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Eco-epidemiology of vectorial *Trypanosoma cruzi* transmission in a region of northeast Brazil

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A R T I C L E I N F O

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A B S T R A C T

Chagas disease (CD) is a parasitic zoonosis endemic in Brazil. Despite virtual control of *Triatoma infestans*, the main domesticated vector of *Trypanosoma cruzi*, vectorial transmission by other triatomine species persists in some rural communities. This study aims to characterize triatomines role in transmitting *T. cruzi* to dogs and humans in the district of Santo Inácio, located in the northwest region of the state of Bahia, Brazil. It also describes environmental factors in housings associated with insect occurrence and assesses the perception, knowledge, and preventive practices adopted by the population regarding CD. Blood samples of humans and dogs, and biological samples of triatomines, were collected between November 2018 and February 2019 and subjected to the detection of *T. cruzi* by serological and molecular biology tests. Also, we applied a questionnaire to research the perception, knowledge, and local practices of people related to CD. The capture of triatomines in households was associated with exploratory variables of the questionnaires using multivariate logistic regression ($p < 0.05$). The 155 triatomines captured in the wild and domestic environment were of the species *Triatoma sherlocki* ($n = 151$), *Panstrongylus sherlocki* ($n = 1$) and *Triatoma sordida* ($n = 3$), and had a natural infection rate for *T. cruzi* by PCR of 18.5%, 100% and 0%, respectively. District residents ($n = 126$) were seronegative for *T. cruzi*, while 17.5% (7/40) of the dogs were seropositive. The fact that residents are aware that triatomines can "cause" CD was configured as a protection factor for residents according to the fitted logistic regression model ($p = 0.04$). However, respondents have limited perception and knowledge about the CD, prevention and control practices for triatomines in a household. The results suggest the existence of a domestic cycle of transmission of *T. cruzi* between triatomines and dogs, configuring a latent risk of infection to the human population of Santo Inácio. Studies that clarify the potential for the establishing of intrusive triatomines in households, surveillance actions for triatomines, and health education in rural communities are indispensable to prevent the reemergence of CD in vulnerable regions of Brazil and other American countries with similar epidemiological characteristics.

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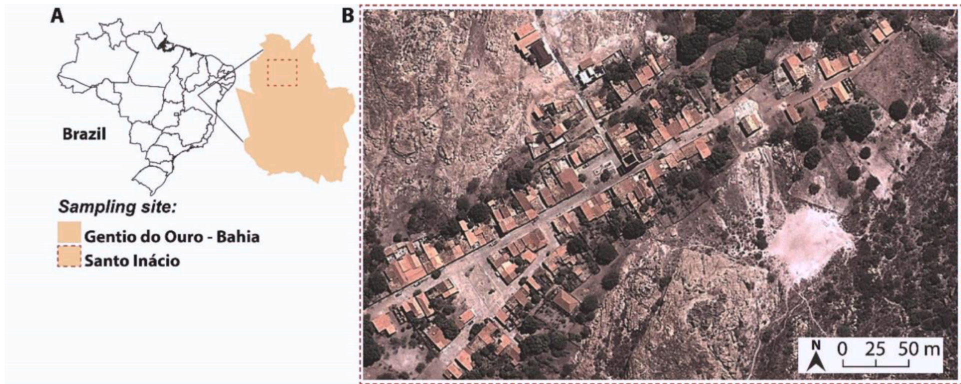

Fig. 1. Characterization of the sampling site. (A) Brazil, state of Bahia, municipality of Gentio do Ouro with the highlight on the district of Santo Inácio (QGis 3.12). (B) Headquarters of the district of Santo Inácio, Gentio do Ouro, Bahia, surrounded by rocky-outcrops (Google Earth®, 2020).

1. Introduction

Chagas disease (CD) is a parasitic zoonosis caused by *Trypanosoma cruzi* and is usually transmitted between vertebrate hosts by triatomine insects popularly known as "the kissing bug" (Sousa et al., 2020). The disease is endemic in Latin America, especially in Brazil (WHO, 2015), with cases recorded in poor rural areas with triatomines frequent occurrence (Daflon-Teixeira et al., 2019; Lima et al., 2012; Vinhaes et al., 2014).

The implementation of the National Chagas Disease Control Program contributed to reducing the population of *Triatoma infestans* (Dias, 2016b), one of the main domiciled vectors in the region, currently with records of residual foci in the state of Bahia (Ribeiro et al., 2019). However, given the diversity of *T. cruzi* vectors, there is a risk of transmission by other intrusive triatomine species (Lima et al., 2012; Ribeiro et al., 2015). Therefore, strengthening the population participation in the notification of vectors in their households in regions vulnerable to infection is essential. This depends on permanent health education actions on the epidemiology of CD, focusing especially on the young population (Yevstigneyeva et al., 2014), seeking to reduce eventual underreporting of cases (Dias et al., 2016a).

According to other authors, vectorial transmission of *T. cruzi* is associated with household invasion by wild triatomines in several countries in the Americas (Waleckx et al., 2015). In the Yucatán Peninsula, Mexico, seasonal invasion of *Triatoma dimidiata* occurs, leading to active infection by *T. cruzi* in its inhabitants (Gamboa-León et al., 2014). In Colombia, the wild triatomine *Triatoma maculata* invades houses, transmitting *T. cruzi* to dogs and humans in a region with the occurrence of synanthropic animals infected by this protozoan, such as *Didelphis marsupialis* (Cantillo-Barraza et al., 2015). The population of the municipality of Apolo, Bolivia, complains of triatomine bites, with the wild species *Eratyrus mucronatus* being the most frequently found in the area (Depickère et al., 2012). Therefore, the presence in households of other triatomine species, previously of less epidemiological importance, infected with *T. cruzi,* represents an important risk of CD reemergence in regions where the main vector species was controlled (Cantillo-Barraza et al., 2020; Carrasco et al., 2014; Waleckx et al., 2015).

In the study area - district of Santo Inácio, municipality of Gentio do Ouro (Bahia) - there are records of triatomine species *Triatoma sherlocki* (Papa et al., 2002), *Panstrongylus sherlocki* (Jurberg et al., 2001), and *Triatoma sordida* (Ribeiro et al., 2014). There are few reports on the latter two species in the region, with *P. sherlocki* being mentioned in a morphological characterization study, without data on *T. cruzi* infection (Jurberg et al., 2001), and *T. sordida* evaluated for the presence of different strains of *T. cruzi* (Ribeiro et al., 2014). *T. sherlocki* species is sylvatic and the most frequent in the region (Costa et al., 2014; Mendonça et al., 2009). Studies report *T. cruzi* infection in these triatomines (Alameida et al., 2009; Ribeiro et al., 2019). Birds were the most prevalent dietary pattern among the targets assessed for sylvatic populations of *T. sherlocki* (Ribeiro et al., 2019), but the high frequency of *T. cruzi* infection in this species suggests the involvement of wild mammals in maintaining the cycle transmission of the parasite.

The occurrence of *T. sherlocki* in households and its possible involvement with domestic animals and humans in the transmission cycle of *T. cruzi* causes concern in the region and constitutes a threat to public health (Alameida et al., 2009). Therefore, this study aims to characterize the vector role of triatomines found in the district of Santo Inácio (Bahia), to estimate the frequency of infection by *T. cruzi* in vectors, dogs, and humans, as well as describing characteristics of the households associated with the occurrence of these insects and evaluate the perception, knowledge and preventive practices adopted by the population regarding CD.

2. Material and methods

2.1. Study area and sample population

The study was carried out in the district of Santo Inácio (Fig. 1), belonging to the municipality of Gentio do Ouro (11°22′49.4″S 42°38′05.5″W), located in the northwest region of the state of Bahia, Brazil. It has an estimated population of 255 inhabitants, living in houses surrounded by large rocky-outcrops with significant fractures. Vegetation and fauna are characteristic of the Caatinga biome (semi-arid), which houses, among others, *Thrichomys* sp. and *Kerodon rupestres*, wild mammals of frequent occurrence in the region and possibly involved as blood-meal sources for triatomines (Papa et al., 2002). The region has an average temperature of 25°C, and precipitation around 750 mm, with rains concentrated in the spring (September to December) and summer (December to March) (SEI-Superintendência de Estudos Econômicos e Sociais da Bahia, 2018).

Between November 2018 and February 2019, visits were made to households in Santo Inácio to invite residents to volunteer in the study. Those who agreed to participate signed an Informed Consent Form. Volunteers were submitted to a questionnaire, blood sample collection, triatomines were captured in their houses and, after data collection, they were informed about the CD. Also, those who owned dogs had their animals sampled for blood. The study was approved by the Research Ethics Committee of the Federal University of West Bahia (protocol 2965700) and by the Ethics Committee for Animal Use of the School of Veterinary Medicine and Zootechnics of the Federal University Bahia (protocol 75/2017).

2.2. Serological survey of *T. cruzi* in humans and dogs

Blood collection was carried out in the volunteers' households by a nursing technician on previously scheduled days. Five ml of blood were collected in aseptic conditions, with dry tubes. The samples were immediately refrigerated and transported to the Laboratory of the Multidisciplinary Center of the *Campus* de Barra (CMB) of the Federal University of West Bahia (UFOB) for centrifugation and serum collection. Two ml serum aliquots were packed in microtubes and kept at −20 °C, being sent to the Advanced Laboratory of Public Health (LASP) of the Gonçalo Moniz Institute (IGM, Fiocruz-BA) under refrigeration, subjected to two commercial indirect ELISA tests for detection of anti-*T. cruzi* antibodies: ELISA Chagas III (BIOSChile, Ingeniería Genética S. A, Santiago, Chile) and Gold ELISA Chagas (batch CHA132A; Rem, São Paulo, Brazil). All results were analyzed in duplicate, and the cutoff values were determined according to the manufacturers.

The consent for sampling the dogs was requested to the tutors through signing of an authorization term. Animals were submitted to physical restraint, clinical evaluation and demographic data collection. Blood samples were collected through the jugular or cephalic veins in aseptic conditions, with dry tubes, in a volume of 3 to 5 ml. They were immediately refrigerated and transported to the CMB/UFOB laboratory. After separation and storage at -20°C, the serum samples were sent to LASP (IGM, Fiocruz-BA). They were analyzed by the modified Gold ELISA Chagas commercial test (batch CHA132A; Rem, São Paulo, Brazil) and *in-house* ELISA, using four *T. cruzi* chimeric recombinant antigens (IBMP-8.1, IBMP-8.2, IBMP-8.3, and IBMP -8.4) according to Leony et al. (2019). Dogs were considered seropositive for *T. cruzi* when reactive in both tests. The samples were also tested for anti-*Leishmania* antibodies, using the protocol recommended by the Ministry of Health: rapid test DPP© and ELISA/EIE [Bio-Manguinhos/Fundação Oswaldo Cruz (FIOCRUZ)]. Dogs were considered seropositive for *Leishmania* sp. when reactive in both tests.

2.3. Analysis of households and triatomines

The manual capture of triatomines in the households took place during the day, with the use of flashlights, gloves and tweezers, being carried out in all places of the house such as under the mattresses, behind furniture and in cracks that could house the vectors, in a clockwise direction. In the peridomicile, the search covered: the annexes, such as chicken coops and sheep corrals; accumulation of construction waste; piles of tiles and bricks; and places of garbage accumulation. The collected triatomines were placed in plastic tubes with a lid, allowing aeration and containing filter paper (Galvão, 2014). The triatomines capture activities in the domestic environment was executed by two people, with an average of 12.5 min/person/home. The research members also filled in a list of household characteristics associated with triatomines, such as roof and wall type, and presence of annexes for raising animals.

The capture of triatomines in the wild took place at dusk, with the aid of flashlights and frequent checking of the surface of rocks that had fissures, hollows of tree trunks and rock burrows of small wild mammals (example *Thrichomys* sp. and *K. rupestres*). The triatomines were collected with tweezers and placed in plastic tubes with aeration. The capture activity involved three to four people per collection in the wild environment, with an average duration of two hours/person. The capture of triatomines was authorized by the Brazilian Environmental Agency (IBAMA/SISBIO protocol 61011-2).

The insects were sent to the CMB/UFOB laboratory for taxonomic identification by optical microscopy of the characters of the head, chest, and abdomen, according to the identification keys of Lent and Wygdzinsky (1979) and Galvão (2014). The insects were also classified into nymphs, males, and females. Entomological indicators were evaluated: (i) the natural infection index, which is the number of triatomines infected by *T. cruzi* divided by the total examined x 100; (ii) the infestation index, which is the number of positive households for triatomines divided by the households surveyed x 100; (iii) the colonization index, which is the number of households with nymphs divided by the number of households with triatomines x 100 and (iv) overall triatomine abundance, which is the number of triatomines captured in the households divided by the number of households surveyed.

2.4. Molecular diagnosis of mammalian hosts and triatomine

The samples obtained from triatomines, humans, and dogs were examined by conventional PCR and sequenced for detection and confirmation of *T. cruzi* at the Laboratory of Pathology and Molecular Biology (LPBM) of the Gonçalo Moniz Institute (IGM/Fiocruz-BA).

DNA extraction from triatomines was adapted from Ribeiro et al. (2019), using abdomen samples from each insect and the DNAzol standard protocol. The purified DNA was eluted in 50 μl of the elution buffer. We used the commercial kit (PureLink Genomic DNA Mini Kit, Invitrogen®) to extract DNA from whole blood samples with EDTA anticoagulant (dogs and humans), following the manufacturer recommendations, and eluted in 30 μl of elution buffer. All DNA concentration of the samples was determined on a NanoDrop™ spectrophotometer, being adjusted to ∼100 ng/μl, and the samples were then stored at -80°C. The entire extraction process was carried out in a security booth, and the material used was sterilized to avoid contamination of the extracted DNA.

Conventional PCR aimed to amplify the histone H2A gene, corresponding to the 16-248 nucleotides of a sequence called SIRE (Pavia et al., 2007), a small interleaved repetitive element presents in *T. cruzi*. Primers TcH2AF (5′- GAGAGTGATCGTGGGAGAGC-3′) and TcH2AR (5′-AGTGGCAGACTTTGGGGTC-3′) were used, specific for *T. cruzi*, and which produce a 234 bp amplicon (Lilioso et al., 2020). In 25 μL volumes, PCR reactions were performed with 11 μl of the Qiagen PCR Master Mix commercial kit (QIAamp, Qiagen, Hilden, Germany), 0.5 μM of each primer, 2 μl of genomic DNA, and DNase free water until the volume is complete. The reaction was performed on the Mastercycler Gradient thermocycler (Eppendorf, Foster City, California, USA), with 94 °C programming for 3 min, followed by 40 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 60 s, with a final extension of 72 °C for 10 min.

The PCR products were stained with SYBR Safe solution (Invitrogen, CA, USA) and submitted to a horizontal electrophoretic run (Micro SSPTM Gel System, One Lambda, USA) in 1.5% agarose gel, in Tris borate EDTA buffer (TBE), together with the 100 bp molecular weight marker (Invitrogen, USA). The results were visualized on a blue light transilluminator and photographed with the MultiDocit Photodocumenter (UVP, Imaging Systems, Upland, CA, USA). The positive control was a triatomine sample positive for *T. cruzi* previously sequenced and with 99% identity with the sequence deposited in GenBank (CP015671.1) and the negative control was ultrapure water. PCR products were purified with a commercial QIAquick PCR Purification kit (Qiagen, Germany) and sent for bidirectional sequencing (Sanger et al., 1977).

2.5. Epidemiological questionnaire

The semi-structured epidemiological questionnaires had objective and subjective questions and were applied according to the profile of the interviewees: (i) addressed to the household provider (adult responsible for the home), containing questions about triatomines, CD, and demographic data (biological, socioeconomic, and cultural); and (ii) addressed to other residents of the households, with questions about demographic data, being applied to individuals over ten years of age.

The interviews were conducted in the interviewees' households by four trained interviewers. The questions were asked without inducing the answer and without reading the alternatives. In cases where respondents were unable to answer, this option was checked in the

Table 1
Natural infection index for *T. cruzi* in triatomines collected in the district of Santo Inácio, Gentio do Ouro, Bahia, Brazil, from 2018 to 2019.

	Triatoma sherlocki					*Triatoma sordida*					*Panstrongylus sherlocki*				
	F	**M**	**N**	**P/A**	**I%**	**F**	**M**	**N**	**P/A**	**I%**	**F**	**M**	**N**	**P/A**	**I%**
INTRA	1[a]	1[a]	1[b]	0/3	0.0	1[a]	0	0	0/1	0	0	0	0	0	0.0
PERI	2[c]	0	1[d]	1/3	33.3	0	0	2[e,f]	0/2	0	0	1[g]	0	1/1	100.0
WILD	21	24	100	27/145	18.6	0	0	0	0/0	0	0	0	0	0	0.0
TOTAL	24	25	102	28/151	18.5	1	0	2	0/3	0	0	1	0	1/1	100.0

F: female; M: male; N: nymph; P: positive triatomines; A: analyzed triatomines; I%: Natural Infection Index - the percentage of positive triatomines for *T. cruzi*; INTRA: inside the house; PERI: peridomicile; WILD: wild environment

[a] captured in the bathroom
[b] captured in the bedroom
[c] captured in the backyard
[d] captured on the balcony
[e] captured in a pile of tiles in the backyard
[f] captured in the backyard where a *T. cruzi* seronegative dog was sleeping; [g] captured in the backyard where a *T. cruzi* seropositive dog was sleeping.

Fig. 2. Characteristics of households and proximity to a wild environment. (A) Rocky-outcrops surrounding the dwellings and considered major ecotopes of *T. sherlocki*. (B) Peridomicile with rock formations serving as a shelter for a dog and triatomines. (C) Dog in the peridomicile, with accumulation of diverse materials in the background, serving as a hiding place for triatomines.

questionnaire.

2.6. Data analysis and geoprocessing

Data from qualitative variables were categorized and their absolute and relative frequencies described. For each analysis, tables were created highlighting the outcome variables and the independent variables, namely: i) demographic and clinical data of the dogs that were associated with the outcome variable "Seropositivity for *T. cruzi* in dogs"; ii) data on the perception, knowledge and practices of providers that were associated with the outcome variable "Infestation rate of triatomines in households". In addition, "Structural and environmental characteristics of households" were associated with the outcome variable "Index of triatomine infestation in households". To analyze the association between the outcome variables and the independent variables, non-fitted model and multivariate analyses were performed using logistic regression. Prevalence ratios (PR) and 95% confidence intervals (95%CI) were estimated. The *forward* variable selection method was used to include the variables in the multiple model. The inclusion criterion was $p < 0.20$ in the fitted model analysis. The criterion for maintaining the variables in the final model was $p < 0.05$. Data analysis was performed using Stata®14 statistical software.

The map that determines the studied area's location was made using the software Qgis 3.12 and Adobe Illustrator CS6.

3. Results

3.1. Demographic data, serological and molecular diagnosis in humans and dogs

In this study, 255 residents were recruited but only 126 individuals agreed to participate. The demographic data of the volunteers are shown in Supplementary Table 1. All volunteers sampled were negative for *T. cruzi* in serological and molecular tests, which is equivalent to 49.5% (126/255) of the population in the district of Santo Inácio.

In the study area, 40 dog samples were collected. Demographic and clinical data of dogs are shown in Supplementary Table 2. 17.5% of the dogs sampled (7/40) were reactive for *T. cruzi* in both the Gold ELISA Chagas and *in-house* ELISA tests (chimeric antigens), and 7.5% (3/40) were reactive to both the DPP© and ELISA/EIE for anti-*Leishmania* sp. A single dog was seropositive for *T. cruzi* (Gold ELISA Chagas and *in-house* ELISA tests) and *Leishmania* sp. (rapid test DPP© and ELISA/EIE), suggesting possible co-infection by both pathogens. Of the dogs sampled, 75% (30/40) were male and 25% (10/40) female. 85.71% (6/7) of males and 14.29% (1/7) of females were seropositive for *T. cruzi*. In addition, dogs older than one year of age were the most seroreactive for *T. cruzi* (85.71%, 6/7). No dog was positive for *T. cruzi* by the PCR technique.

3.2. Analysis of triatomines

The 155 triatomines were captured in the following environments: inside houses ($n = 4$); peridomicile ($n = 6$); and wild environment ($n = 145$). The ten triatomines captured in households ($n = 7$) were of the following species: *Triatoma sherlocki* ($n = 6$), *Triatoma sordida* ($n = 3$), and *Panstrongylus sherlocki* ($n = 1$). All specimens found in the wild ($n = 145$) were of the species *T. sherlocki* and most were captured on the surface of rock formations ($n = 131$). Only one colony was found in a rock burrow ($n = 14$). In this colony, all triatomines were engorged and 12 of them were positive for *T. cruzi*. Twenty-nine (18.7%) triatomines were positive for *T. cruzi* according to PCR testing (Table 1) and confirmed by sequencing.

3.3. Analysis of households

Of the 106 existing households in the district of Santo Inácio, 48 were sampled (45%). Among the information about the infrastructure and organization of the households, it is noteworthy that the 94% (45/48) had ceramic roofs; 88% (42/48) masonry walls with plaster; 88% (42/48) peridomicile materials such as tiles and blocks, 60% (29/48) construction debris and 46% (22/48) accumulated garbage. Forty households (38%) were not inspected, as they were vacant, and in 18 households (17%) there was no permission from the resident.

In 34 households sampled, 351 domestic animals were registered, in the following proportions: 62% (217/351) chickens, 14% (50/351) sheep, 12% (43/351) dogs, 10% (36/351) cats, and 1% (5/351) birds. The presence of annexes for raising domestic animals was verified in 21% (10/48) of the households, of which nine were chicken coops, and one had a chicken coop and a sheep corral. Entomological indicators calculated resulted in an infestation index of ~15% (7/48), triatomine abundance of 0.2 (10/48) and colonization index of 57% (4/7). Fig. 2 shows some households characteristics and their proximity to the rocky-outcrops of the wild environment. Supplementary Table 3 shows the environmental conditions of households positive for triatomines.

3.4. Epidemiological questionnaire

Of the 112 questionnaires applied, 42% (47/112) were answered by the household provider and 58% (65/112) by other residents. The questionnaire was not applied to one specific provider and thirteen other residents for various reasons beyond our control ($n = 14$). The results and frequencies of perception, knowledge, and practices about triatomines and CD are presented in Table 2, and demographic data of the population are in Supplementary Table 1.

3.5. Data analysis

The fitted logistic regression model between "Provider perception, knowledge and practice data" and the "Triatomine infestation index in households" suggests that the fact that the provider has knowledge that the triatomine can cause CD may be configured as a factor of protection for it ($p = 0.04$). Thus, providers with this knowledge may adopt preventive measures to avoid contact with the triatomine (Table 2).

The non-fitted model of the analysis between “Demographic and clinical data of dogs” and “Seropositivity for *T. cruzi* in dogs” was not significant (Supplementary Table 2). Thus, a fitted model was not carried out. Therefore, the exploratory variables do not sufficiently explain

Table 2
Descriptive and statistical analysis of questionnaires on perception, knowledge, and practices in relation to triatomines and Chagas disease obtained from 47 providers and their association with the infestation index of triatomine in households in the district of Santo Inácio, Gentio do Ouro, Bahia, Brazil, from 2018 to 2019.

Independent variables	n/N (%)	[#] Frequency (%)	PR (95% CI)Non-fitted Model	PR (95% CI)Fitted Model
Have you found triatomines at your house? (*N* = 47)				
No/Do not know	14 (29.79)	1/7 (14.29)	1	
Yes	33 (70.21)	6/7 (85.71)	3.21 (0.11-92.79)	
What place in the house did you find the triatomine? (*N* = 33)				
Peridomicile	3 (9.09)	1/6 (16.67)	*	
Inside house	22 (66.67)	3/6 (50.00)		
Both	8 (24.24)	2/6 (33.33)		
[a] **What did you do when you found the triatomine? (*N* = 33)**				
Suitable destination	2 (6.06)	0	*	
Inappropriate destination	31 (93.94)	6/6 (100.00)		
What is the delivery location for the triatomine? (*N* = 47)				
Health Service (person or institution)	13 (27.66)	0	*	
[b] Others/Do not know	34 (72.34)	7/7 (100.00)		
[c] **Which of the insects is a triatomine? (*N* = 47)**				
Incorrectly pointed/Cannot answer	8 (17.02)	2/7 (28.57)	1	1
Correctly pointed	39 (82.98)	5/7 (71.43)	**0.09 (0.00-1.39)****	0.23 (0.03-1.82)
Do you do anything to avoid the triatomine? (*N* = 47)				
No	36 (76.60)	6/7 (85.71)	1	
Yes	11 (23.40)	1/7 (14.29)	0.42 (0.02-7.67)	
Do triatomines cause any disease? (*N* = 47)				
No/Do not know	3 (6.38)	2/7 (28.57)	1	1
Yes	44 (93.62)	5/7 (71.43)	**0.01 (0.00-0.74) ****	**0.04 (0.00-0.66)*****
What disease does the triatomine cause? (*N* = 44)				
Chagas disease	36 (81.82)	5/5 (100.00)	*	
Others	8 (18.18)	0		
Which organ does Chagas disease affect? (*N* = 47)				
Others	10 (21.28)	1/7 (14.29)	1	
Heart	37 (78.72)	6/7 (85.71)	0.12 (0.00-4.91)	
Do you know someone who has Chagas disease? (*N* = 47)				
No/Do not know	24 (51.06)	3/7 (42.86)	1	
Yes	23 (48.94)	4/7 (57.14)	2.54 (0.18-34.23)	
Is Chagas disease curable? (*N* = 44)				
No/Do not know	21 (47.73)	6/7 (85.71)	*	
Yes	23 (52.17)	1/7 (14.29)		
Is there a Chagas disease control service in the region? (*N* = 45)				
No/Do not know	38 (84.44)	6/7 (85.71)	1	
Yes	7 (15.56)	1/7 (14.29)	0.82 (0.04-16.53)	

The name “the kissing bug” was replaced in the text by triatomine; PR = Prevalence ratios; CI = confidence intervals

[#] Frequency of triatomine infestation rate in households in relation to independent variables; * Not included in the non-fitted model due to lack of sufficient data to perform the analysis; ** Criterion used in the non-fitted model was p-value <0.20); *** The criterion for maintaining the variables in the fitted model was p<0.05

[a] Suitable destination (delivered to the university researcher) and Inappropriate destination (killed immediately/ threw in the garbage)

[b] Do not know ($n = 27$), there is no location (= 5), dumping ground ($n = 2$)

[c] Showcase with the presence of three adult hemipteral insects (predator, phytophagous and *T. sherlocki*) was shown to the provider.

Supplementary Table 1
Descriptive analysis of demographic data of residents of the district of Santo Inácio, Gentio do Ouro, Bahia, Brazil, in the period 2018 to 2019.

Variables	n/112* (%)
Sex	
Men	44 (39.29)
Women	68 (60.71)
[a] **Age**	
≤18 years-old	17 (15.8)
>18≤29 years-old	18 (16.07)
>29≤45 years-old	23 (20.54)
>45≤65 years-old	35 (31.25)
>65 years-old	19 (16.96)
Self-reported race/color	
Black	27 (24.11)
Brown	62 (55.36)
Others	21 (18.75)
Do not know	2 (1.79)
Education	
[b] Illiterate and incomplete basic level	39 (34.82)
Complete basic level and incomplete elementary school	24 (21.43)
Complete elementary school and incomplete university graduation	30 (26.79)
Complete university graduation	17 (15.18)
Does not know	2 (1.79)
Do you have a health complaint?	
No	66 (58.93)
Yes	46 (41.07)
Did you have a blood transfusion?	
No	104 (92.86)
Yes	8 (7.14)
Are you in the habit of eating açaí and/or drinking sugarcane juice?	
No	87 (77.68)
Yes	25 (22.32)
Do you eat game meat?	
No	37 (33.04)
Yes	75 (66.96)
Have you ever been stung by triatomines?	
No	57 (50.89)
Yes	41 (36.61)
Does not know	14 (12.50)
Do you use mosquito net?	
No	18 (16.07)
Yes	94 (83.93)

*Information is missing from one provider and thirteen residents (n = 14), because the questionnaire was not applied. [a] Age ranged from 10 to 96 years-old, with an average of 45 years-old and 85% of respondents over 19 years-old
[b] 9% (10/112) of the respondents were illiterate.

the result of serology for *T. cruzi* in dogs in the studied population. The fitted logistic regression model between "Structural and environmental characteristics of households" and the "Infestation index of triatomines in households" was also not significant (Supplementary Table 3). Therefore, the exploratory variables do not sufficiently explain the rate of triatomine infestation in the sampled households. The two statistical analyses mentioned above were purposely not presented here (for more information see Supplemental Tables 2 and 3).

4. Discussion

Three species of triatomines were identified, *T. sherlocki, T. sordida*, and *P. sherlocki,* occurring in different numbers in the researched environments, with 18.7% of the investigated triatomines being infected with *T. cruzi*. It was also observed the presence of seropositive dogs and all human samples were negative for *T. cruzi*.

Adults and nymphs of the *T. sherlocki* species were the most found in households, and the only ones detected in the wild environment. They were positive for *T. cruzi* in both environments. *T. sherlocki* is considered a species of the wild environment (Papa et al., 2002). However, it is believed that at some point, *T. sherlocki*, which cannot fly (Almeida et al., 2012), colonizes households through walking (Alemeida et al., 2009), where it can lay its eggs, with the potential for colonization. The adaptability of the *T. sherlocki* species to the domestic environment was tested in the laboratory, being registered a complete development cycle (egg to adult), low mortality rate, high frequency of blood meal, and resistance to hunger, which can make it possible to remain in households (Lima-Neiva et al., 2017). The infection rate of *T. sherlocki* in this study was 18.5%. Previous studies, also carried out in the district of Santo Inácio, such as Almeida et al. (2009) and Ribeiro et al. (2019), showed infection rates in *T. sherlocki* by *T. cruzi* of 11% and 43%, respectively. Demonstrating that the occurrence of the parasite infecting this species of triatomine is expected in the region, and imposes the need for entomological investigations on the possible colonization probabilities of this vector in households.

Species *T. sordida* and *P. sherlocki* were also captured in households, and the only adult specimen captured of the species *P. sherlocki* in the peridomicile was infected with *T. cruzi*. There is no previous record of infection by *T. cruzi* for this species in the literature consulted. Also, *P. sherlocki* behavioral and occurrence data are scarce (Jurberg et al., 2001), with *P. sherlocki* being considered a chromatic variation of the species *Panstrongylus lutzi* (Garcia et al., 2005). Although the species *T. sordida* is more frequent in the state of Bahia, mostly found in the peridomicile of households, with an infection rate for *T. cruzi* of 4% (Ribeiro et al., 2019), in the present study, few specimens of this species were captured inside households (n = 1; in the bathroom) and peridomicile (n = 2; in the backyard), the same being negative for *T. cruzi*. Species of the genus *Panstrongylus* occupy natural ecotopes associated with hosts (armadillo burrow, rocks with cavy, bird nests) and are found among large tree roots (Sousa et al., 2020), while the natural ecotope for *T. sordida* is the hollow trunk of trees and bird nests (Sousa et al., 2020). Although studies show the occurrence of *T. sordida* (Ribeiro et al., 2014) and *P. sherlocki* (Jurberg et al., 2001) in natural ecotopes of Santo Inácio, both triatomines were not captured in the wild during our study.

Even with a low rate of infestation and low density of triatomines in households, a significant number of the interviewed providers reported finding triatomines in their households (Table 2). Household places with the highest occurrence of insects were the living room (72%), the bedroom (39%), and the kitchen (30%). According to the interviewees, the backyard (n = 6) and chicken coop (n = 1) presented less occurrence of triatomines, perhaps due to the short time that people stay in these areas or, also, because insects find more hiding places in this type of environment. Detection of triatomines is generally low and may vary depending on insect abundance, ecotope characteristics, and capture method (Abad-Franch et al. 2014). We used the manual capture of triatomines as recommended by entomological surveillance as a method. However, capture in households during the day, when the vector hides, where hiding places are diverse, and where the abundance of invasive triatomines is generally smaller, made it difficult to detect the insect. Therefore, we recommend that future studies in this district use dislodging insecticides in households and count on the participation of the residents to capture the insects. In the wild, the capture activity was carried out at night, when the insects are most active and in the natural ecotope of triatomines, allowing for greater capture of vectors (Table 1).

Household invasions and colonization by wild triatomines in Santo Inácio occur for several reasons. Some of them are: i) proximity of the rocky-outcrops to the households; ii) presence of shelters for triatomines [ex: peridomiciles with an accumulation of several materials], and annexes of domestic animals very close to the households; iii) the presence of several domestic animals; iv) artificial light attraction of insects and, finally, v) passive transport by domestic animals and human due to work activities, such as hunting and mining in the wild environment, being able to carry triatomines in their utensils (Almeida et al., 2009). Regarding phototropism, during the stay of the research team in this district, three adult specimens of T. *sherlocki* were found at night inside households, one of which was positive for *T. cruzi* (data not counted). These triatomines are attracted by artificial light (Sousa et al., 2020), which favors the nocturnal invasion of households.

Supplementary Table 2
Descriptive and statistical analysis of the demographic and clinical data of 40 dogs and their association with seropositivity for *T. cruzi* in the district of Santo Inácio, Gentio do Ouro, Bahia, Brazil in 2019.

Independent variables	n/40 (%)	# Frequency (%)	PR (95% CI)Non-fitted Model
Sex			
Female	10 (25.00)	1/7 (14.29)	1
Male	30 (75.00)	6/7 (85.71)	0.51 (0.00-39.64)
Age			
≤5 years-old	29 (72.50)	3/7 (42.86)	1
>5 years-old	10 (25.00)	3/7 (42.86)	3.95 (0.27-57.38)
Does not know	1 (2.50)	1/7 (14.29)	-
Breed			
Undefined race	38 (95.00)	7/7 (100.00)	*
Others	2 (5.00)	0	
Is the dog a hunter?			
No	34 (85.00)	6/7 (85.71)	1
Yes	6 (15.00)	1/7 (14.29)	0.60 (0.02-16.89)
Is the dog fed with game meat?			
No	29 (72.50)	5/7 (71.43)	1
Yes	11 (27.50)	2/7 (28.57)	0.56 (0.03-8.41)
Where does the dog sleep?			
Inside house	3 (7.50)	0	*
Backyard	37 (92.50)	7/7 (100.00)	
Anemic mucous membranes			
No	28 (70.00)	5/7 (71.43)	1
Yes	12 (30.00)	2/7 (28.57)	0.68 (0.04-10.12)
Cardiac arrhythmia			
No	33 (82.50)	7/7 (100.00)	*
Yes	7 (17.50)	0	
Crusts on the ears			
No	24 (60.00)	4/7 (57.14)	1
Yes	16 (40.00)	3/7 (42.86)	2.99 (0.07-124.86)
Hyperkeratosis of the snout			
No	31 (77.50)	6/7 (85.71)	1
Yes	9 (22.50)	1/7 (14.29)	0.51 (0.04-5.97)
Alopecia			
No	26 (65.00)	3/7 (42.86)	1
Yes	14 (35.00)	4/7 (57.14)	2.28 (0.24-21.41)
Lymphadenomegaly			
No	10 (25.00)	0	*
Yes	30 (75.00)	7/7 (100.00)	
Splenomegaly			
No	36 (90.00)	6/7 (85.71)	1
Yes	4 (10.00)	1/7 (14.29)	2.56 (0.11-55.33)
Onychogryphosis			
No	32 (80.00)	5/7 (71.43)	1
Yes	8 (20.00)	2/7 (28.57)	1.26 (0.08-19.04)
Tick			
No	1 (2.50)	0	*
Yes	39 (97.50)	7/7 (100.00)	
Outcome variable: Serology for *T. cruzi*			
Negative	33 (82.50)		
Positive	7 (17.50)		

[#]Frequency of seropositive dogs for *T. cruzi* in relation to independent variables); PR = Prevalence ratios; CI = confidence intervals; * Not included in the non-fitted model due to lack of sufficient data to perform the statistical analysis ($p < 0.20$).

The presence of domestic animals in households may be more attractive for triatomines to feed on than human presence (Daflon--Teixeira et al., 2019; Gürtler et al., 2007). Among the animals raised in the district, the presence of chickens stands out, which are the most accessible blood-meal sources for triatomines (Daflon-Teixeira et al., 2019; Ribeiro et al., 2015). Dogs come in second place, being considered a link in the circulation of *T. cruzi* between the wild and domestic environment (Porfirio et al., 2018). This fact was reinforced in the present study by registering 17% of dogs seropositive for *T. cruzi*.

Dogs generally develop the chronic form of CD, with no apparent clinical signs (Santana et al., 2012) with low parasitemia at this disease stage (Araújo et al., 2002). However, some naturally infected dogs may have detectable parasitemia, depending on the genotype of the parasite circulating in the region and the health status of the animals, as detected by xenodiagnosis, conventional PCR (Enriquez et al., 2013), and qPCR (Enriquez et al., 2014) in other countries (Gürtler and Cardinal, 2015). In this study, dogs infected with *T. cruzi* had a varied clinical picture, compatible with other diseases such as visceral leishmaniasis, making the clinical diagnosis of CD difficult. Only one dog showed seropositivity for *Leishmania* sp. and *T. cruzi*, indicating coinfection. The diagnosis of these diseases in co-endemic regions is a challenge due to the possibility of cross-reaction (Leony et al., 2019; Porfirio et al., 2018), which was discarded in our study because we used two different serological techniques to detect both parasites. In addition, co-infected dogs remain parasitized for long periods, which represents a significant risk in the spread of these zoonoses (Cruz et al., 2020; Porfirio et al., 2018).

Most of the dogs sampled in this study are not used for hunting wild animals and are not fed game meat, even though game meat intake is cultural among people in the region. The two wild animal species most cited as a food source by the interviewees were the rock cavy [*K. rupestre* (87%)] and the armadillo [Dasypodidae (55%)], both being *T. cruzi* reservoirs (Jansen et al., 2020). It is assumed that, during the

Supplementary Table 3
Descriptive and statistical analysis of the structural and environmental characteristics of the 48 households and their association with the rate of triatomine infestation in households in the district of Santo Inácio, Gentio do Ouro, Bahia, Brazil, in the period 2018 to 2019.

Independent variables	n/48 (%)	# Frequency (%)	PR (95% CI)Non-fitted Model	PR (95% CI)Fitted Model
Roof tiles				
Ceramic	45 (93.75)	6/7 (85.71)	1	
Others	3 (6.25)	1/7 (14.29)	29.32 (0.51–1683.04)**	***
Walls				
Masonry with plaster	42 (87.50)	7/7 (100.00)	*	
Masonry without plaster	5 (10.42)	0		
Clay without plaster	1 (2.08)	0		
Construction debris in the peridomicile				
No	19 (39.58)	4/7 (57.14)	1	1
Yes	29 (60.42)	3/7 (42.86)	0.11 (0.01–1.24)**	0.18 (0.02–1.39)
Piles of tiles and bricks in the peridomicile				
No	6 (12.50)	1/7 (14.29)	1	
Yes	42 (87.50)	6/7 (85.71)	1.91 (0.10–33.41)	
Garbage accumulation				
No	26 (54.17)	2/7 (28.57)	1	1
Yes	22 (45.83)	5/7 (71.43)	28.98 (1.21–694.11)**	6.49 (0.81–51.65)
Annexes for domestic animals				
No	38 (79.17)	6/7 (85.71)	*	
Yes	10 (20.83)	1/7 (14.29)		
Presence of dogs				
No	25 (52.08)	2/7 (28.57)	1	1
Yes	23 (47.92)	5/7 (71.43)	4.66 (0.49–44.30) **	4.62 (0.63–33.39)
Presence of cats				
No	41 (85.42)	6/7 (85.71)	1	
Yes	7 (14.58)	1/7 (14.29)	1.55 (0.09–25.14)	
Presence of chickens				
No	37 (77.08)	5/7 (71.43)	1	
Yes	11 (22.92)	2/7 (28.57)	4.58 (0.38–54.21)	
Outcome variable: Households with triatomine capture				
No	41 (85.42)			
Yes	7 (14.58)			

Frequency of triatomine infestation rate in households in relation to independent variables; PR = Prevalence ratios; CI = confidence intervals; * Not included in the non-fitted model due to lack of sufficient data and/or collinear variable with other variables to perform the analysis; ** Criterion used in the non-fitted model was p-value <0.20; *** Removed from the fitted model as it is a confounding variable that modifies the effect of the other variables; The criterion for maintaining the variables in the fitted model was p<0.05.

preparation of the game meat, the remains of these animals are given to the dogs. Two dogs seropositive for *T. cruzi* fed on wild animals, which suggests that oral transmission of the parasite could occur, as reported by Porfirio et al. (2018), who detected 76% of dogs in a rural community in the state of Mato Grosso do Sul infected with *T. cruzi*, 40% of which were fed with wild mammals.

All *T. cruzi* seropositive dogs slept in backyards. However, there was no association between the serological result and the variable "Where does the dog sleep?" (Supplementary Table 2), even knowing that the overnight stay of dogs in the peridomicile increases exposure to triatomines because of the more significant number of vectors in these places (Walter et al., 2005). We recorded an adult triatomine positive for *T. cruzi* living with a seropositive dog in the same peridomicile, and a negative nymph captured in the place where a seronegative dog slept. This scenario shows the severity of CD in this region, where triatomines and dogs share the same space (Fig. 2.B,C).

However, we did not find any PCR-positive dog for *T. cruzi*, which would confirm the infection in tested animals. Some points can be raised in this regard: i) the tested aliquot does not contain parasite DNA (*T. cruzi* is not present in the peripheral circulation of the animal); ii) the fact that the *primer* does not detect some *T. cruzi* strains (Barrera et al., 2008); and iii) the difference in the objectivity of serological tests in relation to molecular testing (antibodies/immunological memory x DNA/infection). The sensitivity of the PCR reaction using TcH2AF/TcH2AR primers was 0.1 ng DNA/μL, which was determined using *T. cruzi* positive control DNA in 10x serial dilutions, ruling out the possibility of negative results due to problems of PCR sensitivity. Regarding possible reaction inhibitors present in the DNA sample, this problem was ruled out by satisfactorily amplifying all negative samples after adding 1.0 μl (50 ng) of parasite DNA (positive control) to them, following the methodology of Gil et al. (2007). The sensitivity of PCR with other *primers* (for example kinetoplast target) in naturally infected dogs ranged between 40-90% (Araújo-Neto et al., 2019; Enriquez et al., 2013; Porfirio et al., 2018). However, the study by Cantillo-Barraza et al. (2020) showed PCR negativity in *T. cruzi* seropositive dogs, even using more sensitive primers than those used here, which shows the importance of using different diagnostic tests to expand the possibilities of reactive and/or detecting the parasite (antibodies and/or DNA). The PCR test has high sensitivity and specificity to detect infected dogs (Araújo-Neto et al., 2019), being used as a complementary diagnosis to serology and allowing the detection of circulating *T. cruzi* lineages (DTUs TcI-TcVI) and other species of *Trypanosoma* (Malavazi et al., 2020; Porfirio et al., 2018).

Diverse studies deal with human seroprevalence of *T. cruzi* in several regions considered endemic for triatomines in northeast Brazil (Aras et al., 2002; Borges-Pereira et al., 2006; Borges-Pereira et al., 2008; Brito et al., 2012; Lima et al., 2012; Santos et al., 2015). In the present study, all people tested negative for *T. cruzi*. According to Cerqueira et al. (1998), previous studies carried out in 1975 and 1995, in this same study area, registered 3% and 4% of seropositivity for *T. cruzi*, respectively. The low prevalence of people infected in these two periods was attributed to better conditions in the infrastructure of households, not being attractive for colonization of triatomines, and preservation of the wild environment (Cerqueira et al., 1998). These conditions were also observed, which may have contributed to the absence of human cases of *T. cruzi* infection in the present study. Furthermore, this seronegativity may be associated with the significant elimination of *Triatoma infestans* in the 1990s through the frequent application of insecticides in households, which reduced the occurrence of new cases of CD in several regions of Brazil (Dias, 2016b).

The species *T. sherlocki* is known and called by the population of Santo Inácio as "bicudo", and it is a vector of the CD protozoan (Almeida et al., 2009), which was identified in the hemipteral insects showcase by 83% of the providers. The correct identification of triatomines by the population allows the notification of these insects to the government agencies responsible for disease control and prevention programs (Dias et al., 2016a). However, there was a lack of information from the population regarding the correct procedure for capturing and disposing of triatomines captured in their households, with most providers immediately killing the insect when they found it and 57% of providers not knowing where to forward them. Similar results have also been reported in other studies (Dias et al., 2016a; Rosecrans et al., 2014).

To avoid triatomines in their households, 77% of providers use some preventive measures. Of these, 50% are insecticide spraying and 42% seek to improve hygiene aspects to avoid an accumulation of garbage and materials (data not shown). These precautions are also the most cited in other studies (Rosecrans et al., 2014; Urioste-Stone et al., 2015). Spraying households with insecticides was suggested by 31% of providers to improve the region's CD control service. However, for this to happen, the resident needs to notify entomological surveillance of the occurrence of triatomines in their household (Dias et al., 2016b), which does not happen in this population as previously reported, even though they have a health post in the district that receives the insects and sends them to the responsible sector.

In the present study, most individuals used a mosquito net attached to the mattress to sleep (Supplementary Table 1). This palliative action protects people from the blood meal of triatomines when they are sleeping. However, 37% of people reported having been bitten by insects, 29% with a sting frequency greater than ten times (data not shown), demonstrating that this occurs when individuals are awake, thus being able to protect themselves from insects. In general, the questionnaire analysis revealed several gaps in respondent knowledge about CD and about the set of practices associated with reducing exposure to triatomines. However, logistic regression analysis showed a protective factor for providers who know that triatomines "cause" CD, as they avoid favorable conditions for invasion and colonization of these insects in their homes (Table 2). Thus, the questionnaire can help to produce educational materials that reinforce aspects still deficient about CD in this population.

The epidemiological scenario revealed is worrying and also occurs in other regions of Brazil (Araújo-Neto et al., 2019; Lima et al., 2012; Sangenis et al., 2015) and in other countries in the Americas (Waleckx et al., 2015), with the aggravation of detecting active cases of *T. cruzi* in humans in the context of invasions by the triatomines *Triatoma vitticeps, Triatoma maculata, Triatoma dimidiata* and *Eratyrus mucronatus* in Brazil (Sangenis et al., 2015), in Colombia (Cantillo -Barraza et al., 2015), in Mexico (Gamboa-León et al. 2014) and in Bolivia (Depickère et al., 2012), respectively. These studies generally show that people have socioeconomic and cultural characteristics that favor the invasion of these insects in their houses (Daflon-Teixeira et al., 2019). In Bolivia, for example, the population of the municipality of Apolo has the habit of building walls with clay to delimit the peridomicile of their dwellings, an ideal hiding place for triatomines (Depickère et al., 2012). While in a certain region of Colombia, the transmission of *T. cruzi* to humans and dogs is related to the occurrence of synanthropic animals such as *Didelphis marsupialis* (Cantillo-Barraza et al., 2015) that finds shelters in the peridomicile of households. Thus, the control of transmission of T. cruzi, in the current scenario, must be analyzed from a holistic perspective, engaging the residents of these regions in the recognition and notification of triatomines (Rosecrans et al., 2014; Yevstigeyeva et al., 2014), as well as the use of prevention and control methods for these insects inside residents households (Urioste-Stone et al., 2015).

5. Conclusion

Based on previous reports and the detection of anti-*T. cruzi* antibodies in dogs and *T. cruzi* infection in specimens of *T. sherlocki* collected in the wild and domestic environment, the presence of the etiological agent of CD in the district of Santo Inácio is verified. Although uncommon in the studied area, the species *T. sordida* and *P. sherlocki* can contribute to the maintenance of the *T. cruzi* transmission cycle. We also report the first record of the infection of *P. sherlocki* by *T. cruzi*. Though limited, the knowledge about CD and the adoption of some preventive measures against the vector practiced by a portion of the population seems to have contributed temporarily to prevent the transmission of *T. cruzi* to humans. However, the data reveal a latent risk of infection and spread of CD in the population studied. The epidemiological scenario observed has already been reported in other regions of Brazil and in other countries with similar characteristics. This situation can only be overcome with frequent actions of entomological surveillance, active search for cases, and the inclusion of health education content in school materials, with further dissemination of these contents to the general population.

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CRediT authorship contribution statement

Flavia Santos: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Supervision, Writing – original draft, Writing – review & editing, Visualization. **Jairo Torres Magalhães-Junior:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Ianei de Oliveira Carneiro:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Fred Luciano Neves Santos:** Methodology, Supervision, Writing – review & editing. **Ângelo Antônio Oliveira Silva:** Methodology, Validation, Investigation. **Joane Maíra Cavalcante Braga Novais:** Methodology, Investigation. **Jessica Samile Sousa Santos:** Methodology, Investigation. **Gilmar Ribeiro-Jr:** Methodology, Supervision, Writing – review & editing. **Mitermayer G. Reis:** Methodology, Supervision, Writing – review & editing. **Carlos Roberto Franke:** Conceptualization, Funding acquisition, Project administration, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary Table 1, Supplementary Table **2**, Supplementary Table 3.

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