REVISTA DO INSTITUTO DE MEDICINA TROPICAL DE SÃO PAULO

JOURNAL OF THE SÃO PAULO INSTITUTE OF TROPICAL MEDICINE

ORIGINAL ARTICLE

http://doi.org/10.1590/S1678-9946202062074

Triatoma brasiliensis Neiva, 1911 and *Triatoma pseudomaculata* Corrêa and Espínola, 1964 (Hemiptera, Reduviidae, Triatominae) in rural communities in Northeast Brazil

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ABSTRACT

Chagas disease is an important endemic morbidity in Latin America affecting millions of people in the American continent. It is caused by the protozoan *Trypanosoma cruzi*, and transmitted through the feces of the insect vector belonging to the subfamily Triatominae. The present conducted an entomological survey of triatomines and analyzed entomological indicators, such as the rate of infestation, colonization, triatomine density and natural infection in rural communities in the municipality of Campinas do Piaui, Piaui State, in the Northeast region of Brazil. Data on the search of triatomines performed in 167 domiciliary units (DUs), harvested during the period of February to July 2019, in 12 rural communities were analyzed. The capture of triatomines occurred in all studied communities, being 76 the number of positive DUs, of the 167 surveyed, presenting a global rate of infestation of 45.51%. Two triatomines species were collected: *Triatoma brasiliensis* (98.49%) and *T. pseudomaculata* (1.51%), the first was found in the domiciliary and peridomiciliary areas, while the second was captured only in peridomiciliary areas. The index of colonization was 17.1%. Natural infection was observed only in 5.44% of *T. brasiliensis* samples. The entomological survey was conducted in rural communities, showingthe risk of transmission of Chagas disease to the local population, requiring continuous entomological surveillance and vector control.

KEYWORDS: Chagas disease. Surveillance. Epidemiology. Complex *Triatoma brasiliensis*. *Triatoma pseudomaculata*. *Trypanosoma cruzi*.

INTRODUCTION

Chagas disease is endemic in Latin American countries. It is caused by the protozoan *Trypanosoma cruzi* Chagas, 1909 (Kinetoplastida: Trypanosomatidae), it is transmitted mainly through infected feces of the insect vectors belonging to the subfamily Triatominae (Hemiptera: Reduviidae)[1-3]. It is estimated that the disease affects, approximately, from 6 to 8 million people in the world and that 13% of the Latin American population is at risk of infection due to active transmission, with Brazil being among the countries with the highest risk of infection[4]. In Brazil, approximately one million people are infected with *T. cruzi*, the majority coming from rural areas, where the vector of transmission is still active and represents the main form of infection of the disease, being possible to find it in intradomiciliary environments that offer the conditions for the vectors colonization[5].

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Received: 6 July 2020

Accepted: 22 August 2020

Beyond the vector transmission, other forms of infection may represent important mechanisms to the disease dissemination, as is the case of the oral transmission, with a large number of cases in the Northern region of Brazil, due to the consumption of açai and sugarcane juice[5]. In addition, there are other means of transmitting the disease, such as: transfusion, organ transplantation, congenital and laboratory accidents.

Currently, there are 151 existing species t of triatomines and three fossils assigned to five tribes and 19 genera, classified in the subfamily Triatominae; 65 of these species occurs in Brazil[3,6-10]. Approximately 10 of these species have epidemiological importance, because they are often found in the home environment[2,3]. With the elimination of disease transmission by *Triatoma infestans* (Klug, 1834) in Brazil, other species, previously considered of minor epidemiological importance, but with sufficient capacity to maintain the disease cycle, took on a more active role in the transmission of the parasite. Even with the eradication of the main species, the Northeast region of Brazil hosts a large number of secondary species with high potential for transmitting the etiological agent[11], such as *Triatoma brasiliensis* Neiva, 1911, *Triatoma sordida* (Stål, 1859), *Triatoma pseudomaculata* Corrêa & Espínola, 1964 and *Panstrongylus megistus* (Burmeister, 1835). *Triatoma brasiliensis* is the main vector of Chagas disease in Northeastern Brazil, due to their habitat being held frequently in the home environment[12].

The Piaui State is endemic for Chagas disease, with the finding of 1.9% of seropositivity in a study conducted in 2002 in rural areas across the State. The highest rates of infection occurred in the municipalities of Cajazeiras do Piaui (10.8%), Capitao Gervasio de Oliveira (11.5%), Campinas do Piaui (11.5%) and Sao Joao do Piaui (11.6%), municipalities located in the Southeastern region of the state[13]. The entomological data recorded for this region found a large amount of vectors in the home environment.

According to an entomological survey carried out in Piaui State, 11 species of triatomines have been registered. The most representative species in the peridomiciliary and intradomiciliary environments is *T. brasiliensis* which is often found infected by *T. cruzi*[11].

Due to the absence of immunization strategies to protect populations at risk of infection and the efficiency of existing drugs for the etiological treatment of Chagas disease that has been proven, only, in cases of recent infection, in addition to the large number of reservoir animals, making it impossible to exhaust sources of infection; it is necessary to carry out specific educational actions aimed at home hygiene and permanent entomological surveillance, through combating the domiciled vector, installations of Triatomine Information Stations (PIT), housing improvement and spraying houses in endemic areas[14].

In the present study, we carried out an entomological survey of vectors of Chagas disease in rural communities in the city of Campinas do Piaui, Piaui State, as well as an evaluation of entomological indicators, such as infestation index, colonization rate, triatomine density and natural infection index.

MATERIALS AND METHODS

Field of study

The research was carried out in twelve communities in the municipality of Campinas do Piaui, Piaui State, during the period corresponding of February to July 2019. The municipality is located in the Southeastern region of Piaui State, 368.1 km from the capital Teresina and has 5.603 inhabitants, more than half of whom are residents of rural areas[15]. It is a region of warm, semi-arid tropical climate, with Caatinga trees and shrub vegetation, with the Tranqueira and Caninde rivers as the sources of water[16].

The selection of the rural communities was carried out by the municipal supervision of the Chagas Disease Control Program (PCDCH) in Campinas do Piaui, Piaui State, according to established criteria and epidemiological priority for carrying out the previously scheduled activities.

Collection of triatomines

The visits to Domiciliary Units (DUs), in the locations chosen for the research, were accompanied by community health agents and combat endemic disease agents. The search for triatomines took place after authorization of a person responsible for the residence and in their presence. The search in intradomiciliary and peridomiciliary areas took place in an exhaustive way; every single room was thoroughly searched, including places like behind pictures, cracks on the walls, under mattresses, or heaps of clothes, as well as in the annexes, such as heaps of tiles, chicken coops, pigsties, fences and food stores. All of the houses in the surveyed locations were georeferenced and classified as "searched" or "not searched" (if the person responsible did not authorize the search for the triatomine or the house was closed at the time of the visit).

Bugs were collected by manual searches using forceps and lantern, in peridomiciliary and intradomiciliary areas, during daytime. All of the specimens found in the DUs were captured and were packed in plastic tubes lined with filter paper and paper cutouts folded in a "Z" shape to increase the contact surface of the insects. All of the packages were labeled and identified.

Data analysis

The specimens collected at each point were organized according to the instar of development, sex, species and capture ecotope. All of the data were tabulated in spreadsheets. The identification of triatomine species was performed at the Research Laboratory in Parasitic Biology (LaBioPar), located in the Department of Parasitology and Microbiology/CCS of Universidade Federal do Piaui (UFPI), according to the dichotomous key described by Dale *et al*.[17]

The entomological indicators adopted were the family infestation index, the colonization index, the triatomine density and the natural infection by *T. cruzi*-related flagellates. Infestation is defined as the presence of any specimen of triatomine, detected by entomological search. It may be referred to any site or ecotope in relation to the total number of units investigated or covered by surveillance. Infestation rates related to DU, intradomicile (ID) or peridomicile (PD) are more commonly used. The Domiciliary Units is defined as the set consisting of human housing, its attachments and the space next to the house. The intradomicile corresponds to the dwelling, not only the internal space, but also the external walls; the peridomicile is defined as the external space, close to the house, which includes attachments and any other possible shelters for triatomines[18].

The infection rate of triatomines for trypanosomatid forms, found in their feces, was performed only for adult specimens and nymphs of the fourth and fifth instar. The parasitological technique applied was the abdominal compression of the insect to collect fecal material in a drop of saline solution (NaCl 0.9%), on a slide and examined under an optical microscope at 400 x magnification[19].

For entomological indicators, the recommended guidelines from the Pan American Health Organization were used[20]. To calculate entomological indicators, the following formulas were adopted:

$$\text{Infestation Index (INFEST\%)} = \frac{\text{number of DU positive}}{\text{number of DU surveyed}} \times 100$$

$$\text{Colonization Index (COLON\%)} = \frac{\text{number of DU with triatomine ninphs}}{\text{number of DU with triatomine}} \times 100$$

$$\text{Triatomine density per DU (TD)} = \frac{\text{number of captured triatomine}}{\text{number of DU surveyed}} \times 100$$

$$\text{Natural Infection Index (NI\%)} = \frac{\text{number of triatomine infected with } \textit{T. cruzi}}{\text{number of triatomine examined}} \times 100$$

RESULTS

Between February and July 2019, 12 rural communities in the municipality of Campinas do Piaui, Piaui State, were visited, among them Aroeiras, Carnaibas, Castelo, Chapadinha, Joaquim Pequeno, Moco de Cima, Olho D'Agua das Ovelhas, Olho D'Agua dos Bois, Peixe, Retiro Velho, Vaca Brava and Volta do Campo Grande. Altogether, 167 DUs were investigated (Figure 1).

A total of 1,063 specimens were collected, 1,047 (98.49%) identified as *T. brasiliensis* and 16 (1.51%) as *T. pseudomaculata*. *Triatoma brasiliensis* was found in the 12 communities surveyed, while *T. pseudomaculata* was collected in the communities of Aroeiras, Chapadinha,

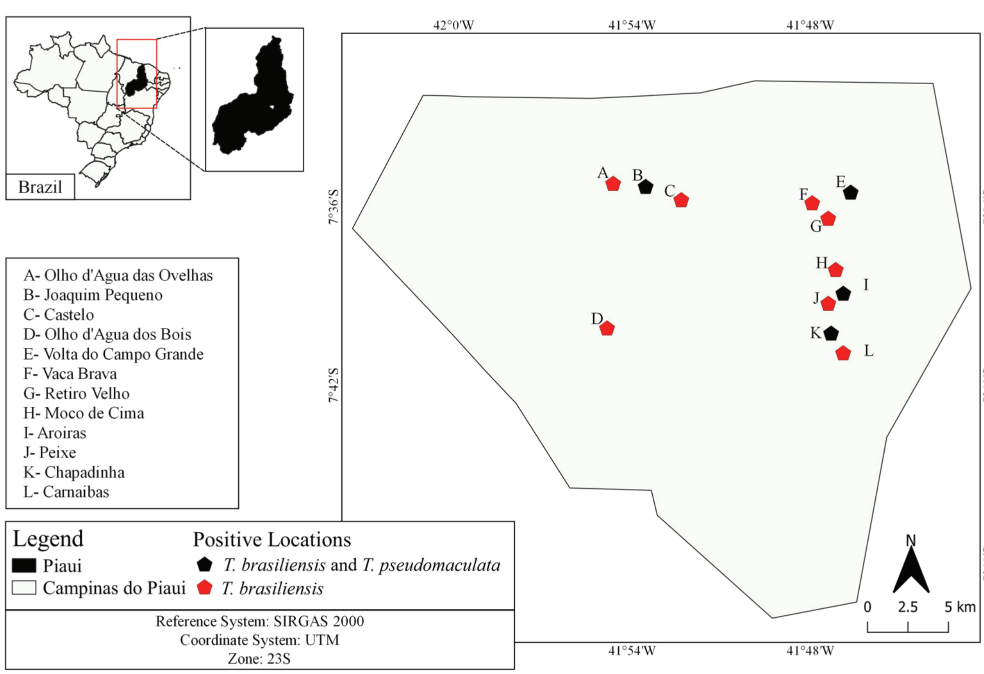

Figure 1 - Map of the municipality of Campinas do Piaui, Piaui State, showing the geographical location of the studied areas.

Joaquim Pequeno and Volta do Campo Grande. Out of the 167 DUs surveyed, triatomines were collected in 76, and in 13, the presence of vectors in the intradomicile was confirmed (Figure 2).

Out of the total specimens collected in the rural communities of Campinas do Piaui, 917 came from the peridomicile and 146 from the intradomicile (Table 1).

In three DUs, two in Castelo and one in Aroeiras, triatomines were found in both, inside and around the intradomicile.

In relation to the surveyed peridomestic environments, a larger number of specimens were found in chicken coops and pile of tiles. Other ecotopes surveyed with the presence of triatomines were: pig pens, cattle pens, debris, piles of bricks and piles of wood (Table 2).

In relation to the total number of triatomines collected, a global index of infestation of 45.51% was observed, with the locations Olho D'Agua dos Bois, Castelo, Carnaibas and Moco de Cima having the highest indexes. The observed general triatomine density was 6.36, with Olho D'Agua dos Bois, Vaca Brava and Carnaibas having the highest indexes. Out of the 13 positive DUs for the presence of *T. brasiliensis* in the intradomicile, 11 had nymphs inside, representing a general colonization rate of 17.1% (11/76). For the species *T. pseudomaculata*, all of the captured specimens were collected in the peridomicile (Table 3).

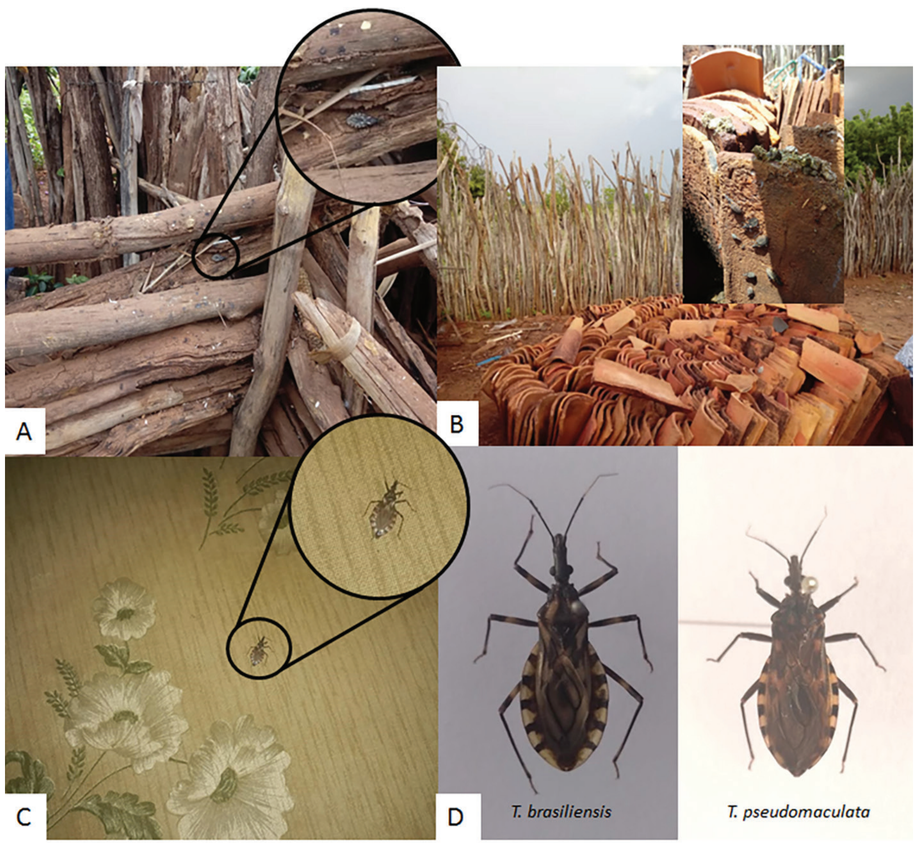

Figure 2 - (A and B) Peridomiciliary ecotope (A = Chicken coops; B = Pile of tiles); (C) Intradomiciliary ecotope (under the bed); (D) Collected species (*T. brasiliensis* and *T. pseudomaculata*).

Table 1 - Triatomine species collected in relation to the capture ecotopes.

Species	Ecotopes			
	Peridomicile		Intradomicile	
	N (%)	DU +	N (%)	DU +
Triatoma brasiliensis	901 (86)	66	146 (14)	13
Triatoma pseudomaculata	16 (100)	5	-----	-----
Total	917 (86.26)	66	146 (13.74)	13

N = number of specimens; DU+ = positive domiciliary units.

Table 2 - Peridomestic ecotopes investigated during research in rural areas of the city of Campinas do Piaui, Piaui State.

Type of annex	Ecotopes	
	Nº	%
Pile of tiles	20	27.03
Chicken coops	19	25.68
Other type of annex	12	16.22
Stones (house)	7	9.46
Pigsty	5	6.76
Rubble	4	5.40
Pile of Woods	3	4.05
Pile of bricks	3	4.05
Bovine corral	1	1.35
Total	74	100

In the analysis of natural infection (NI) by trypanosomatid forms similar to *T. cruzi*, only adult individuals and nymphs of the fourth and fifth instar were evaluated. The parasitological research was carried out in 211 specimens and presented an IN of 5.44%, making it possible to observe *T. cruzi* only in specimens of *T. brasiliensis* (Table 4).

DISCUSSION

Despite the elimination of Chagas disease transmission by *T. infestans* in Brazil, Piaui State still has a wide distribution of triatomines, remaining a risk area for vector transmission, a condition evidenced by autochthonous and potentially vector species, especially *T. brasiliensis*, found colonizing households in rural communities, as shown by the results of this study.

The high number of *T. brasiliensis* captured in this work are in accordance with the study by Costa *et al.*[12] in which, among all the States in the Northeast region, Piaui had the highest index of home infestation by this species. Gurgel-Gonçalves *et al.*[11] performed collections in Piaui State and, as in this work, *T. brasiliensis* (65%) was the most captured species, followed by *T. pseudomaculata* (28%). These are the species most widely distributed in the State and with the highest colonization index. Similar results to those presented here were reported by Coutinho *et al*.[21], in a similar study developed in Russas (CE), which found a predominance of *T. brasiliensis* (77.1%) followed by *T. pseudomaculata* (19.8%) among the collected species.

As in the study by Gurgel-Gonçalves *et al.*[11], our results points to *T. brasiliensis* that is still the most captured species in the home environment, and the second most captured species remaining *T. pseudomaculata*.

The two species occupy a variety of domestic, peridomestic and wild ecotopes, with *T. brasiliensis* frequently associated with small rodents found in rocky outcrops and *T. pseudomaculata* most commonly associated with bird nests and hollow trees[22].

In the present study, *T. brasiliensis* and *T. pseudomaculata* were found cohabiting different artificial peridomestic structures (chicken coop, bovine corral and piles of tiles).

Table 3 - Investigated and positive DUs, infestation indexes, colonization index and triatomine density by studied locations in the 12 rural communities of Campinas do Piaui, Piaui State.

Communities	DUs		ECOTOPES		COLON %	TD
	RES/POS	INFEST %	Peri	Intra		
Aroeiras	13/6	46.15	146	1	16.7	11.31
Carnaibas	5/4	80	69	32	33.3	20.2
Castelo	10/9	90	74	15	33.3	8.9
Chapadinha	25/6	24	25	1*	-	1.04
Joaquim Pequeno	49/21	42.85	191	67	9.5	5.26
Moco de Cima	10/8	80	66	18	37.5	8.4
Olho D'Agua das Ovelhas	10/1	10	-	2	100	0.2
Olho D'Agua dos Bois	5/5	100	143	-	-	28.6
Peixe	3/2	66.67	2	10	50	4
Retiro Velho	7/3	42.85	7	-	-	1
Vaca Brava	4/3	75	97	-	-	24.25
Volta do Campo Grande	26/8	30.77	97	-	-	3.73
TOTAL	167/76	45.51	917	146	17.1	6.36

DUs = domiciliary units; RES = researched home units; POS = positive home units; INFEST % = infestation index; COLON % = colonization index; TD = triatomine density); *presence of adults.

Table 4 - Natural Infection Index by trypanosomatid forms similar to *T. cruzi* in captured species.

Species	Examined	Infected	NI%
Triatoma brasiliensis	202	11	5.44
Triatoma pseudomaculata	9	0	0

This phenomenon can be justified by the juxtaposition of natural and artificial ecotopes, which constitute a unique environment in which species benefit from a good food supply, provided by domestic animals, mainly chickens, and still live in their natural substrates[23].

On the other hand, the lower occupation of triatomines in the home environment should not be ignored and serves as an indication of the need of routine surveillance in homes in locations with high amounts of triatomines in the peridomicile[24]. Thus, analysis of the invasive capacity of vector species is of great importance to assess and monitor the domiciliation process and also to direct control measures against Chagas disease vectors[12].

In accordance with the statement by Oliveira-Lima *et al.*[25] that triatomines have a significantly higher prevalence in animal shelters and debris, in the present study the results showed chicken coops (25.68%) and pile of tiles (27.03%) as the most infested annexes in the surveyed communities. Still, in this same study, the highest incidence of triatomines was observed in piles of wood, tiles and bricks (46%), followed by animal shelters (28%)[25].

In this study, the home infestation indicator (ID) showed that all communities had high indexes, ranging from 30.77 to 100%, and the home colonization index (COLON %) from 9.5 to 100%, with an average index of colonization of 17.1%. These indicators evaluated in the rural communities studied in Campinas do Piaui are inferior to the results of Costa *et al.*[12] for Piaui State. In this study, the home infestation indicator was equal to 125.7% and the colonization index was equal to 46.3%, for 626,290 DUs investigated in 71 municipalities in the State, between 1993 and 1999.

The relationship between infestation and home colonization rates showed that the 12 communities are affected by adult triatomines and, with the exception of Chapadinha, Olho D'Agua dos Bois, Retiro Velho, Vaca Brava and Volta do Campo Grande communities, the others are also affected by triatomine nymphs. Although the communities had a general triatomine density index of 6.36, the results indicated a high prevalence of vectors in the studied areas, when compared to the general results of the studies by Barreto *et al*.[26], in the Western mesoregion of Rio Grande do Norte State, where they presented a general density household triatomine of 0.11, a much lower value compared to the general result of the present study.

The prevalence of nymphs among the captured specimens characterizes the adaptation of triatomines to the artificial ecotope, consolidating the domiciliation process. Usually, the finding of insects in the DUs is associated with the lack of hygiene, indoor disorder and the presence of animals inside the dwellings, which are conditions that favor the persistence of triatomines in rural areas[27].

The general analysis of the results showed that the correct interpretation of the data through entomological indicators helps to determine the risk of home transmission of *T. cruzi*[12]. According to Soares *et al.*[28], *T. brasiliensis* and *T. pseudomaculata* are the most important vectors of *T. cruzi* in the arid Caatinga region of Northeastern Brazil. Epidemiological studies indicate that *T. pseudomaculata* is less efficient than *T. brasiliensis* as a vector for *T. cruzi*, however, it is still one of the pertinent concerns in relation to the vector transmission of Chagas disease, considering the degree to which vector species potentially colonize new areas and are, thus, capable of expanding risk areas of transmission[29].

The analysis of natural infection (NI) by parasitological research showed an IN of 5.44%, a very high value when compared to the general average of natural infection by *T. cruzi*-like flagellates found by Gurgel-Gonçalves *et al.*[11] for Piaui State (0.8%).

The absence of natural infection in *T. pseudomaculata* can be explained by its frequent feeding in birds and, probably, by eliminating few parasites in the feces, in addition, the lower number of individuals of this species that were captured must be considered[30,31].

Xavier *et al.*[32], described that the human impact on the local environment can lead to an increase in the rate of infection by *T. cruzi* in certain species of mammals, reinforcing the need to obtain detailed information on specific local conditions to effectively evaluate the potentials risk factors for the occurrence of the disease.

The entomological and reservoir investigation process must be associated with sustained environmental surveillance actions[33], making it necessary to understand the habitat selection processes by triatomines for the construction of epidemiological evidence, aiming at planning and developing the local epidemiological surveillance systems and control[14,33,34], so that the improvement of the estimates of eco-epidemiological indicators may significantly reinforce the strategies of integrated surveillance and vector control[34-36].

The results found in the 12 rural communities of Campinas do Piaui, Piaui State, showed the colonization of domestic environments by *T. brasiliensis* and *T. pseudomaculata*, both species with great epidemiological importance in Southeastern Piaui regarding the vector transmission of

Chagas disease, due to the high indices of infestation, colonization and density of triatomines in households, aside from the presence of vectors infected with trypanosomatid forms similar to *T. cruzi*, modifying the risk of transmission of Chagas disease to residents of the region.

ACKNOWLEDGMENTS

To the Health Department of the Campinas do Piaui city for making available community health agents and combat endemic diseases agents.

AUTHORS' CONTRIBUTIONS

ALSF, MAS, JHFC, LLJS, VJM: performed the collection of material; ALSF, MAS, LVBS, DPM: performed the experiments; ALSF, MAS, LVBS, DPM: analyzed the data; ALSF, VJM: wrote the paper.

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