Seroepidemiological Investigation for Chagas Disease in Two Municipalities of Goiás, Brazil

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Abstract— *This study evaluated the risk of transmission of Chagas disease following implantation of the Brazilian National Health System (Sistema Único de Saúde – SUS) in two municipalities considered endemic risk areas. A seroepidemiological and entomological survey was conducted as part of the Triatoma infestans eradication program. According to a previous survey conducted in 1980, seroprevalence of Chagas disease was around 0.82% in São Luís dos Montes Belos and around 2.35% in Novo Brasil. In the present serological survey conducted in 303 schoolchildren born after the control phase in these regions, one of the children tested positive for the disease. In the 236 homes surveyed (150 in São Luís dos Montes Belos and 86 in Novo Brasil), all of which were infested by triatomine bugs, none of the triatomine bugs captured tested positive for Trypanosoma cruzi. Although Triatoma infestans is under control, there has been a considerable increase in secondary vectors such as Triatoma sordida; therefore, continuous epidemiological surveillance is fully justified within the current context of the SUS, and should be considered for inclusion as routine practice within the Family Health Program.*

Keywords— Chagas disease, serology, epidemiology, triatomine bugs, surveillance.

I. INTRODUCTION

As part of a Chagas disease control program, the Brazilian Ministry of Health conducted a serological and triatomine survey throughout most of the country between 1975 and 1983. Data on the prevalence of the infection and on the distribution of its vectors were obtained. The areas in which the disease was endemic were identified, allowing control actions to be defined and prioritized [1, 2].

Chagas disease is an endemic disease caused by the protozoan parasite *Trypanosoma cruzi*, transmitted to humans and other animals by triatomine bugs. The condition is characterized by a chronic generalized infection. Chagas disease is found throughout the entire American continent from the southern United States to the south of Argentina [3].

Domestic transmission of the disease depends on the following factors: the vector must be present, it must be infected and it must have colonized human households. This situation results primarily from circumstances related to the environment, to man's effect on the environment and to the attributes of the vector itself, all of which will end up favoring the presence of the vectors in households [4].

American trypanosomiasis, or Chagas disease, is the most prevalent zoonosis in Goiás, a Brazilian state that is considered a region at risk of vector transmission. Natural transmission has always been associated with close contact between humans and triatomine bugs, with rural populations being those most affected [5, 6].

Triatoma infestans was the principal household vector of Chagas disease in Brazil between 1975 and 1980 and it is now believed to be eradicated from the country. In 2006, the Pan American Health Organization/World Health Organization certified that, as a result of the Southern Cone Initiative against Chagas Disease, transmission by the principal household vector, *Triatoma infestans*, was halted [7].

From an epidemiological viewpoint, the state of Goiás ranked third with respect to the prevalence of Chagas disease in a nationwide survey conducted in 1980. In the municipality of São Luís dos Montes Belos, seroprevalence was around 0.82%, while in the municipality of Novo Brasil, the rate was around 2.35%. In Novo Brasil, a serological survey was conducted in children up to 10 years of age in 1980; however, no such survey was conducted in São Luis dos Montes Belos. At that time,

the most prevalent species of triatomine bug were *Triatoma sordida*, *Rhodnius neglectus*, *Panstrongylus diasi*, *Panstrongylus megistus* and *Triatoma pseudomaculata*. [1].

Oliveira et al. reported that of 32,437 triatomine bugs examined in Goiás between 2000 and 2003, 276 (0.85%) tested positive for *Trypanosoma cruzi*. Of these 276 triatomine bugs, 234 (84.78%) were of the *T. sordida* species (50 intra- and 184 peridomestic) and 21 (7.61%) were of the *Rhodnius neglectus* species (18 intra- and 3 peridomestic) [4]. A triatomine survey conducted in April 2013 found six specimens of *T. sordida* infected by trypanosomatids in the town of Trombas (in northeastern Goiás), corresponding to a rate of infection of 0.6%. These trypanosomatids were morphologically similar to *T. cruzi*. [8].

Based on these previous serological and entomological data recorded by the regional offices of the National Health Foundation, this study aimed at evaluating the seroepidemiological and entomological patterns in towns considered areas of risk, although located within areas in which the transmission of Chagas disease has been controlled. The results of this study will be helpful when making decisions on the implementation of low-cost, effective and viable control measures, as well as the establishment of continuous entomological and epidemiological surveillance.

II. MATERIAL AND METHOD

The present methodology complied with the technical guidelines of the Ministry of Health's nationwide Chagas disease control program [3] and with the routine fieldwork conducted by the National Health Foundation. All municipal rural schools in the selected towns were included in the study. Consent for the children to participate in the survey was obtained from their parents at meetings held at the schools.

2.1 Eligibility criteria with respect to the towns and to the study sample

Based on seroepidemiological and entomological parameters established by the official government agency between 1995 and 1999, for logistical reasons (proximity, the support provided by the municipal councils, the active presence of the Family Health Program in the town), and by consensus, two out of a total of twenty towns were selected for inclusion in the present study: São Luís dos Montes Belos and Novo Brasil. (Fig. 1).

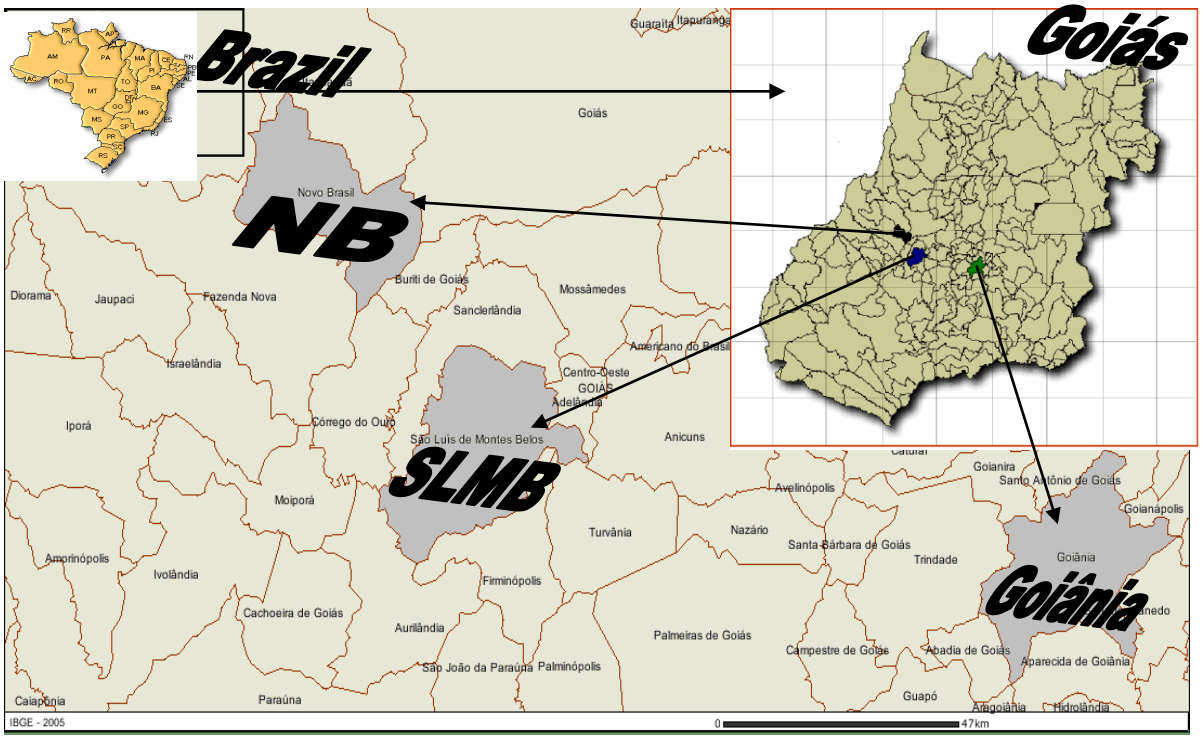

FIGURE 1 – LOCATION OF THE MUNICIPALITIES INVESTIGATED IN RELATION TO THE STATE CAPITAL, GOIÂNIA.

NB: Novo Brasil; SLMB: São Luís dos Montes Belos.

TABLE 1
LOCALITIES INVESTIGATED AND THOSE INFESTED BY TRIATOMINE BUGS: NOVO BRASIL AND SÃO LUÍS DOS MONTES BELOS, 2003-2005

	2003	2004	2005
Municipality	**Novo Brasil**		
Localities investigated	82 / 86	21/86	11/ 86
Percentage of localities investigated	95.35%	24.42%	12.79%
Localities infested	21/ 82	11/21	09/12
% of localities infested	25.61%	52.38%	75.00%
Municipality	**São Luís dos Montes Belos**		
Localities investigated	50/69	50/69	36/69
Percentage of localities investigated	72.46%	72.46%	52.17%
Localities infested	69/93	69/81	69/97
% of localities infested	74.19%	85.19%	71.13%

TABLE 2
LOCALITIES INVESTIGATED AND LOCALITIES INFESTED BY TRIATOMINE BUGS: NOVO BRASIL AND SÃO LUÍS DOS BELOS MONTES, 2003-2005.

Novo Brasil							
Year	**Domestic infestation**		**Peridomestic infestation**		**Households investigated**	**Households infested**	**Households infested (%)**
	T. sordida	*R. neglectus*	*T. sordida*	*R. neglectus*			
2003	2	0	0	0	86	02	02.33%
2004	6	13	10	5	86	11	12.79%
2005	1	10	0	3	86	9	10.46%
São Luís dos Montes Belos							
Year	**Domestic infestation**		**Peridomestic infestation**		**Households investigated**	**Households infested**	**Households infested (%)**
	T. sordida	*R. neglectus*	*T. sordida*	*R. neglectus*			
2003	41	0	346	22	91	58	63.74%
2004	45	1	677	8	91	77	84.62%
2005	32	6	594	3	91	54	59.34%

2.2 Description of the study area

The town of São Luís dos Montes Belos (latitude 16.57868°, longitude 50.31041°) is located at an altitude of 320 feet above sea level, 150 kilometers east of the state capital, Goiânia (latitude 16.7267°, longitude 49.25481°, altitude 2,780 feet above sea level). The town is within an endemic risk area. According to data supplied by the municipal council, the principal economic activities consist of agriculture, animal farming, and commercial activities.

São Luís dos Montes Belos had a population of 26,383 inhabitants in 2005, distributed over 114 localities and consisting of 2,513 household units. *T. infestans* was not detected during previous study years, with a prevalence of Chagas disease of 0.82% being found in a serological survey conducted in 1980. However, children under ten years of age were not included in that serological evaluation [1].

Novo Brasil, also considered an endemic risk area, is situated at 320 feet above sea level, 199 kilometers to the east of the capital city, Goiânia. According to the city council, its principal economic activities are related to agriculture, animal farming and commerce. In 2005, its population consisted of 4,086 inhabitants distributed over 82 localities and 1,515 households. *T. infestans* was not detected during the previous study years; however, the secondary vector rate was high, reaching 33.33% in 1998 [4]. In Novo Brasil, 30.8% of the population lives in rural areas (1,153 inhabitants or 395 families) [10].

2.3 Study sites

It was decided to focus on rural areas in the municipalities due to the higher domestic infestation by secondary vector species there. The municipal schools were strategic in the project, since schools serve as a link between the community, children, parents, educators, healthcare professionals (the National Health Foundation and the Family Health Program) and local community leaders.

Two reference laboratories conducted the study tests: a) the *Fundação Nacional Ezequiel Dias* of the Minas Gerais State Health Department (FUNED), a national reference laboratory for Chagas disease situated in Belo Horizonte, Minas Gerais screened filter paper blood samples from schoolchildren under 10 years of age; while b) the Chagas Laboratory of the Federal University of Goiás Teaching Hospital in Goiânia, Goiás tested the full blood samples collected in tubes. These full blood samples were obtained from a random sample of the same schoolchildren under 10 years of age and were used as quality control.

2.4 Study sample

The sample consisted of 303 children ≤ 10 years of age enrolled at municipal schools in rural or urban areas (Fig. 2).

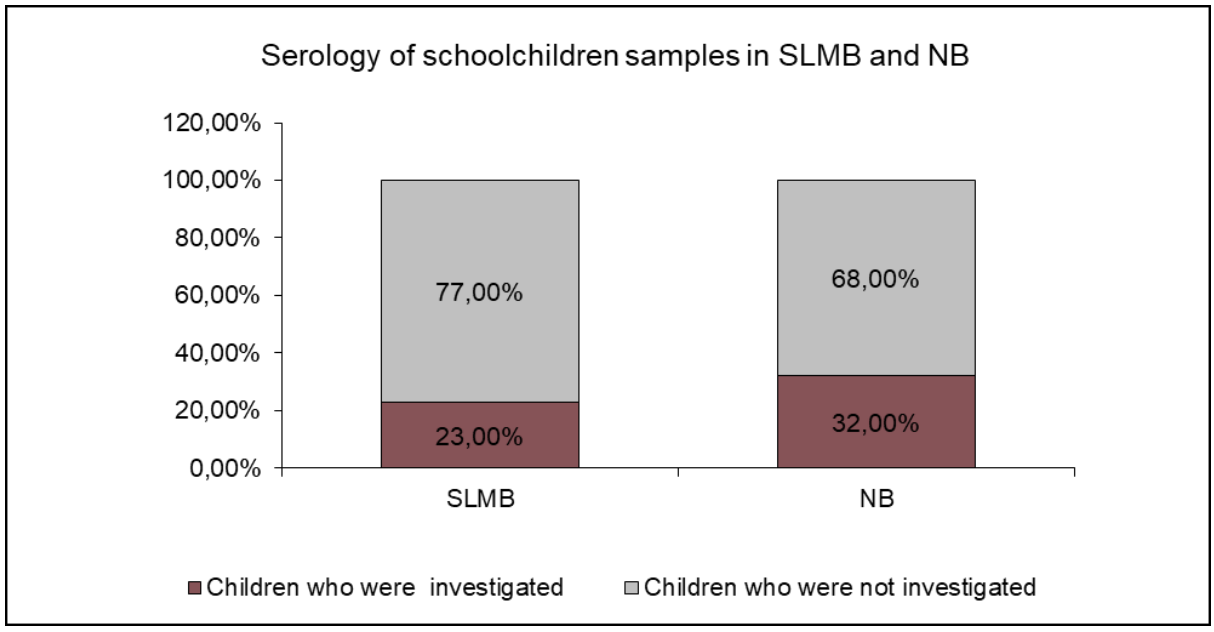

FIGURE 2: SEROLOGY PERFORMED IN THE SAMPLE OF SCHOOLCHILDREN EVALUATED IN THE MUNICIPALITIES.
SLMB: São Luís dos Montes Belos; NB: Novo Brasil.

In São Luís dos Montes Belos, 175 blood samples were collected (140 on filter paper and 35 in tubes), representing 23% of the children ≤ 10 years of age living in rural areas and attending school regularly. These children were living in 69 of the rural household units included in the study.

In Novo Brasil, 128 blood samples were collected (103 on filter paper and 25 in tubes), representing 32.41% of the children ≤ 10 years of age living in rural areas and also attending school regularly. These children were living in 82 of the rural household units evaluated.

2.5 Instruments used in the study

2.5.1 Mapping and description of the study area

The townships of São Luís dos Montes Belos and Novo Brasil are located east of the state capital, Goiânia, and are endemic risk areas. According to the respective council authorities, the principal economic activities of the region are related to agriculture, animal farming and commerce.

In São Luís dos Montes Belos, 15.9% of the population (4,109 inhabitants or 1,182 families) lives in a rural area compared to 30.8% in Novo Brasil (1,153 inhabitants or 395 families).

2.6 Techniques and Procedures

After each child's parent or legal guardian had provided authorization for his/her participation in the study by signing the informed consent form, the following procedures were carried out.

A trained clerk from the National Health Foundation captured triatomine bugs manually for entomological evaluation. A minimal surveillance unit was established and the study forms were completed. In addition, triatomine bugs detected by chance by the inhabitants were collected in a plastic bag attached to a minimal surveillance unit consisting of an illustrated detector calendar and a collection device. These were then duly identified with the investigator's code, the micro-area to which the household belonged, the family, date, and the name and address of the inhabitant, and sent to the triatomine bug data collection point. The data were recorded (triatomine bug notification record) and the material was then sent to the state entomologic center or to the National Health Foundation for detailed evaluation. All the households infested with triatomine bugs were then scheduled to be sprayed by personnel from the municipal health department. Overall, 150 minimal surveillance units were established in São Luís dos Montes Belos and 86 in Novo Brasil, making a total of 236 household units (Fig. 3).

FIGURE 3: MINIMAL SURVEILLANCE UNIT. THE PICTURE SHOWS A TRIATOMINE BUG CAPTURED USING THE INSTRUCTIONS PROVIDED IN THE MINIMAL SURVEILLANCE UNIT. GOIÁS, 2003-2006.

Samples were progressively collected from the target population (children under 10 years of age born after the massive attack phase involving insecticides and individuals with symptoms compatible with those of Chagas disease). In the first phase, conducted with samples collected on filter paper (Watman #1) for use with the techniques with greater sensitivity (IFA or IHA), 103 samples were collected in Novo Brasil and 140 in São Luís dos Montes Belos. Later, 25 and 35 samples, respectively, were collected in tubes for the techniques with greater specificity (ELISA was used in all inconclusive cases). The dilution used in indirect IFA was 1:20, while the cut-off point used in ELISA was <0.9 (Fig.4).

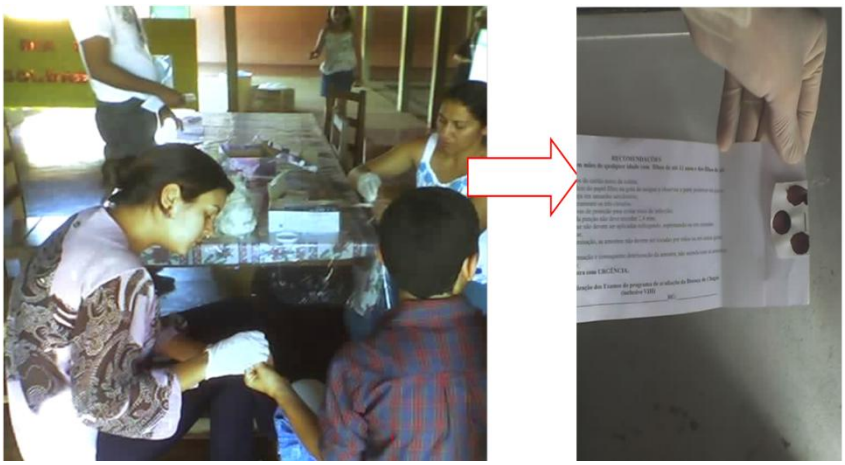

FIGURE 4: COLLECTION AND PROCESSING OF THE SAMPLES ON WHATMAN FILTER PAPER NO. 1.

2.7 Community participation

A health education program was implemented to encourage the communities to participate actively in the survey. A campaign was initiated to increase community awareness of the study and to involve the community in the process by implementing a program in the rural schools, with the collaboration of the teachers, in the form of lectures, fliers and posters. The family health units (7 healthcare units in São Luís dos Montes Belos and 2 in Novo Brasil), together with the National Health Foundation, were transformed into health multipliers. Twelve community leaders from Novo Brasil and 54 from São Luís dos Montes Belos participated in the study. Initially, they, together with the community health agents and, later on, the teachers from the municipal schools, the schoolchildren themselves and, finally, the general population, formed the components of each family health unit (Fig. 5).

FIGURE 5: A HEALTH EDUCATION PROGRAM WITH THE COMMUNITY PARTICIPATION

2.8 Referral of infected patients

Any infected patients were to be referred to one of the municipal health clinics within the National Health Service (SUS) network. If necessary, they would then be referred to a state referral center for treatment of the disease, preferably the Chagas laboratory at the Federal University of Goiás Teaching Hospital, for clinical evaluation and follow-up. The health authorities responsible for health surveillance in the municipality would be notified.

III. DISCUSSION

The results of the present survey are of considerable value, since they provide a picture of the current situation in the two municipalities evaluated, both Novo Brasil where the prevalence of the infection in this age group fell from 42.5% to 0% between 1996 and 2006 [9] and São Luís dos Montes Belos where no previous survey had been conducted with that age group. Nevertheless, investigation into this population found one single case that tested positive for the disease. Confirmation of the positivity of that single sample from a 10-year old child in the São Luís dos Montes Belos region leads us to believe that although Chagas disease is under control, it has yet to be completely eradicated.

In Goiás, the epidemiological profile of Chagas disease changed following implantation of the *T. infestans* eradication program in 1991; however, in 2003, 20 municipalities were considered to be at risk of transmission of the disease in the state, from an original total of 221 in 1980. Traditionally, the regional office of the National Health Foundation in Goiás was responsible for maintaining this program, with the Goiás State Health Department having inherited this responsibility in 1998 following decentralization of the National Health Service [9].

The choice of the municipality of São Luís dos Montes Belos for evaluation in this study was due to the fact that no serological investigation had been conducted in children under 10 years of age there; because *T. sordida* had been found there in all the years evaluated, with its prevalence reaching 17.89% in 1998; and because of the presence of 25 community

health agents who would be available to help in the study. On the other hand, in Novo Brasil, *Triatoma infestans* was not detected in any of the years covered by the study; however, the index of the secondary vector was high, reaching 33.33% in 1984 [4, 9].

Evaluating the entomological situation in the municipality of Novo Brasil, the presence of secondary vectors such as *T. sordida* and *R. neglectus* is notable. However, in São Luís dos Montes Belos, the risk is even greater, with nymphs of *T. sordida* being found in 60% of the cases of infestation. In the municipality of Posse in Goiás, the peridomestic indexes of infestation by *T. sordida* were also found to be high, particularly in henhouses; nonetheless, no nymphs were found in any of the homes [8].

According to Diotaiuti, the epidemiological importance of triatomine bugs is defined by their degree of association with humans. Among the species of secondary importance, *T. sordida* is the most important due to its high peridomestic density. In fact, this is currently the most commonly captured species throughout the entire country [11]. In the municipality of Posse in the state of Goiás, of the 1,059 specimens of triatomine bugs captured, 99% consisted of *T. sordida* [8]. In towns in the southeast of Bahia, *T. sordida* was also the most common species found (96.5%, n=8,657) and of all the specimens captured, 19 were found to be infected by *T. cruzi* [13]. Epimastigote forms of *T. cruzi* were found in *T. sordida* specimens in the district of Santo Inácio in Bahia. Inoculation of BALB/c mice showed low infectivity, with results revealing only mild inflammation, with no sign of parasitism in the tissues [14].

There was great interest in the results of this study from all the sectors involved - the population, healthcare professionals and municipal councils, all of which form the basis for the control and prevention of the disease. In the municipality of Totolga in Nicaragua, the integration of primary healthcare into the services was also the key to improving the surveillance and response system required to eliminate Chagas disease in the country [15].

IV. CONCLUSION

In conclusion, the measures required to control the transmission of Chagas disease consist of simultaneous seroepidemiological and entomological surveillance, continuous spraying of residual insecticides in endemic areas, and now the continuation and strengthening of health education by the Family Health Program.

In general, the data show how the control measures adopted up to the present time have been effective in controlling the disease; however, according to data obtained from the Epidemiological Surveillance Department of the Ministry of Health in December 2006, acute cases of the disease had been confirmed in Brazil.

Although the state of Goiás has been certified as free from *T. infestans*, the principal vector in previous years, the fact that other species of synanthropic triatomines have been captured that tested positive for *T. cruzi* (Chagas, 1909) is a warning that the natural transmission of Chagas disease continues in the state.

Further serological studies need to be conducted to increase the effectiveness of the program and its results, since in some municipalities no investigation has yet been carried out. Failure to conduct such studies may result in the non-identification of some areas in which Chagas disease continues to be transmitted, with the result that attack efforts to eradicate the disease may focus on the wrong strategic points in the state or even in the transfer of a large number of personnel to a region that is not so badly infested. It is vital to prioritize careful epidemiological surveillance, including the involvement of the population and local health authorities, and to have sufficient resources available to do so.

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