Article/Artigo

Study on Chagas disease occurrence in the municipality of Monte Negro, State of Rondônia, Brazilian Amazon

Estudo da ocorrência da doença de Chagas em Monte Negro, Estado de Rondônia, Amazônia Brasileira

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ABSTRACT

Introduction: Studies on Chagas disease deal with the perspective of its occurrence in the Amazon region, which is directly correlated to the population growth and the spread of the bug biotope. The state of Rondônia has an immense source of vectors (Triatomine) and reservoirs of *Trypanosoma cruzi.* Environmental changes brought forth by the deforestation in the region may cause vector behavior changes and bring these vectors to a closer contact with humans, increasing the probability of vector infection. **Methods:** This study was carried out to check the occurrence of Chagas disease in the municipality of Monte Negro, Rondônia, Brazil, based on a random sampling of the farms and people wherein blood collection from the population and capturing triatomines were done. The blood samples were submitted to serologic tests to detect antibodies of the IgG class against *T. cruzi*. The triatomines that were collected had their digestive tract checked for the presence of trypanosomatidae with morphology resembling that of the *T. cruzi.* **Results:** The population examined was mostly from other states. From the 322 bugs examined on the microscope, 50% showed parasites with morphology compatible with *T. cruzi.* From the serology of 344 random samples of human blood, 1.2% was found positive, 6% showed inconclusive results, and 92.8% were negative. **Conclusions:** Monte Negro shows low prevalence of human infection by *T. cruzi* and none active vector transmission; however, preventive and surveying measures, which are not performed until now, shall be taken due to the abundance of vectors infected by trypanosomatidae.

Keywords: Chagas disease. Amazon. *Trypanosoma cruzi*. Triatomines.

RESUMO

Introdução: Estudos anteriores sobre doença de Chagas tratam da perspectiva da ocorrência endêmica da parasitose na região Amazônica, que se correlaciona diretamente com o aumento populacional e a invasão do ecótopo pelos triatomíneos. O Estado de Rondônia possui um imenso manancial de vetores e reservatórios de *Trypanosoma cruzi*. As modificações ambientais ocasionadas pelo desmatamento na região podem gerar mudanças nos hábitos dos vetores, trazendo-os para um contato mais próximo do homem, aumentando as possibilidades de infecção vetorial. **Métodos:** Este estudo foi realizado para verificar a ocorrência da doença de Chagas em Monte Negro, Rondônia, equivalente à amostragem aleatória das propriedades rurais e de pessoas residentes no município, onde foram realizadas coletas sanguíneas dos moradores e coleta de triatomíneos. As amostras sanguíneas foram submetidas a testes sorológicos para detecção de anticorpos da classe IgG contra *T. cruzi* e os triatomíneos coletados foram analisados para verificar a presença de tripanosomatídeos em seu trato digestivo. **Resultados:** A população estudada, randomicamente selecionada, é em sua maioria advinda de outros estados. Dos 322 barbeiros avaliados microscopicamente, 50% apresentavam flagelados com morfologia compatível com *Trypanosoma cruzi.* A sorologia das 344 amostras de sangue humano coletadas randomicamente apresentou 1,2% de positividade para *T. cruzi*, 6,1% de resultados inconclusivos e 92,8% negativos. **Conclusões:** Monte Negro apresenta baixa prevalência de infecção por *T. cruzi* em humanos, em torno de 1,2% e sem transmissão vetorial ativa, porém há a necessidade de medidas preventivas de vigilância, não existentes atualmente, devido à abundância do vetor, e a presença de vetores infectados por tripanosomatídeos.

Palavras-chaves: Doença de Chagas. Amazônia. *Trypanosoma cruzi*. Triatomíneos.

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INTRODUCTION

Chagas disease is endemic in the American continent, and it is more prevalent in South America. In Brazil, this parasitosis has contaminated about 5 million people, while 20 million are at risk of being infected[1-3]. Typically, Chagas disease occurs in people who live in rural areas and in poor quality houses where the vector insects can easily lodge and remain out of sight. The infection in humans may be very severe. It may cause a significant mortality rate among children in its acute phase and seriously compromise the heart and/or the digestive tract in adults with chronic disease[4].

Triatomines are hematophagous insects that transmit *Trypanosoma cruzi* by the time they have their blood meal when they excrete fecal matter and urine containing parasites over the mucosa or in small lesions in the skin or by contaminating food with their feces. Other mechanisms of transmission are vertical, accidental transmission, by blood transfusion, and organ transplantation[5-6]. Most of these insects are sylvatic and are associated with a broad variety of habitats and vertebrate hosts. Some of them may be adapted to peridomestic and domestic habitats[5-6].

The epidemiology of Chagas disease in the Amazon region is still being understood in a superficial and fragmental way. Despite the broad Amazon biodiversity, with the presence of diverse triatomines and many mammalian classes that may host *T. cruzi,* this region has been spared from the disease. Today, some works have considered the disease as emerging around Brazilian Amazon, with some autochthonous cases and some triatomines being reported around the dwellings[2,7-10].

Up to now, there are no ongoing preventive programs and/or specific disease control measures being implemented in Amazon. A recent research carried out in Monte Negro by the investigators of the V Biomedical Science Institute of the University of São Paulo (ICB5/USP) in the past years showed a large occurrence of infected triatomines (on palms) around the rural area of the municipality and an indication of none vector transmission[9] in a small area of the municipality.

METHODS

The study was carried out in the municipality of Monte Negro (S 10º 15'35" e W 63º 18'06") in the State of Rondônia, which has a population of about 14,010 inhabitants, stretches over an area of 1,413.4km^2, and is located 250km away from the state capital, the city of Porto Velho[11]. The weather is equatorial and super humid, with an annual rain precipitation of 2.020mm (1,800-2,200mm), an average temperature of 25.8°C ranging from 12°C to 37°C, and air humidity ranging between 70% to 80% over most part of the year[9,12]. There are considerable anthropogenic activities along the BR421 road, which crosses the municipality and its vicinal districts. The original forest was replaced mainly by coffee plantations and pastures.

In this study, 100 sampling farms were randomly chosen out of the 1,093 existing farms located along the vicinal roads of the municipality (**Figure 1**), and 344 people out of a total of 398 inhabitants in these randomly sampled farms were included. Two visits were done in each farm with the aim of catching bugs inside the dwelling and around them, and dissection of two specimens of *babassus* (*Orbginya speciosa)* for each visit, besides the collection of blood samples from the inhabitants, was carried out. These visits were done during the rainy season and during the dry season to determine the influence of the rain precipitation on the diversity of the triatomine species (**Figure 1**).

Capture of triatomines

With the owners' authorization, domestic and peridomestic inspections were carried out for manually catching the bugs without using any dislodging chemical product. In addition to the manual search, samples of triatomines and collecting kits (gloves, tweezers, and collecting bottle) were given to the general population and to every farm owner to catch and keep any suspicious specimen in the absence of the researchers for future identification.

Four *babassus* samples were dissected at each farm, two for each visit with the aim of finding bugs. The distance of each *babassu* being dissected from the dwelling house was measured by a GPS (Global Positioning System) device.

The existing fauna around the dwellings, peridomestic areas and facilities, and *babassus* was described, along with the information on rain precipitation. The insects captured were placed in collecting bottles, labeled, and taken to the laboratory where they were examined and randomly split into two portions — one to be checked for infection by trypanosomatidae specimens and another to be forwarded to the Biologic Sciences Department of the Pharmaceutical Sciences College of Araraquara for morphological identification of the species based on the key elaborated by Lent & Wygodzinsky[13]. The nymph stage was identified based on the works of Correa et al.[14] and Rosa et al.[15]

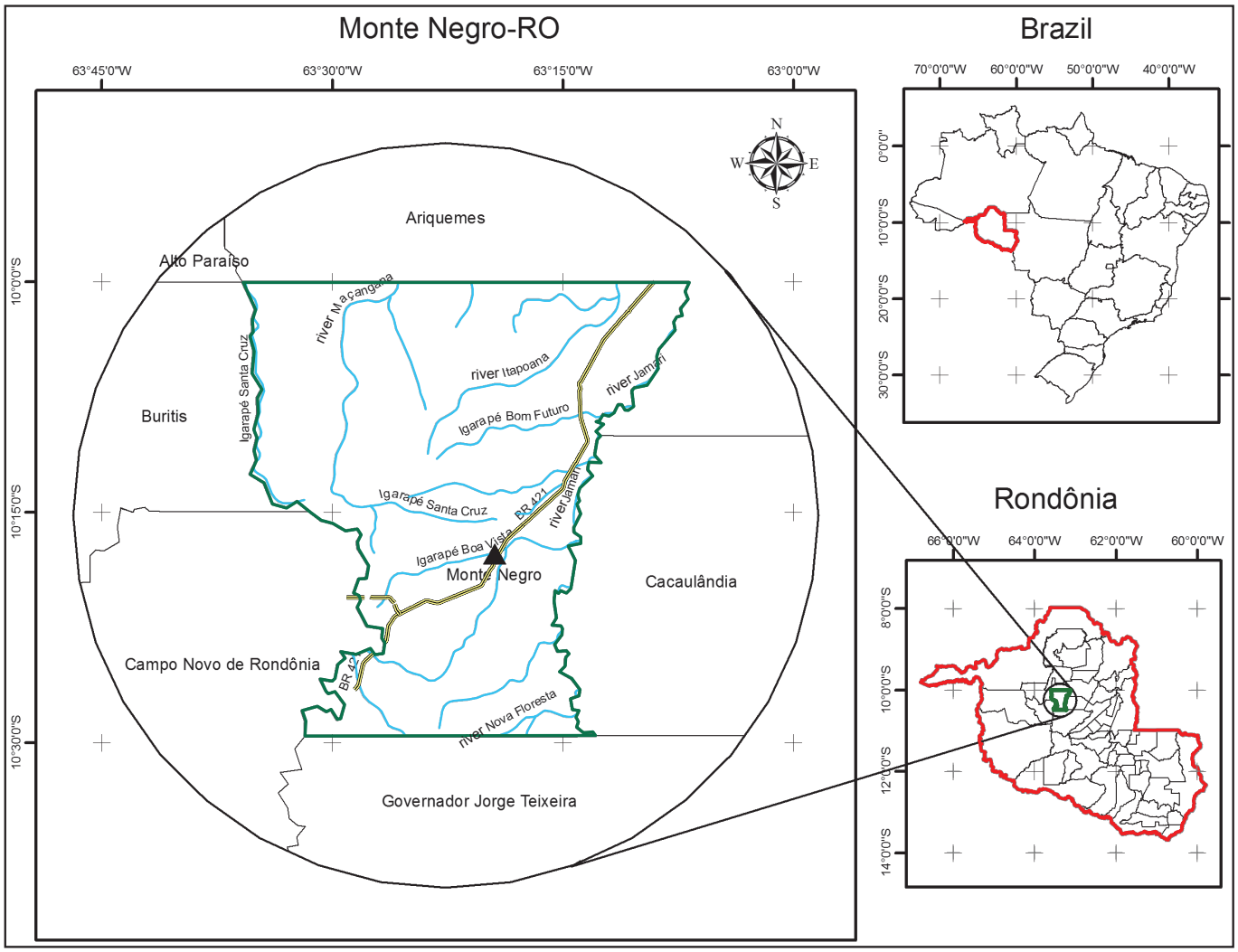

FIGURE 1 - Monte Negro municipality localization, Rondônia, Brazil.

The triatomines selected for infection analysis were anaesthetized with ethyl acetate, rinsed with a 70% ethanol solution, and had their terminal digestive tube dissected, from which the smears were prepared on slides diluted in saline solution. The checkout of the slides for trypanosomatidae presence was carried out by optical microscope examination (400X).

The approach of human beings

After signing the written consent form, the inhabitants of the visited farms were subjected to medical examinations and blood collection where a physician filled out the epidemiologic clinical cards of the patients.

Serologic exams

A 10mL blood sample was collected from each inhabitant, which was then centrifuged for 8min at 2,500rpm. The blood serum was separated into 3 portions of 1mL each and then frozen at -20°C. A portion of each person's sample was forwarded to the laboratory of the Federal University of Goiás for checking of anti-*T. cruzi* antibodies of the IgG class by the ELISA method and the Indirect Immunofluorescence method. For the ELISA test, a Chagatest ELISA Kit (Wiener®) and a PaGIA DiaMed Kit (DiaMed®) were used. For the immunofluorescence, an anti-IgG Human globulin was used, which was conjugated with fluorescein (Biomerieux®). Evans Blue (Bioshop®) and *T. cruzi* microscope slides (Y strain prepared from LIT cultures in house) were used. For ELISA, the cutoff test optical density ratio was considered as negative when it was below 0.9, undetermined when it was between 0.9 and 1.1, and positive for values above 1.1. For the immunofluorescence, the positive values were considered when the titles were above 1/20.

Statistical analysis and data storage

The information found was added to a databank in Microsoft Access 2.0. The statistical analysis of the epidemiologic data was carried out by using Epilnfo 6.04 b and Excel 2007 software. Kolmogorov-Smirnov test with Lilliefors correction, Chi-square test, Fisher's exact test, and Wilcoxon-Mann-Whitney U test were performed. In all cases, a significance level of 5% was considered (α=0.05).

Ethical considerations

This study has been submitted and approved by the Research Ethical Commission on Human Beings of the College Sao Lucas (Letter AP/CEP/173/08) (Originally, *Comitê de Ética em Pesquisa em Seres Humanos da Faculdade São Lucas — Carta AP/CEP/173/08* in Portuguese). The cutting down of palms was authorized by the State Department of Environmental Development (Originally, *Secretaria de Estado do Desenvolvimento Ambiental* — SEDAM), with Special Authorization nr. 195/2007, and by the Renewable Resources — IBAMA (Licence nr. 05/2007, process nr. 02024.001826/2006-47).

RESULTS

Property, dwelling, and population profiles

The properties visited had an average colonization time of 16.3 years, ranging from 2 months to 38 years. The areas of the properties ranged from 33 to 231ha, and the inhabitants were engaged mostly in the cattle farming business. Most of the dwellings had a uniform shape, while the walls were made of wood and bricks, and the roofs were covered by ceramic tiles or cement asbestos roofs. The peridomestic improvements (cattle-shed, granary, hennery, and pigsty) were usually made of wood and covered with cement asbestos roofs or ceramic tiles (95%), with very few exceptions, covered with wood of *babassus* tree feathery leaves.

The population studied was mostly people who were from other states (57%). The average age was 34.2 years, ranging between 2 and 78 years.

When dissecting 393 *babassus*, a wide diversity of animal species was found and observed, and they were sorted into 2 Phyla (*Arthropoda* and *Chordata*), 9 classes (*Insecta, Arachnida, Chilopoda, Diplopoda, Amphibia, Repitilia, Avian, and Mammalia*), and 23 orders, among which bats, rodents, and marsupials were reported.

Triatomine quest

Eight hundred fifty-three triatomines were collected; 5 were found inside the dwellings and 848 on the *babassu* (around 1 triatomine/*babassu*). No bug colony was found inside the dwellings and around their peridomestic area, which indicated that there were no bug lodging occurrences in the region researched.

Most of the triatomine specimens collected in this study were found on *babassus* (*Orbignya speciosa*) (99.4%), and the others were not lodged in the dwellings. All the dissected *babassus* had an average distance from the dwelling of 234.7m. The shortest distance was 76.6m, while the longest was 392.8m.

The relation between the quantity of triatomines collected and the distance the *babassus* were found from the dwelling was confirmed by the Chi-square statistical test, where $\chi 2$=15.3 and p=0.0321, that is, the closer to the dwelling, the higher is the amount of bugs found on each *babassu*. Also noticed was the clear relationship between the presence of mammalians and/or birds found on *babassus* and the presence of collected bugs, as the mammalians and birds are different feeding sources for triatomines and live on the *babassu*. The Fisher's exact test confirmed such a relation, where p=0.0000000000.

By using the Kolmogorov-Smirnov test with Lilliefors correction, it was found that the distribution of a variable quantity of triatomines for the samples collected over the dry and rainy seasons was not regularly distributed, p<0.01. To compare the two populations, the Wilcoxon-Mann-Whitney U test was used. It was found that there was no statistically significant difference between the rainy season and the dry season, U=4,799.5 and p=0.851.

Out of the 853 bugs collected, 652 specimens were selected for the analysis, and the others were discarded. Others were discarded due to the death of the insects, which impaired their morphology.

The triatomines analyzed showed the predominance of specimens in nymph stage (63.2%), and 36.8% were adult insects, out of which 46.6% were male.

The triatomine identification showed a uniform outcome in which all the specimens were identified as *Rhodnius robustus.*

Three hundred twenty-two triatomines were microscopically analyzed for trypanosomatidae specimens with resembling *T. cruzi* morphology in their digestive tract, and 161 (50%) triatomines were found to be positive.

Among all locations studied, the one found with the highest average number of triatomines positive for trypanosomatidae was the LC-40 locality, where 87.5% of the insects analyzed were found positive. There were four locations where no bug was found to be positive in localities LC-0, LC-52, LC-12,5, and TB-24.

Human being quest

The serology carried out at the Federal University of Goiás for the 344 samples collected showed that 1.2% were positive for *T. cruzi,* 6.1% gave inconclusive results, and 92.7% of the samples of serum were negative. All the people found positive were migrants from the States of Paraná, Goiás, and Minas Gerais (endemic states for Chagas disease), were older than 23 years (24, 50, 54, and 57 years old), and had lived in Rondônia for at least 22 years.

DISCUSSION

The studies carried out in the locations considered as endemic such as Jaguarana, in the State of Ceará, and Berilo, in the state of Minas Gerais, showed an average of over 50% of poor quality dwellings built of bricks but with no lath-and-plaster wall[1,16].

Typically, Chagas disease contaminates people who live in the rural areas or used to live in poor quality dwellings where the vector insects are easily lodged and settle colonies[4].

Despite the existence of animals around the peridomestic area, according to the works carried out in other Brazilian places[16-17], no triatomine was reported in peridomestic area. Such information is consistent with the reports of Dias et al.[18] who also found the triatomine specimens lodging in the Amazon region.

Such a correlation was also noticed by Massaro et al.[9] in studies previously carried out in the same region (Monte Negro). The short time that the area has been populated by humans may be the reason why the vector has not yet settled around it.

The *babassus* dissected were mainly found over the pastures (95.6%) and 4.4% in the forest, plantations, and along rivers. The relation between the presence of animals on *babassus* and the presence of triatomines is significant (Fisher´s Exact Test, p=0,0000000000) and, is pursuant to Gurgel-Gonçalves et al.[19] and Massaro et al.[9].

The *babassus* may be considered as the ecological indicators of risk areas of Chagas disease and are useful in indentifying the vectors and hosts present in the sylvan transmitting cycles of *T. cruzi*. Studies carried out in many different regions of Brazil have shown high triatomine infestation on palms, chiefly by bugs of the genus *Rhodnius spp.*[9,19,20].

In their study, also carried out in Monte Negro, Massaro et al.[9] found the existence of the species *R. robustus, R. pictipes, R. prolixus, R. milese*, and *Panstrongylus geniculatus*; the latter being found during soaring and intradomiciliarily (dormitory). It is possible that the existing difference between the results found in this study from the study carried out by Massaro et al.[9] is the non-random characteristic of their samples and because the samples were obtained in a limited area (10 sites) and in the surrounds of urban areas[9].

Fifty percent of the 322 bugs were found positive for trypanosomatidae, *T. cruzi,* or others in the microscopic analysis. This is considerably a high percentage when compared with similar studies in an endemic area in the State of Rio Grande do Sul, where only 4.2% of bugs were found positive for trypanossomtidea[21].

Massaro et al.[9] found 23.7% trypanosomatidae-positive results for *T. cruzi*, and this work was carried out in the same location. According to the work carried out by Martins et al.[22], *R. robustus* shows a considerable susceptibility to some *T. cruzi* strains, and, therefore, it is quite possible that part of the triatomines found positive in this study may be infected by *T. cruzi*.

The work of Massaro et al.[9] showed that 3% of the serum collected was found to be positive by using the ELISA tests and immunofluorescence. No reagent serum (neither inconclusive reactions) was found in children and youths, which suggests that the vector transmission is not occurring in the region. Contrasting this data, in the City of Barcelos, State of Amazonas, eighteen children under 10 years of age were found positive for *T. cruzi*[23]. Two autochthonous cases were found in Rio Negro, Amazon, and both died from chronic Chagas myocardiopathy[24].

Cases of oral and congenital transmission have not been reported in the studied region as described in the States of Pará and Santa Catarina[25], as the population examined is not used to drinking *açaí* and sugarcane juice.

According to the Information System on Aggravating Notices (SINAN), 42 acute Chagas disease cases were reported and confirmed in Rondônia between 2001 and 2006, but the infecting mechanism was not differentiated.

Although such a study has not found any autochthonous case of Chagas disease in Monte Negro and because the State of Rondônia has not been considered endemic for this disease, it is necessary to implement an epidemiological surveillance program (that actually does not exist), while the existing program used for malaria may be also used for Chagas disease surveillance. The higher presence of triatomines near households and in *babassus* with sylvatic animals and the high percentage of infected triatomines with possible *T. cruzi* reinforce this point of view. In this way, it would be possible to monitor the sylvatic triatomine dwelling infestation rates and also the disease transmission risk, mainly by vector and also by oral means.

The studies carried out in 2007 in the municipality of Monte Negro, State of Rondônia, showed results that allow for some conclusions.

Monte Negro, State of Rondônia, shows a low infection prevalence for *T. cruzi* in humans, which is near 1.2%. Up to now, there is no vector or oral transmission, and only few cases were identified as alochthonous and originated from other Brazilian endemic regions.

Altogether, the triatomines collected inside the dwellings and on the *babassus* were of the genus *Rhodnius*.

The invasion of dwellings by the triatomines is not frequent in the region and accounts for only 0.6% of the specimens collected. No triatomine was found in the peridomestic facilities, nor colonizing them.

There is a high rate of triatomines infected by trypanosomatidae in the area examined, and they may have been infected by *T. cruzi* and/or other trypanosomatidae.

Despite the low endemic contamination in the region, the abundance of sylvatic vectors and the growing human occupation suggest a high vector and oral transmission probability.

It is necessary to implement an epidemiological surveillance program along with the ongoing malaria control program implemented by FUNASA by carrying out thick smear analysis, collecting triatomine samples, and launching education campaigns aimed at preventing the Chagas disease transmission in the state.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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