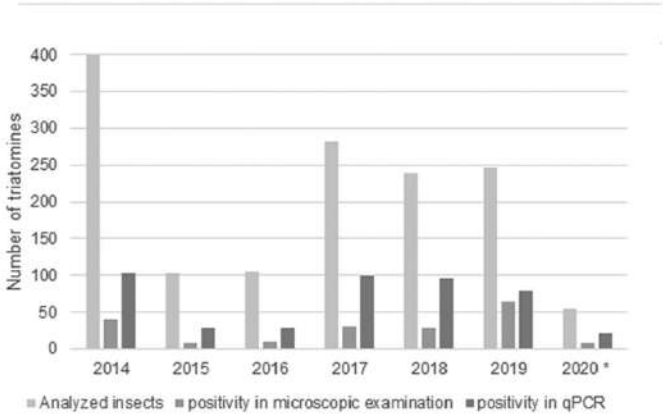


Fig. 2. Triatomine analysis from 2014 to 2020. Number of analyzed triatomines and number of positive specimens in the microscopic examination and quantitative polymerase chain reaction (qPCR) from 2014 to 2020* in Regional Superintendence of Health of Alfenas. *: data until June.

Fig. 3. Moreover, the percentage of infected triatomines has also showed an upward trend since the beginning of these analyses (2014). The data referring to 2020 include only statistics up to June. Nonetheless, the number of human cases in only a portion of that year nearly reached the total observed in the entire previous year (2019), indicating that Chagas disease cases are increasing at a higher rate in 2020 when compared to another period.

Our data showed that canine bloodmeal was frequently observed in collected triatomines. In the first year, the percentage of this source of feeding varied around 8% in the collected insects, according to qPCR canine *GAPDH* detection. In recent years, it has greatly increased, reaching 91% in 2018 and remaining elevated in the following years (Fig. 4). However, when analyzing human bloodmeal, the results were remarkable. According to the analyzed year, we observed that at least 11.9% of the insects fed on humans, as shown by human β-globulin detection by qPCR. An increase in this kind of bloodmeal has been observed since 2017; the percentage varied over the investigated years, reaching the highest value (85.7%) in 2020. However, in every investigated period, human blood was detected in intestinal content of *T. cruzi*-infected triatomines (from 11.90% to 85.7%), evidencing the risk of vectorial Chagas disease transmission.

From the 26 municipalities included in the Regional Superintendence of Health of Alfenas, 25 presented *T. cruzi*-infected insects. In 17 of them, triatomines that had ingested canine blood were captured. Moreover, human bloodmeal was found in insects from 13 municipalities. Fig. 5 shows these municipalities' identity and also the frequency that *T. cruzi*-infected insects that fed on human blood were found in all the study period. Frequency varied between 4.5% in Cabo Verde and 53.3% in Carmo do Rio Claro. These data show a high dispersion of the potential risk of vectorial Chagas disease transmission in Southern Minas Gerais.

4. Discussion

The risk of vectorial transmission of Chagas disease is maintained in the Minas Gerais state until the current year. It was pointed out by these paper's results, about the findings of *T. cruzi*-infected triatomines that fed on human blood.

The number of captured insects greatly varied each year of the investigation period (2014–2020). In developing countries, some infectious diseases receive more financial investments in some occasions, while others such as Chagas disease, can be neglected for a longer time. Variation in captured bugs throughout the years could be attributed to political-administrative responses to the emergence of other pathogens during this period, such as the dengue fever virus.

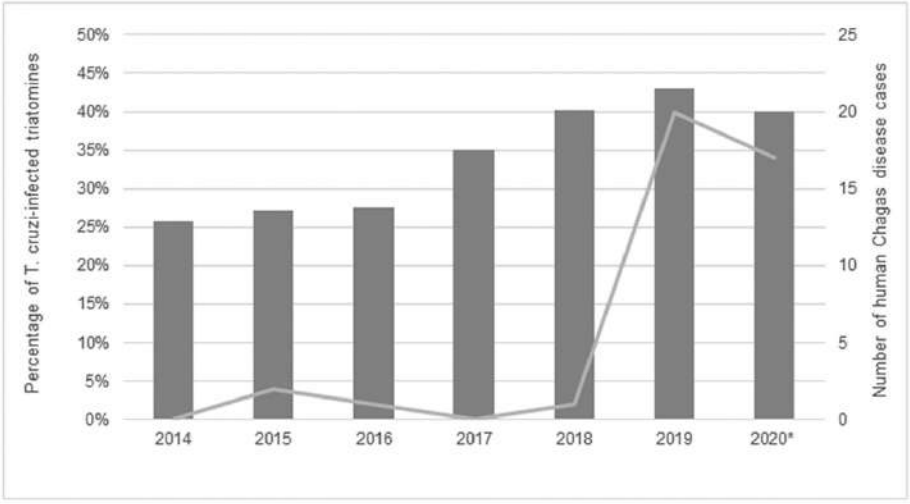


Fig. 3. *T. cruzi*-infected triatomines and human Chagas disease. Comparison between qPCR positivity rates of *T. cruzi*-infected triatomines and the number of confirmed human Chagas disease cases from 2014 to 2020* in the studied region, available in www.tabnet.saude.mh.gov.br. *: data until June.

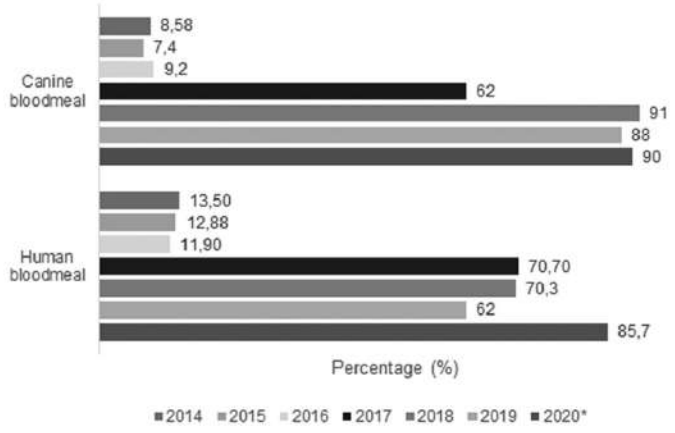


Fig. 4. Triatomine feeding habits. Percentage of *T. cruzi*-infected triatomines that fed on canine and human blood, in the studied region, from 2014 to 2020* in each analyzed year. Some insects were positive for both bloodmeal sources. *: data until June.

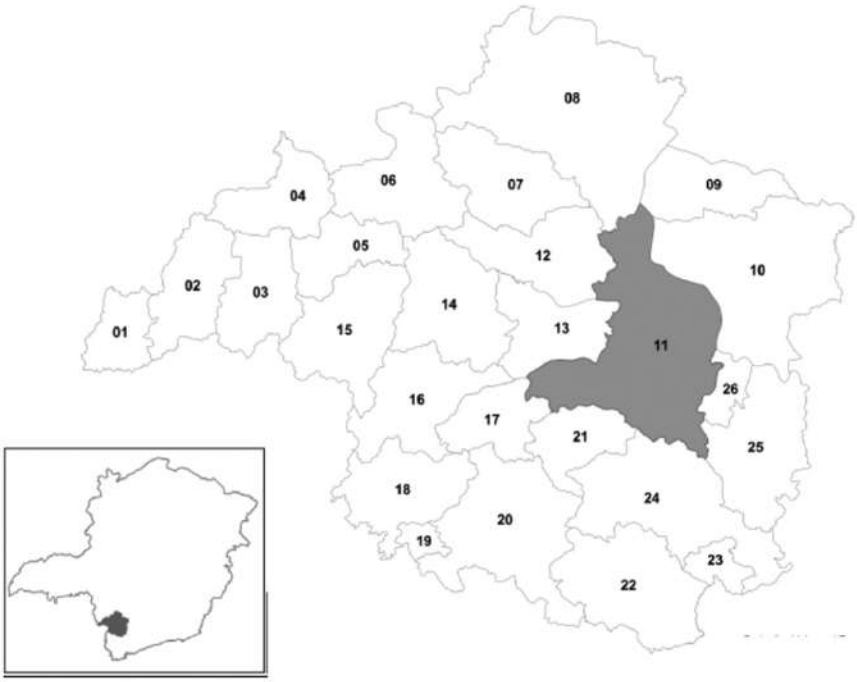
All the investigated triatomines were identified as *P. megistus*, evidencing this species predominance in the study region. Similar results were also found by other authors investigating the Minas Gerais and São Paulo states (Barbosa et al., 2012; Fernandes and Costa, 2012). This specie has great epidemiological importance owing to the high rates of natural infection and the capacity of home colonization when compared to others (Diotaiuti, 2000). Moreover, evidence that triatomines can be found in densely populated urban environments has already been published elsewhere (Dye-Braumuller et al., 2019), which was consistent with our findings because insects were not only collected in rural environments but also in urban areas.

Southern Minas Gerais is a well-developed region, as shown by the Municipal Human Development Indexes (MHDI). This statistical tool is used to assess the degree of well-being and quality of life of a municipality as well as countries' indexes on a scale from 0 to 1 (total human development) (Messias, 2003; PNUD, 2020). The 26 municipalities that compose the Regional Superintendence of Health of Alfenas exhibit MHDI ranging from 0.66 to 0.76, which classifies them as medium to high development (IBGE, 2020). This evidence indicates that the risk of vectorial Chagas disease transmission can occur in developed regions as well as in low-income ones.

Some differences were observed when microscopic and qPCR analyses were carried out on triatomine intestinal content. It was expected, because microscopic analysis sensitivities are usually lower than those of molecular methods in *T. cruzi* detection, as previously stated by other authors (Dorn et al., 2001; Pizarro et al., 2007). Furthermore, infection rates observed at the beginning of our study (±10% in microscopic analysis) were similar to those obtained in other investigations carried out in Bahia (Mendonça et al., 2015), Mato Grosso do Sul (Cominetti et al., 2014), and Pernambuco (Silva et al., 2012). However, the rates observed in the latter investigated years (around 40% in qPCR analysis) appear to be higher than those observed in the literature, evidencing the elevated risk of vectorial transmission in this region.

P. megistus can feed on a great variety of sources, such as humans, birds, cats, marsupials, pigs, and dogs (Carcavallo et al., 1998). Indeed, the results of this work suggest that dogs take part in the biological cycle of triatomines in the study region, frequently acting as a blood source. These animals seem to be an important link in the domiciliation process of triatomines, as they are the feeding sources in peridomestic and domestic habitats as pets in many households. Moreover, as previously stated by Ribeiro Jr et al. (2019), dogs usually sleep in places that are more accessible to triatomines, increasing the probability of infecting an initially uninfected insect. Other authors have already found canine blood in triatomine intestinal content in Brazil and other countries (Orantes et al., 2018; Ribeiro Jr et al., 2019), evidencing the role of this animal in the Chagas disease transmission cycle. Indeed, a limitation of this study is that other animal sources of bloodmeals were not tested; in other investigations, some authors have found that birds are a frequent source of blood to triatomines in Brazil (Ribeiro Jr. et al., 2015; Ribeiro Jr et al., 2019).

In this study, an increasing percentage (from 13.5% in 2014 to 85.7% in 2020) of *T. cruzi*-infected insects fed on humans, according to human β-globulin detection. Furthermore, analyzing each municipality individually, some of them have presented an elevated percentage (up to 53.3%) of these infected insects which were also positive to human blood. These findings highlight the considerable risk of Chagas disease vectorial transmission and show the need of better interventions in the study region. Between 2016 and 2020, an increase in confirmed acute cases of Chagas disease was observed among the investigated municipalities, along with an increase in the number of *T. cruzi*-infected insects. Therefore, the parasite cycle has been maintained in this region, and the risk of parasite transmission still exists, as our results provide further evidence in support of this idea.



Identity	Municipality	Frequency of infected bugs that fed on HB
02	Guaranésia	38.0%
04	São Pedro da União	11.1%
05	Juruaia	19.1%
06	Nova Resende	38.6%
07	Conceição da Aparecida	46.6%
08	Carmo do Rio Claro	53.3%
11	Alfenas	33.3%
12	Alterosa	6.4%
14	Monte Belo	13.8%
16	Cabo Verde	4.5%
17	Divisa Nova	25.0%
18	Botelhos	40.0%
22	Poço Fundo	18.7%

Fig. 5. Distribution of infected triatomines. Municipalities belonging to the Regional Superintendence of Health of Alfenas which presented triatomines with *T. cruzi*-infection and also human blood DNA presence, from 2014 to 2020*. Percentage shows the frequency with which these insects were found. HB: human blood. *: data until June.

5. Conclusion

In summary, the present findings show that the risk of Chagas disease transmission in Southern Minas Gerais exists, as *T. cruzi*-infected triatomine may often feed on humans. The trend of increasing Chagas disease in humans of this area could be attributed to vectorial transmission. Moreover, our results revealed that dogs could be an important link in the transmission cycle. These findings support the suggestion that Chagas disease vectorial transmission is still a hazard in Southern Minas Gerais. Further interventions in this area concerning to triatomine control are needed.

Ethical statement

No animal or human studies were carried out by the authors.

Declaration of Competing Interest

None.

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