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Major Article

Evaluation of natural foci of *Panstrongylus megistus* in a forest fragment in Porto Alegre, State of Rio Grande do Sul, Brazil

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ABSTRACT

Introduction: *Panstrongylus megistus* is commonly found in wild environments of the State of Rio Grande do Sul, Brazil. The aim of this study was to characterize the network of refuges used by triatomine in a forest fragment of Porto Alegre and to identify *Trypanosoma cruzi* infection, associated hosts and the epidemiological importance of both hosts and triatomines. **Methods:** Techniques including the spool-and-line method and active searching (transects) were used to identify natural foci. **Results:** The food source for each triatomine was determined using the precipitin test, and the infection of marsupials was determined by xenodiagnosis. A total of 33 adults (domestic environment) and 27 nymphs (wild environment) of *P. megistus* were found in addition to 43 *Didelphis albiventris* specimens. The infection rates of triatomine adults, triatomine nymphs and opossums with *T. cruzi* I were 64%, 73% and 69%, respectively. Birds, rodents and opossums were the main resources used by triatomine. **Conclusions:** This work presents the first characterization of a natural focus of *P. megistus* in Rio Grande do Sul. The natural characteristics of this focus and its implication in the transmission of *T. cruzi* are discussed.

Keywords: *Panstrongylus megistus*. *Didelphis albiventris*. *Trypanosoma cruzi*. Sylvatic foci. Rio Grande do Sul.

INTRODUCTION

Chagas disease was discovered in 1909 and remains one of the main neglected diseases in South America. In Brazil, it is estimated that approximately 1.9 million individuals are infected with Chagas disease, and an additional 23 million are at risk of acquiring it[1]. Due to the success of the Chagas Disease Control Program in 2006, Brazil was certified as free of disease transmissions by *Triatoma infestans* (Klug, 1834) and blood transfusion[2]. Among the main species of autochthonous triatomines of Brazil, *Panstrongylus megistus* (Burmeister, 1835) (Hemiptera, Reduviidae, Triatominae) is singularly known for its high capacity to adapt to domestic environments and wide distribution throughout the country, being present in 20 Federal Units[3]. Based on genetic and paleobiogeographic characteristics, several authors have demonstrated that the States of Bahia (BA), Minas Gerais (MG), Pernambuco (PE), Rio de Janeiro (RJ) and São Paulo (SP) are probable areas of species endemism[4-6]. Currently, some of these states, particularly BA, MG, PE, SP and Alagoas, are areas of greatest epidemiological importance for *P. megistus*[7].

The presence of this triatomine in natural environments represents possible sources of infestation and re-infestation of residences and also contributes to the maintenance of the sylvatic cycle of *Trypanosoma cruzi*[3]. Multiple authors have observed cohabitation of *P. megistus* and small mammals in other natural shelters since the beginning of the last century.

The first occurrence of *P. megistus* in natural environments was reported by Gomes[8] in State of São Paulo (SP), citing the capture of a female trying to feed from her captor in the forest. In 1920, Oliveira[9] presented evidence on possible relationships among the triatomine and *T. cruzi* natural reservoirs in Rio Grande do Sul (RS). However, he did not describe sylvatic foci. The first significant sylvatic focus was reported by Leal et al.[10], who found numerous colonies in nests of opossums, rodents and cuícas in bromeliad clumps as well as holes in trees on an island in State of Santa Catarina (SC). In the same decade, Barretto et al.[11] found a large number of *P. megistus* nymphs and adults in hollow trees, anfractuous agave clumps and roots of the fig tree *Ficus euomphala* in SP, recording a higher rate of infection in

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triatomines associated with opossums (39%) than rodents (17%). Work performed in the 1970s by Forattini et al.[6,12,13] showed *P. megistus* foci in hollow trees inhabited by bats, rodents, birds and, mainly, opossums as well as in epiphytic bromeliads, pine *Cryptomeria* sp. and palm *Attalea* sp. inhabited by opossums and rodents. In a study at Horto Forest (Rio de Janeiro-RJ) in 1982, Miles et al.[14] found 12 nymphs infected by the parasite in a hollow tree inhabited by *Didelphis marsupialis*, and in 1985, Schlemper-Jr et al.[15] detected an 84.5% rate of infection with *T. cruzi* in *P. megistus* associated with opossums and rodents in hollow trees and bromeliad clumps in SC. Grisard et al.[16] found nymphs in hollow trees in the same state. The most recent work, performed by Santos-Jr et al.[17] in the Bambuí, MG, municipality, reported the first finding of a *P. megistus* sylvatic colony in 69 years of research. This colony was discovered in the hollow of a large tree (*Dimorphandra mollis* Benth, popularly known as faveira).

Unlike the Northeast and Southeast Brazilian states, in RS, *P. megistus* is predominantly sylvatic and rarely colonizes the domestic environment. However, adult insect visits are common in dwellings in close proximity with sylvatic areas during the warmer months[18]. The first reports of Chagas disease vectors in RS were associated with the discovery of *Triatoma rubrovaria* (Blanchard, 1843) in 1911 and *T. infestans* in 1913 in the Pelotas municipality and surrounding areas[19,20]. Although vectors were detected during the early 20th century**,** the first acute case was only described in 1939 at the boundary with Uruguay[21]. RS was one of the last states to be certified as free of Chagas disease transmission (2005) in Brazil[22]. Recently 11 species of triatomines have been recognized in the state; markedly, *P. megistus*, *T. infestans* (currently in residual foci) and *T. rubrovaria* have notable *T. cruzi* infection rates of 15%, 4% and 0.9%, respectively[22-27]. In Porto Alegre, four triatomines species, *T. circummaculata, T. oliverai* (both rarely), *T. infestans* (last observed in 1950) and *P. megistus* (observed since 1931), have been reported and are found mainly in the southern municipality region[28-30].

In addition to elucidating the trophic relationships between species, the identification of the various natural habitats shared by triatomines and their hosts allows for the characterization of potential risk areas related to *T. cruzi* transmission, leading to better Chagas disease control planning[31]. Various techniques have been used to identify these habitats, including light trapping, live-baited adhesive capture (Noireau trap)[32], spool-and-line methods[33] and precipitin tests[34].

This study aimed to characterize the network of refuges used by *P. megistus* in an ombrophilous dense forest fragment of Porto Alegre with an emphasis on *T. cruzi* infection, associated hosts and the epidemiological importance of both hosts and triatomines for the municipality.

METHODS

The municipality of Porto Alegre, the largest City of Rio Grande do Sul, Brazil, is situated at 100km from the Atlantic Ocean and has a humid subtropical climate. Samples were collected in an ombrophilous dense forest fragment (30^{o}12'05''S, 51^{o}12'43''W, 50m) of approximately 14ha in the Ponta Grossa neighborhood, located in the Guaíba lake basin **(Figure 1A, 1B, 1C** and **1D)**. This region is formed by three forest areas: Alto Uruguay Subtropical Forest, Pines Forest and Atlantic Slope Rain Forest[35]. The inclusion or proximity of forests in urban areas make this region vulnerable to environmental changes.

Specimens were collected between October 2005 and September 2006. Four residences near the 10m from the forest were investigated monthly for the presence of triatomines. Searches using flashlights and tweezers were performed in each domiciliary environment, and a light trap was installed (mercury lamp focused on a white wall) at a frequency of three nights per month. In the peridomiciliary, two doghouses, one pigsty, one hennery, firewood, tiles and two barns were searched. In the intradomiciliary, all rooms were investigated. In the sylvatic environment, marsupial refuges were investigated using transect and spool-and-line methods. Transects were traversed from north to south in the studied area, inspecting shelters up to 5m away from the stipulated line. For the spool-and-line search, 20 wood traps (25 x 25 x 40cm) were installed for capturing opossums **(Figure 1E)**. The traps were baited with banana for two consecutive nights/month. At each capturing point, a mixture of codfish liver oil (Scott Emulsion™), papaya, banana and water was spread to attract marsupials. Captured opossums were subjected to xenodiagnosis (7 nymphs of *T. infestans*/20min), marked and equipped with spool-and-line (IBAMA: 02023.002615/05-25). The design of these spool devices was adapted from that of Miles[33]; each device consisted of a cylindrical plastic pot with a screw cap (4 x 7cm) and a central axis (total weight: 54g). A yellow or orange colored 914m polypropylene line was used (Kooban™). When refuges were found, they were manually examined, and the internal materials were collected (e.g., leaves and twigs) for screening in the laboratory. Noireau traps were installed in inaccessible locations[32]. The distance traversed by the opossums was verified by the difference in the spool-in-line weight after a night route. Refuges with nymphs, eggs and/or exuviae were considered *P. megistus* foci. Refuges with triatomines and/or opossums were characterized.

Fresh feces collected from triatomines for xenodiagnosis were submitted to parasitological examination (diluting in saline solution) to verify trypanosome infection under an optical microscope (400x). Xenodiagnosis was assessed at 15, 30 and 45 days after exposure. Triatomine feces were stored on sterile filter paper (air dried and stored at -20°C) for the molecular characterization of *T. cruzi* (positive samples) and food source analyses (all samples). *T. cruzi* samples were identified by the presence of a nontranscribed spacer of the mini-exon gene. For deoxyribonucleic acid (DNA) extraction, round pieces of filter paper (6mm) containing feces were boiled with 50µL of ultra-pure distilled water (Gibco™) for 10min[36]. The polymerase chain reaction (PCR) amplification and reaction conditions were performed according to the protocol of Souto et al.[37], which used the following pooled primers: TC (5' - CCC CCC TCC CAG GCC ACA CTG), TCI (5' - GTG TCC GCC ACC TCC TTC GGG CC) and TCII (5' - CCT GCA GGC ACA CGT GTG TGT G). Amplification products were subjected to polyacrylamide gel (8%) electrophoresis and observed by silver staining. Parasites were classified according to the procedures of Anonymous[38] and Zingales et al.[39].

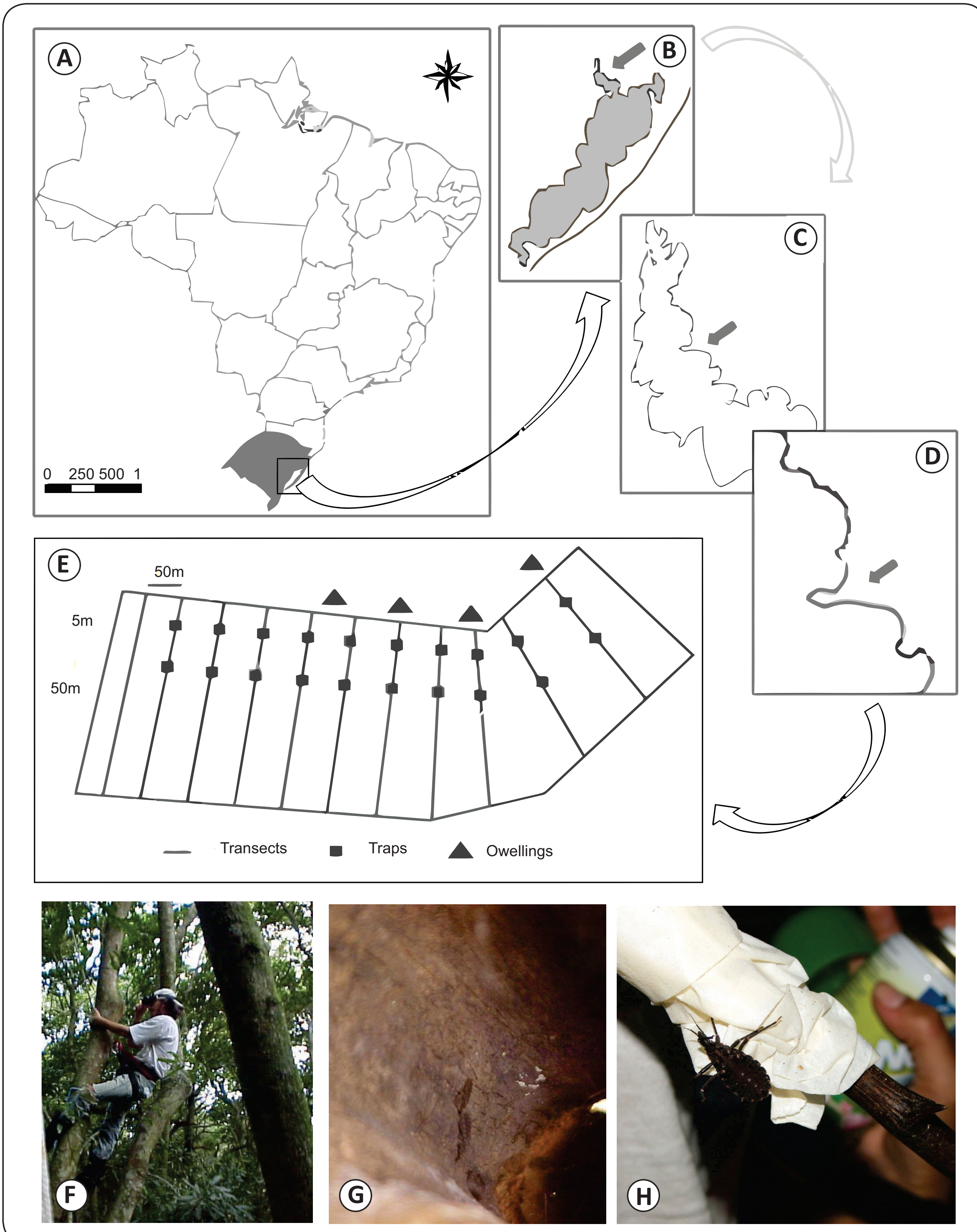

FIGURE 1 - Location of studied area: A: Brazil (arrow indicating the Figure 1B); B: Region of Patos lagoon and surroundings (arrow: Guaíba lake basin); C: Ponta Grossa neighborhood (arrow); D: Study area (arrow); E: *Croquis* drawing of the study area showing the opossum traps, transects and investigated dwellings; F: Investigation of a hollow tree with the aid of ropes; G: *Panstrongylus megistus* found inside the hollow; H: Nymph (N5) captured by adhesive tape.

Food sources were determined by the precipitin test according to the methods of Lorosa et al.[40]. Anti-sera from the following species were used: armadillo, bird, cat, dog, horse, lizard, opossum, ox, pig and human. Statistical analyses were performed using an one-way ANOVA and the post-hoc Tukey test for the evaluation of technique efficacy (transects and spool-and-line). The sex ratio of infection by *T. cruzi* in the reservoirs was evaluated using the chi-square test with BioStat 4.0 software.

RESULTS

There were no colonies found inside residences or in peridomiciliary areas, and no triatomines were obtained using light traps. Peridomiciliary inspections revealed only the presence of adult insects, which were found on outside walls of residences visited between November 2005 and February 2006. The largest *P. megistus* captures occurred in December **(Figure 2)**. Among the 33 triatomines collected, corresponding to 26 (79%) males and 7 (21%) females, 28 were analyzed, and 18 (64%) were infected with *T. cruzi*. Molecular analysis characterized the parasites as *T. cruzi* I.

A total of 27 nymphs were collected: 26 (96%) were analyzed and 19 (73%) were infected. Different instars of *P. megistus* were found among the nymph samples at different times of the year: 26% were 2nd instars (7 of 27), 44% were 3rd (12 of 27), 4% were 4th (1 of 27) and 26% were 5th (7 of 27). However, 4th instars were only observed in March **(Figure 3)**. All negative nymphs were found in the same refuges (dead tree hollow). Triatomine nymphs, eggs and/or exuviae were found only in tree refuges between 10 and 205m from the forest fragment edge near the residences **(Figure 1F, 1G** and **1H)**.

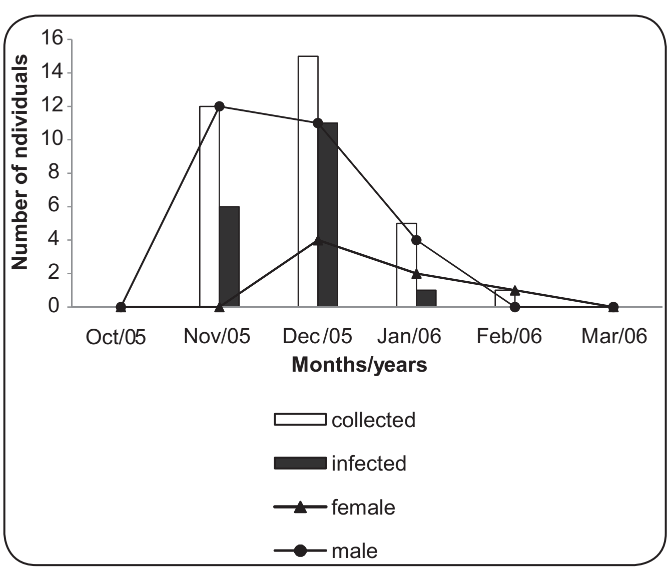

FIGURE 2 - Capture of *Panstrongylus megistus* adults throughout the months (October 2005 to March 2006) showing the number of infected individuals and the sex ratio of specimens from Porto Alegre, State of Rio Grande do Sul, Brazil.

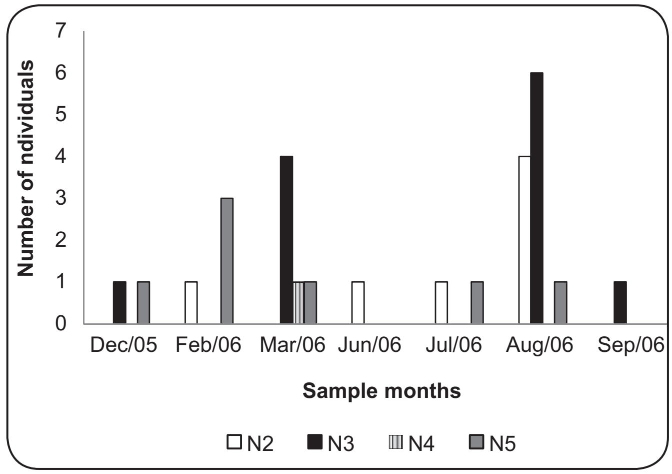

FIGURE 3 - Initial instars of *Panstrongylus megistus* (N2 to N5: 2nd to 5th instar) captured in the sylvatic environment in Porto Alegre, State of Rio Grande do Sul, Brazil. N: Nymph.

The Noireau traps captured two *P. megistus* nymphs (3rd and 5th instars) in opossum nests. The only adult triatomine obtained in this environment was a female found among the branches of a tree (in February 2006) **(Table 1)**. Using the transect method, 33 refuges were found, of which 18% (6 of 33) demonstrated the presence of *P. megistus*. Utilizing the spool-and-line method (with 27 opossums), 28 refuges were found, and this triatomine was present in 14% (4 of 28). There were no significant differences (p>0.05) between the sampling methods. However, the spool-and-line method allowed for a better understanding of the shelters used by marsupials. A total of 61 refuges were observed and classified into five categories: 10 (16%) rock shelters, 10 (16%) dead trees, 11 (18%) ground shelters, 13 (22%) roots and 17 (28%) live trees. Among the examined trees, seven species revealed a high number of refuges for *P. megistus* and *D. albiventris*, including *Casearia sylvestris* (chá-de-bugre), *Coussapoa microcarpa* (figueira-mata-pau), *Ficus organensis* (figueira-de-folha-miúda), *Myrsine guianensis* (capororoca), *Diospyrus inconstans* (maria-preta), *Luehea divaricata* (açoita-cavalo) and *Trichilia elegans* (pau-de-ervilha). The spool-and-line results indicated that triatomine foci occur between 5 and 893m from the animal capture/release location. The opossum demonstrated a wide distribution in the area. In six refuges (four hollow trees and two rock shelters), only opossum nests were found (no triatomines foci) between 8m and 259m from the closest residences, representing a potential focus of *P. megistus*. A total of 462 traps were installed per night, resulting in 98 captures and recaptures of 43 different *D. albiventris*. Among these animals, 39 (91%) were analyzed (26 males and 13 females), and 27 (69%) were infected with *T. cruzi.* There was no significant difference (p>0.05) between the sexes that were caught. The parasites isolated from nymphs and opossums were characterized as *T. cruzi* I. Opossum characteristics, such as semi-nomadic habits, the use of the same nest by different individuals and two females sleeping together in a hollow tree, were observed in field specimens.

TABLE 1 - Characteristics of hollows found in the sylvatic environment (October 2005 to September 2006) showing the different stages of *Panstrongylus megistus*, *Trypanosoma cruzi* infection and associated food sources in Porto Alegre, State of Rio Grande do Sul, Brazil.

Methods	Ecotopes	*Height	**Traverse	***Distance	#Hosts	*+*P. megistus*	*T. cruzi*	Food sources
Transect	Dead tree	2.3	-	30	Hair opossum	2 eg/exv	-	-
						3 N3#*	Pos	Opossum, rodent
						1 N4	Pos	Armadillo
						4 N5#*	Pos	Rodent/bird, opossum, bird
Transect	Live tree	4.0	-	145	Fly feather	2 eg/exv	-	-
						1 N2	Pos	Opossum
Transect	Live tree	2.2	-	186	-	3 eg	-	-
Transect	Live tree	4.0	-	205	Rodent	4 eg	-	-
Transect	Dead tree	2.8	-	36	Rodent	3 N3	Pos	Rodent, opossum
						1 N5	Pos	Bird
Transect	Dead tree	1.4	-	92	-	2 N2	Neg	Bird
						5 N3	Neg	Rodent, opossum, bird/opossum
Spool and line	Live tree	3.8	322	148	-	7 eg/exv	-	-
						4 N2++	Pos	Bird
						1 N3	Pos	Rodent/bird
						1 N5	Pos	Bird
Spool and line	Dead tree	2.5	835	140	-	5 eg/exv	-	-
Spool and line	Live tree	1.2	167	10	Opossum	exv	-	-
Spool and line	Live tree	1.0	12	10	Opossum	1 N5	Pos	Bird
Spool and line	Live tree	3.3	5	59	Opossum	♀##	Neg	Rodent

P. megistus:. Panstrongylus megistus; T. cruzi: Trypanosoma cruzi. *height of hollows from the ground (m); **distance between the point of capture/release and opossums found (m); ***distance between the hollows and nearest residence (m); #hosts found in hollows or traces observed; +two female opossums; *+foci found: instars bugs found in the hollows, N2 to N5: nymphs of 2nd to 5th instar; eg: eggs hatched; exv: exuviae; #*one individual caught with Noireau traps; ++one individual was not examined for *T. cruzi* and food source; ##female found in the tree; Pos: (positive) and Neg (negative) for *T. cruzi* infection.

The precipitin test was conducted in 26 *P. megistus* nymphs and 29 adult insects. The nymphs were associated with four different hosts (bird, rodent, opossum and armadillo) in a manner that indicating that 88% used only one feeding source and 12% used two feeding sources. The adults were related to seven hosts (bird, rodent, opossum, armadillo, cat, dog and lizard) such that 77% used one food source, 20% used two hosts and only one (3%) individual used three different host species. The bird, rodent and opossum showed the highest frequency of serving as hosts in both environments (sylvatic and peridomiciliar). There were no positive reactions for human blood **(Table 2)**.

DISCUSSION

This paper reports the first discovery of a *P. megistus* sylvatic focus in RS. The occurrence of triatomines in residences during the warmer months was reported in Porto Alegre[25,29], with males being more frequently present[28]. Even with the presence of early instar nymphs throughout the year, as observed in natural ecotopes, *P. megistus* showed only an annual life cycle, with adults emerging in the last months of spring. This feature is likely attributed to a prolongation of the 5th instar due to low mobility and decreased hematophagism in the cold of winter. With the nearing of summer, there is an increased activity of individuals followed by a rise in the search for food, thereby leading to the dispersion of adult triatomines[41,42]. Although domestic *P. megistus* colonies were not found in the evaluated houses, the proximity of natural foci to residences could facilitate triatomine infestation[15].

Although intradomiciliary triatomine foci were not found, the *Programa de Controle da Doença de Chagas* (PCDCh) has maintained continuous epidemiological surveillance.

TABLE 2 - Frequency of the hosts associated with sylvatic and domestic *Panstrongylus megistus* in Porto Alegre, Rio Grande do Sukl, Brazil, as demonstrated by the reaction of precipitin.

Host found	Reactions observed				Total frequency of the host by environment	
	nymphs		adults		sylvatic*	peridomiciliary**
Bird	10/26 (38%)	88%+	7/29 (25%)	77%+	13/30 (43%)	13/36 (37%)
Rodent	6/26 (23%)		5/29 (18%)		9/30 (30%)	8/36 (23%)
Opossum	6/26 (23%)		2/29 (7%)		7/30 (24%)	4/36 (11%)
Armadillo	1/26 (4%)		3/29 (10%)		1/30 (3%)	3/36 (8%)
Cat	-		2/29 (7%)		-	3/36 (8%)
Dog	-		1/29 (3%)		-	3/36 (8%)
Lizard	-		2/29 (7%)		-	2/36 (5%)
Bird/opossum	1/26 (4%)	12%+	2/29 (7%)	20%+	-	-
Rodent/bird	2/26 (8%)		3/29 (10%)		-	-
Dog/cat	-		1/29 (3%)		-	-
Bird/rodent/dog	-		1/29 (3%)		-	-

*nymphs and one female found in the natural environment; **only adults; +frequency (%) of one or two feeding sources identified.

The few studies performed in recent years have reported that only adults of *P. megistus* have been found in homes. Currently, the PCDCh has four PITs (*Ponto de Informação de Triatomíneos*) in various parts of the city and advises residents regarding the presence of triatomines in houses near natural areas, the cleaning of peridomiciliary environments (avoiding potential foci) and the installation of screen doors and windows[43].

In Bambuí State of Minas Gerais, Santos Jr et al.[17] demonstrated that even with a low rate of infestation in sylvatic habitats, the colonization of domiciliary environments has been extensive during the last three decades. The presence of triatomines in sylvatic or, occasionally, domiciliary environments threatens to interrupt the progress of disease control measures in many areas given the focus of control programs on resident species [44]. These programs should implement new strategies to prevent human-vector contact using materials impregnated with insecticide, such as curtains and mosquito netting, and create alternatives for the proper management of the environment and maintenance of peridomiciliary areas[45].

The lack of housing foci found in this work corroborates data from classic publications reporting that populations of the species in southern areas have a lower tendency to occupy artificial environments[6,46,47]. In an evaluation of populations in the States of Bahia, Minas Gerais and Santa Catarina, Barbosa et al.[48] observed that the occupation of artificial environments may be related to a greater efficiency in resource utilization, presenting a demographic cline where the lowest fitness was observed in SC. Additionally, genetic and paleogeographic studies have demonstrated that the most recent geological events may have acted differently on the various *P. megistus* populations, leading to the observed behavioral differences in these species[49].

According to Forattini[6], *P. megistus* have a less invasive tendency of artificial ecotopes in more humid climates with rainy winters and larger preserved natural areas. Aragão[46] showed that humidity followed by lower temperatures produces a greater wet surface permanence in residences, making such habitats unsuitable for these triatomines. However, the presence of *P. megistus* in artificial habitats in the States of Santa Catarina and Paraná demonstrates the species' ability to colonize this environment in the southern region of the country, even at a low intensity[50,51]. In the State of Rio Grande do Sul, the first domiciliary species focus was described by Di Primio[30] in the municipality of Viamão. Here, colonies of the species were occasionally found in artificial environments, maintaining its sylvatic characteristics. According to Bedin et al.[22], 88% of the *P. megistus* found in RS between 1996 and 2008 occurred with residences, and 96% of these were adults.

Although found in different types of habitats, *P. megistus* demonstrates a preference for arboreal habitats[11,13,18]. This preferred ecotope provides an opportunity for greater contact between this triatomine and various species of rodents, marsupials and birds[31]. According to Barretto et al.[12], sylvatic colonies of *P. megistus* may have a high rate of infection by

T. cruzi, especially when associated with *Didelphis* sp. The presence of nymphs in the reservoir nests implies a significant circulation of the parasite between vector and host, as observed in this present study in which most of the sylvatic colonies showed *T. cruzi* infection. The omnivorous habit of opossums allows for the ingestion of insects as a nutritional alternative[52]. This insectivorous diet may act as an important route of *T. cruzi* infection when associated with the triatomines present in their burrows[53], potentiated by semi-nomadic habits that enhance the possibility of encountering this vector in the natural environment[54]. *T. cruzi* oral infection experiments demonstrated a high infectivity toward marsupials, especially those that ingested infected triatomines[54,55].

According to Patterson et al.[44], high infection rates might be an indicator of close proximity to reservoir hosts and high susceptibility to *T. cruzi.* In this paper, infection rates were higher than in other studies conducted in RS (41%[40] and 50%[18] for *P. megistus* and 18%[56] for *D. albiventris*). However, the results presented herein are in agreement with studies in other states (39-85% for *P. megistus*[12,14] and 11-91.7% for *D. albiventris*[57-59]). The parasite characterization performed here allowed us to observe the involvement of *D. albiventris* and *P. megistus* in the maintenance of the *T. cruzi* sylvatic cycle. In contrast, Fernandes et al.[56] demonstrated that although *P. megistus* showed only *Trypanosoma cruzi* Z1 (TCI[39]) in Porto Alegre, *D. albiventris* was found with *Trypanosoma cruzi* Z2 (TCII[39]), suggesting that the involvement of these marsupials in domestic cycles may serve as a carrier mechanism for this group in the natural environment.

The evaluation of food sources revealed that the main resources used by adult and nymph triatomines were birds, rodents and opossums. These three animals may use the same shelters in trees, as observed by Barretto et al.[11,60]. Although not analyzed in this study, the association of rodents with infected triatomine colonies highlights their epidemiological potential as loci, as rodents are considered potential reservoirs in the *T. cruzi* sylvatic cycle. Birds, although refractory to parasites, enable the maintenance of colonies and can act as population growth vectors, thereby increasing the risk of disease transmission, especially if the triatomines had previous contact with an infected mammal[61]. In an analysis of populations in the State of Minas Gerais Midwest, Villela et al.[62] also found a higher level of blood feeding on birds in triatomines captured in peridomiciliary environments; however, the majority (90.4%) of individual triatomines fed on only one source (bird and others species). The same authors showed that 44% of infected bugs had this source.

The adults showed three food sources that were lacking nymphs: cats, dogs and lizards. Cats were found living only in the woods due to the hostility of the local people; thus, dogs were the only domestic animals used as a food source by triatomines. The number of food sources indicated the eclecticism of *P. megistus*[40,63].

The presence of anti-armadillo serum in a nymph found in a hollow tree suggests a considerable mobility of these triatomines in the natural environment. Forattini et al.[64] observed the mobility of 4th and 5th instar *Triatoma arthurneivai* nymphs, which walked up to 10m between the studied habitats. A similar behavior was indicated for *P. megistus*[13]. The lower occurrence of dogs and absence of other animals, such as pigs, cattle and humans, resulted in a low rate of infestation, confirmed by the absence of peridomestic colonies and a similarity of food sources between adults and nymphs.

The forest fragment analyzed here represents the typical arboreal species in the granite hills of Porto Alegre and, considering its natural conditions, contributes to the maintenance of the sylvatic characteristics of *P. megistus*. Thus, based on the data presented in this study, it is suggested that sites with similar characteristics in the city can present similar rates of infection. Thus, sylvatic areas at greatest risk of urban effects tend to connect humans and domestic animals to the sylvatic cycle of the parasite. Despite the absence of colonization, the high number of infected opossums and triatomines demonstrates the potential risk of *T. cruzi* transmission in the municipality, which suggests the importance of continued epidemiological surveillance and management strategies in environmentally important areas of the native city.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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