

Surveillance of *Trypanosoma cruzi* transmission by serological screening of schoolchildren

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The seroprevalence of Trypanosoma cruzi infection among children is a sensitive indicator for assessing the effectiveness of programmes for control of Chagas disease. In this study we report the result of a cross-sectional serological survey carried out among schoolchildren living in a poor rural area in central Brazil. Eluates of blood collected on filter-paper were tested for anti-T. cruzi antibodies using immunofluorescence, haemagglutination, and enzyme-linked immunosorbent assays. The overall seroprevalence of T. cruzi infection was 7.9%, which compared with the findings of the national survey carried out in 1975–80 indicates that a twofold-to-threefold reduction in prevalence has occurred over the last 10 years. This is consistent with a reduction of transmission in the area, probably related to vector control efforts. Based on our results, the incidence of new cases was estimated to be 44 per annum in the study region. In rural areas with a scattered population, surveillance of T. cruzi transmission by serological screening of children at school entry is more practical and economical than entomological evaluation for assessing both the risk of transmission in the community and the efficacy of vector control measures. A sample size of around 1000 schoolchildren is sufficient to detect prevalences as low as 2%, and such an approach would be practical and applicable to most areas where Chagas disease is endemic.

Introduction

The WHO Expert Committee on the Control of Chagas Disease has stated that Chagas disease control is scientifically and technologically feasible (1). The prevention of *Trypanosoma cruzi* infection relies on chemical control of the vector with conventional insecticide spraying, improvements in housing, and serological screening of blood donors. Chemical vector control programmes have been successfully implemented in Argentina, Brazil, and Venezuela, resulting in a significant reduction in the domestic population of vectors and interruption of transmission in large parts of these countries (2–4).

The environmental changes resulting from deforestation, mechanization of agriculture, extensive replacement of natural flora by cattle pastures, and

the dislocation of rural workers to urban areas that have occurred in some areas in Brazil have also been cited as factors contributing to the control of triatomine bugs (5, 6).

In Brazil, because of political and economic constraints, as well as frequent shifts of resources to different public health problems, it has not been possible to implement regular chemical control of *T. cruzi* vectors in all the endemic areas. Also, the replacement of the domestic vector, *Triatoma infestans*, by secondary species has called attention to the importance of the peridomestic habitat in the transmission of Chagas disease and to the need for environmental management as part of the control effort.

Assessment of the impact of programmes for the control of Chagas disease is usually based on entomological monitoring of house infestation and on serological evaluation of selected cohorts of the population (7). The seroprevalence of *T. cruzi* infection among children is a sensitive indicator of household rates of seropositivity, and for the assessment of the risk of transmission in the community is more practical and economical than entomological evaluation (8, 9). In sparsely populated rural areas, where house-to-house surveys are operationally difficult, an alternative approach is the serological screening of schoolchildren (10, 11).

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Here we report the result of a cross-sectional survey of schoolchildren living in a poor rural area in central Brazil, where very few economic and environmental changes have occurred in the last two decades, and where vector control activities have been kept as regular as possible. The study was designed to provide baseline information on *T. cruzi* transmission and to select individuals for participation in further studies on risk factors for the infection.

Materials and methods

Study area

The survey was carried out between March and September 1991 in rural communities in north-east Goiás State, central Brazil. A population census and housing plan of the study areas were provided by the Ministry of Health. The counties of Posse (25 295 inhabitants), Simolândia (6242 inhabitants) and Guarani de Goiás (4766 inhabitants) were selected because about 50% of their population was rural and they had a high seroprevalence of *T. cruzi* infection in the national rural survey conducted in 1975–80 (12). Over the last 5 years, house spraying with insecticide has been greatly reduced in this area, as well as in Brazil as a whole.

The rural population in the study area is fairly stable and depends on subsistence farming, while the dwellings are mainly made of mud and wattle or unplastered adobe bricks.

Study population

A total of 60 village schools with 20–40 students each, i.e., a total of 1990 schoolchildren aged 7–12 years, corresponding to 82% of the estimated number of 7–12-year-olds in the three counties, were included in the study. The schools were randomly selected from a list provided by the local education department.

Data collection and laboratory tests

A blood sample was collected from all participants by finger-prick onto filter-paper (Whatman No. 1), filling two circles of 1.6-cm diameter (area, 2 cm²) (13). After being dried at room temperature, the filter-papers were stored at 4 °C according to standard procedures (14) and sent weekly for examination to the Reference Laboratory for Chagas Disease, Goiânia (15) (600 km from the study area). Eluates from the filter-paper were tested simultaneously for anti-*T. cruzi* antibodies using indirect immunofluorescence (IIF) (16), indirect haemagglu-

tionation (IHA) (17) and enzyme-linked immunosorbent assays (ELISA) (18), as described previously (19). A sample was considered positive when at least two serological tests were positive, i.e., under the following conditions: IIF titre $\geq 1:20$; ELISA absorbance ≥ 1.2 times the cut-off value; and IHA $\geq 1:10$. Borderline results were retested using the sample on the stored filter-paper and taken to be negative if the results were persistently borderline.

Data processing and analysis

The data were analysed using the *Statistical package for the social sciences* (SPSS/PC, 1987).^a The significance of differences between proportions was determined using χ^2 tests. *P*-values ≤ 0.05 were considered to be statistically significant. The association between seropositivity, age, and sex was assessed by calculating χ^2 tests for trend, estimates of relative risks (odds ratios), and 95% confidence limits (95% CL). Tests for interaction were performed using the *Epidemiological graphic, estimation and testing package* (EGRET) software.^b

Results

Of the 1990 schoolchildren aged 7–12 years who were tested, 52.6% were males and 53.9% were aged 7–9 years. The overall seroprevalence of *T. cruzi* infection was 7.9% (95% CL: 6.8–9.1%) and there were no statistically significant differences between the prevalences estimated for each county. For a given age group, males had a higher prevalence of infection than females, the overall prevalence for males being 9.3% versus 6.5% for females (*P* = 0.02) (Table 1). The seroprevalence increased with age, ranging from 3.9% for children aged 7 years to 11.5% among 12-year-olds (test for trend, *P* < 0.01). The same pattern was observed in the three counties. Table 2 shows the odds ratios for antibodies against *T. cruzi* among 10–12-year-olds compared with those aged 7–9 years, after controlling for sex. For 10–12-year-olds, males had a significantly higher risk of infection than females (3.3 versus 2.2). No significant difference in the risk of infection was observed between younger males and females, and for positive serological tests there was no statistically significant interaction with age and sex.

^a SPSS Inc., Chicago, IL, USA.

^b SERC, Seattle, WA, USA.

Table 1: Seroprevalence of *Trypanosoma cruzi* infection, by age and sex, among schoolchildren in central Brazil^a

Age (years)	Males		Females		Total ^b
	Pos/n ^c	(%) ^d	Pos/n	(%)	Pos/n
7	10/229	(4.4) ^d	7/210	(3.3)	17/439 (3.9)
8	11/164	(6.7)	8/160	(5.0)	19/324 (5.9)
9	11/150	(7.3)	8/159	(5.0)	19/309 (6.1)
10	24/169	(14.2)	10/150	(6.7)	34/319 (10.6)
11	20/154	(13.0)	15/150	(10.0)	35/304 (11.5)
12	21/180	(11.7)	13/115	(11.3)	34/295 (11.5)
Total	97/1046	(9.3) ^e	61/944	(6.5) ^e	158/1990 (7.9)

^a Based on the results of indirect immunofluorescence, indirect haemagglutination, and enzyme-linked immunosorbent assays.

^b χ^2 for trend adjusted for sex = 24.2; $P < 0.01$

^c Pos/n = No. of positive samples/total number of samples.

^d Figures in parentheses are percentages.

^e $\chi^2 = 5.3$; $P = 0.02$.

Table 2: Odds ratios for antibodies against *Trypanosoma cruzi* among schoolchildren, according to sex and age group

Age group (years)	Odds ratio		
	Males	Females	All ^a
10-12	3.3 (1.9-5.5) ^b	2.2 (1.2-3.9)	2.3 (1.6-3.3)
7-9	1.4 (0.8-2.5)	1.0	1.0

^a Odds ratio for 10-12-year-olds versus 7-9-year-olds, adjusting for sex.

^b Figures in parentheses are the 95% confidence limits.

Discussion

Serological screening is a useful tool for monitoring *T. cruzi* transmission and for evaluating the effectiveness of vector control programmes for Chagas disease (10, 20). The development of simplified methods for blood collection and sensitive techniques to detect anti-*T. cruzi* antibodies have made the diagnosis of *T. cruzi* infection easy and affordable in many large-scale field studies in urban and rural areas (12, 21). An overall *T. cruzi* seroprevalence of 13.1% has been reported among 5425 unskilled urban workers in central Brazil, reflecting the level of infection among rural migrants (21). Serological screening for *T. cruzi* infection among blood donors in this region indicated a decreasing trend of infection for the period of 1980-90 (22, 23).

Few serological studies of *T. cruzi* infection have been conducted on representative samples of

rural populations. In Brazil, the only such national serological survey, carried out between 1975 and 1980, indicated an overall seroprevalence of infection of 4.2%, although a wide regional variation was observed (12); an estimated 2 million people were infected in rural areas (24). For the rural counties of Goiás State, a mean prevalence of 7.4% was reported in the national survey (range, 0.2-26.3%). The prevalence of *T. cruzi*-positive serology among 7-14-year-olds was around 23% for Posse and Simolândia and 12% for Guarani de Goiás (Ministry of Health, unpublished data, 1980). In the current study, which was carried out approximately a decade later, the 7.9% positive serology to *T. cruzi* was estimated for a representative sample of 7-12-year-olds, indicating that transmission was still occurring.

Comparison of the results of the national survey with those from the present study shows that the prevalence of *T. cruzi* infection decreased by 2-3 times over the last 10 years. However, it should be borne in mind that for the national survey no specific age-sex prevalences and denominators are available to permit adequate comparison. The increasing seroprevalence with age found in our study is similar to that described in other cross-sectional studies in rural areas (25, 26), and arose through either a cumulative opportunity for infection or a cohort effect. Children born between 1979 and 1981 (10-12-year-olds) possibly had a higher risk of infection than those born in the period 1982-84 (7-9-year-olds) (odds ratio = 2.3; 95% CL = 1.6-3.3). This suggests that the reduction of transmission in the area was probably related to vector control.

The cohort of schoolchildren studied represents a sample of the population born before the attack phase of the vector control programme (1984-85) in the study area. When the study was carried out, the three counties were under entomological surveillance. Although serological surveys among children below the age of 6 years would have provided more accurate information on the impact of the programme, since only incidence cases of infection would have been detected, such surveys are much more difficult to conduct.

The incidence of *T. cruzi* infection in the study area is difficult to estimate. Based on the approach suggested by Hayes & Schofield (27) for an estimated rural population of 21 563, an overall prevalence of infection of 7.9% and a crude birth rate of 25.6% (national census data, 1990), 44 new cases of Chagas disease would be expected to occur annually in the study area; however, this is probably an underestimate, since the prevalence of *T. cruzi* infection in children is generally lower than that in adults.

Vector control operations using insecticidal paints and fumigant canisters have resulted in favour-

able outcomes. These novel approaches, together with community-based bug monitoring using sensor boxes, form the basis of alternatives for affordable vector control activities (15). Evaluation of the impact of alternative approaches on the control of Chagas disease has as a prerequisite the development of indicators of efficiency to facilitate comparison of different strategies and to perform trend analyses. Serological screening is a valuable tool in all phases of vector control since it can provide baseline information and age-prevalence data for monitoring the impact of control programmes (9). The challenge is to design an affordable surveillance system within vector control programmes that includes both serological and entomological evaluations.

In scattered populations the screening of schoolchildren is more feasible than efforts to screen infants—which would, however, reveal more accurately the incidence of *T. cruzi* infections over the last 1–2 years. Also, it is cheaper and easier to determine the risk of transmission in the community than to detect triatomine bugs in dwellings.

Serological screening of children at school-entry age in a random sample of rural schools, every 4–5 years, would permit reliable assessment of the impact of Chagas disease control programmes. The number of schools and children to be examined would depend on the prevalence of infection in the area under surveillance. A sample size of around 1000 schoolchildren would be sufficient to estimate prevalences as low as 2% (95% CL, 1.1–2.8), which should be practical and applicable to most endemic areas. A network of laboratories with appropriate quality control standards is required for a serological evaluation programme.

Surveillance by serologically screening schoolchildren for *T. cruzi* could also be useful for mapping morbidity patterns and for detecting any changes in the time trend, thereby permitting comparisons between regions. The decrease in the incidence of *T. cruzi* infections estimated from prevalence data may reflect the efficacy of the vector control measures; however, changes in environmental factors should also be taken into account when assessing the impact of such programmes.

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Résumé

Surveillance de la transmission de *Trypanosoma cruzi* par des tests de dépistage sérologique chez les écoliers

Le présent article analyse les résultats d'une enquête transversale menée auprès de 1990 écoliers âgés de 7–12 ans, soit 82% du nombre estimé des enfants d'âge scolaire de 3 districts ruraux du centre du Brésil. L'étude a porté sur un total de 60 écoles de village. Une goutte de sang était recueillie sur papier filtre après piqûre au doigt et la recherche des anticorps anti-*Trypanosoma cruzi* dans l'éluat était faite simultanément par immunofluorescence indirecte (IF), hémagglutination indirecte (IHA) et titrage immuno-enzymatique (ELISA). Un échantillon était considéré comme positif lorsque deux épreuves sérologiques au moins donnaient un résultat positif.

Globalement, la séroprévalence de l'infection à *T. cruzi* a été évaluée à 7,9%, les garçons étant plus atteints que les filles (9,3% contre 6,5%, $P = 0,02$). D'autre part, dans le groupe des 10–12 ans, le risque d'infection était significativement plus élevé chez les garçons que chez les filles (3,3 contre 2,2). La comparaison des résultats de cette étude avec ceux de l'enquête nationale effectuée dans la même région en 1984–1985 montre que la prévalence de la maladie de Chagas a été divisée par deux ou trois au cours des dix dernières années, ce qui semble indiquer une réduction de la transmission, probablement liée aux efforts accomplis en matière de lutte antivectorielle.

On peut s'attendre à ce qu'au moins 44 nouveaux cas de maladie de Chagas se déclarent annuellement dans la région à l'étude. Afin d'évaluer l'efficacité des programmes de lutte, nous estimons qu'il serait possible de détecter une prévalence de l'ordre de 2% avec une limite de confiance de 95% en pratiquant des tests de dépistage sérologique tout les 4 à 5 ans sur environ 1000 enfants au début de leur scolarisation dans des écoles rurales choisies au hasard. Une telle approche serait applicable en pratique dans la plupart des régions où la maladie de Chagas est endémique.

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