Am. J. Trop. Med. Hyg., 52(2), 1995, pp. 183–187

RISK FACTORS FOR *TRYPANOSOMA CRUZI* INFECTION AMONG CHILDREN IN CENTRAL BRAZIL: A CASE-CONTROL STUDY IN VECTOR CONTROL SETTINGS

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Abstract. This population-based case-control study was conducted in northern Goias State, central Brazil, in rural settings under vector control surveillance. One hundred forty-nine children seropositive for *Trypanosoma cruzi* antibodies, selected in a cross-sectional survey carried out in village schools, were compared with 298 seronegative classmate controls matched for age, sex, and place of residence. Information on potential environmental, familiar, and social economic risk factors for *T. cruzi* infection was collected during household visits, and interviews with parents and entomologic inspections of domestic and peridomestic environments were conducted. The presence of triatomines in dwellings or evidence of triatomine colonization was found to be statistically associated with seropositivity in children. The presence of exuviae and a report of triatomines indoors or outdoors by householders in the past were strong predictors of an infected child. Children from seropositive mothers had a 3.9-fold increase in the risk of having anti-*T. cruzi* antibodies after adjusting for the confounding variables, including triatomine capture, mother's age, and family size in multivariate analysis. Parent's report of vector presence showed a 97.7% sensitivity in identifying a dwelling with at least one seropositive child. The possibility of transplacental *T. cruzi* transmission and its implication for Chagas' disease control were considered.

Conventional insecticide spraying has been the most critical public health intervention for the control of vectorial transmission of Chagas' disease in endemic countries. The impact of chemical vector control varies in different ecoepidemiologic conditions, due mostly to the characteristics of the vectors and housing, coverage, and efficiency of the health services, and human behavior. In some countries, the implementation of programs for house improvement and health education has proven to be very efficacious in controlling triatomine infestation.[1] In rural areas, Chagas' disease control programs have focused on vector surveillance and selective insecticide spraying in residual foci and on occasional house colonization by sylvatic vectors.[2] Alternative tools for vector control and community-based entomologic surveillance strategies are under evaluation in several countries.[1,3] Socioeconomic improvement and environmental changes are considered essential for long-lasting vector control and interruption of transmission.[4]

Few attempts have been made to assess a reduction or interruption of *Trypanosoma cruzi* transmission through the correlation of entomologic indicators of control with epidemiologic data on infection. Long-term prevalence trends of positive serology to *T. cruzi* have been studied among schoolchildren and blood donors, considered potential sentinel populations to measure the impact of Chagas' disease transmission control.[5–7] These evaluations, however, have not been routinely incorporated into the control programs. The identification of risk factors associated with *T. cruzi* infection may help assess the impact of the interventions and may provide useful information for planning public health activities.

The present population-based, case-control study was conducted in a rural endemic area under routine vector surveillance. The objective was to investigate the role of environmental characteristics, as well as family and household factors, in determining the risk of *T. cruzi* infection in childhood. An attempt was also made to identify predictors of families with seropositive children.

MATERIALS AND METHODS

The study was carried out in three contiguous rural counties in northern Goias State in central Brazil, with an estimated population of approximately 36,000 inhabitants, and which is highly endemic for Chagas' disease. A serologic survey for antibodies against *T. cruzi* was conducted from March to September 1991, and included 1,990 schoolchildren, 7–12 years of age, corresponding to 82% of students enrolled in 60 village primary schools. A detailed description of this investigation has been reported elsewhere.[7] Blood samples collected on filter paper were tested by indirect hemagglutination assay (IHA), indirect immunofluorescence assay (IIF), and enzyme-linked immunosorbent assay (ELISA) following standard procedures.[8–10]

Selection of cases and controls. Study cases consisted of 149 of the 158 children (7.9%) who tested positive for anti-*T. cruzi* antibodies by at least two immunologic tests on filter paper eluates, and were further confirmed by testing venous blood samples. For each case, two seronegative controls were selected among classmates that were matched for age (± one year), sex, and community. To exclude samples with a borderline result, an increased serologic cutoff point was adopted for each serologic technique used to confirm the results on sera samples (IHA ≥ 1:8, IIF ≥ 1:20, and ELISA ≥ 1.2 [optical density values]). All controls tested were negative with the three techniques on eluates and also on sera.

Data collection. Socioeconomic and environmental factors possibly associated with the risk of *T. cruzi* transmission were recorded by interviewing parents of cases and controls regarding land ownership, number of family members, years

TABLE 1

Baseline features of seropositive (cases) and seronegative (controls) children

Characteristics	Cases (n = 149)	Controls (n = 298)
Mean ± SD age (years)	9.9 ± 1.6	10.0 ± 1.7
Males, no. (%)	89 (59.7)	178 (59.7)
History of child illness		
Moderate illness, no. (%)*	23 (15.4)	59 (19.8)
Hospitalization, no. (%)	35 (23.5)	71 (23.8)
Mean ± SD family size†	7.5 ± 2.1	6.9 ± 2.3
Family owns dwelling, no. (%)	136 (91.3)	272 (91.3)
Permanent settlement, no. (%)	134 (89.9)	277 (92.9)

* Moderate illness was that which required medical attention but not hospitalization, e.g., measles, respiratory disease, diarrhea.
† $P = 0.01$, $t = 2.53$, by Student's t-test.

of residence in the community, history of blood transfusion, presence of domestic animals, and any past report of a triatomine in the house or environs. The interviews were carried out by trained community health workers blinded to the serologic results and to the purpose of the study. In addition, a domestic and peridomestic entomologic evaluation was done using pyrethrum as a flushing-out agent. The presence of triatomine adults, nymphal stages, or traces of triatomines were recorded. For analysis, each house was classified as suitable or not suitable for triatomine colonization, according to the characteristics of the floor, roof, wall, the presence of animals, and the storage of agricultural products. Householders were also requested by the interviewers to identify the true vectors of Chagas' disease among eight different types of triatomines included in a special preparation to validate their reports of triatomines. A venous blood sample was taken from parents of cases and controls to be tested for anti-*T. cruzi* antibodies by IHA, IIF, and ELISA, with the same criteria of a positive and negative results adopted for cases and controls.

Statistical analysis. Cases (n = 149) and matched controls (n = 298) were compared regarding three groups of variables: parents' serology, housing characteristics, and peridomestic environment. Baseline characteristics of cases and controls were compared. Differences between means were evaluated by the Student's t-test and differences between proportions were evaluated by the chi-square test. Matched odds ratios (OR) and 95% confidence limits (CL) were estimated by the Mantel-Haenszel method[11] to assess the association between exposure variables and positive serology. Conditional logistic regression models were applied to control for confounding variables using the Epidemiological Graphic, Estimation and Testing Package (Statistical and Epidemiological Res. Corp., Seattle, WA).

To control for common exposures among household members and avoid clustering of seropositive results, further analyses were also performed excluding cases and controls who lived in the same household, and including only one seropositive child per household, the first one recruited for serologic testing (dataset 1). This strategy of analysis yielded 89 houses with an infected child and 178 houses of matched controls (dataset 2). Building dataset 2 was necessary to ensure that the risk estimates obtained in dataset 1 were not biased by entering the same exposure variable more than once in the statistical model. The sensitivity in identifying a house with a positive child was estimated for the following variables: parents' serology, report of triatomines indoors or outdoors, and presence or evidence of a vector. Although all the houses of controls were visited, they did not represent the totality of houses of seronegative children; therefore, the specificity in identifying a house with a negative child could be biased.

TABLE 2

Matched odds ratio (OR) of vector variables associated with Trypanosoma cruzi *infection among children*

Vector variables	Cases (n = 149)	Controls (n = 298)	Crude OR	95% confidence limit
Report of triatomines indoors or outdoors*				
No	3	41	1	
Yes	144	256	7.7	2.3–25.5
House infestation†				
No	66	188	1	
Yes	83	110	2.4	1.5–3.8
Triatomine				
No	138	277	1	
Yes	11	21	1.0	0.5–2.2
Exuviae				
No	93	247	1	
Yes	56	51	3.5	2.1–5.8
Eggs				
No	106	256	1	
Yes	43	42	2.8	1.6–4.7
Feces				
No	95	220	1	
Yes	54	78	1.9	1.1–3.1

* 147 matched sets (three were missing). A report of triatomines indoors or outdoors in the past by the householders.
† House infestation = current triatomine capture or evidence (exuviae, eggs, feces) in the dwellings.

RESULTS

Cases and controls did not differ with respect to age and sex distribution, family economic activity, ownership of houses, medical history, and length of residence in the area (Table 1). Most of the families were involved in agricultural activities and owned their plot of land. Ninety per cent of the children were born in and had always lived in the study area. The average number of family members was slightly higher among cases than controls (7.5 versus 6.9; $P < 0.01$).

Parents who reported triatomines inside or outside the house were 7.7 times more likely to have an infected child according to the crude OR in matched set analysis (Table 2). Triatomine infestation or evidence of triatomine colonization in houses was found to be statistically associated with children's seropositivity, and the presence of exuviae was the strongest predictor of infected children (OR = 3.5, 95% CL 2.1–5.8). Cases and controls shared similar environmental and peridomestic characteristics; therefore, no statistical association was found between outbuilding characteristics and seropositive test results.

A strong association was found between mother's serology and her children's anti-*T. cruzi* antibodies even when the adjusted OR was calculated (OR = 3.9, 95% CL 2.2–6.9, $P < 0.01$). This effect was independent and did not

TABLE 3

Matched odds ratio (OR) and 95% confidence limit (CL) of the association of parents' serology and seropositivity for Trypanosoma cruzi *among children*

Parents' serology	Cases (n = 149)	Controls (n = 298)	OR* (95% CL)	OR† (95% CL)
Mother‡				
Negative	24	109	1	1
Positive	114	170	3.6 (2.1–6.5)	3.9 (2.2–6.9)
Father§				
Negative	37	97	1	1
Positive	88	151	1.6 (1.0–2.7)	1.5 (0.9–2.5)

* Crude odds ratio.
† Adjusted odds ratio for mother's or father's age, family size, and triatomine capture as appropriate.
‡ 136 matched sets (30 were missing).
§ 124 matched sets (74 were missing).

interact with the sex or age of the child, mother's age, number of siblings, and house infestation (current triatomine capture or evidence of triatomine colonization in the dwelling). Although an OR greater than 1.0 was found between seropositivity of fathers and their child's serology, it was not statistically significant at a 95% CL (Table 3).

After excluding cases and controls living in the same household, and building a dataset 2 with just one seropositive child per household, the report of vector by householders showed a 97.7% sensitivity, with a correct identification of 265 of 267 dwellings with at least one seropositive child. The actual capture of triatomine vectors in the dwelling was not correlated with a seropositive house, having only a 4.5% sensitivity. The seroprevalence of mothers and fathers among the control groups, 56% (92 of 165) and 59% (88 of 148), respectively, were taken as being representative of the seropositivity of women and men in the area (Table 4). Similar results of the OR of parents' serology and vector variables were obtained by using this reduced model. The relative risk of paternal seropositivity still was not statistically associated with seropositive children (OR = 1.5, 95% CL 0.8–2.8). A report of triatomines indoor or outdoors (OR = 6.5, 95% CL 1.5–28.6) and seropositivity of the mother (OR = 3.3, 95% CL 1.6–6.8) persisted as significant predictors of families with a *T. cruzi*–seropositive child.

TABLE 4

*Sensitivity of variables related to vector and parents' serology for predicting a house with a seropositive child**

Variables	House: Case (n = 89)	House: Control (n = 178)	Sensitivity (%)
Report of triatomines indoors or outdoors†			
Yes	85	151	97.7
No	2	26	
Triatomines in dwellings			
Yes	4	15	4.5
No	85	163	
Mother's serology‡			
Positive	63	92	78.7
Negative	17	73	
Father's serology§			
Positive	50	88	69.4
Negative	22	60	

* Reduced model (dataset 2). Only one child per household
† Three were missing. Report of triatomines indoors or outdoors in the past by the householders.
‡ Twenty-two were missing.
§ Forty-seven were missing.

DISCUSSION

This study showed that *T. cruzi* infection in childhood was associated with the presence or evidence of triatomines inside dwellings, and that *T. cruzi*–seropositive children were more likely to have a seropositive mother than those with seronegative test result, even when controlling for house infestation and environmental characteristics.

The presence of exuviae, eggs, or triatomine feces, which are considered as entomologic indicators of past infestation, was found to be statistically associated with antibodies to *T. cruzi*. This was not observed with the actual capture of live insects, which occurred only in 7.1% of case and control dwellings. It has been demonstrated that infestation rates are underestimated when based only on the manual capture of live triatomine bugs.[3]

A consistent association between seroprevalence of *T. cruzi* antibodies in children, house construction, and vector density was reported in northeastern Brazil in areas with high infestation rates (18–25%) by *Panstrongylus megistus* 10–20 years ago.[12–14] Vector to human transmission was also well-documented by the association of infected bugs with infected children. Nevertheless, there are important differences within endemic regions regarding vector species and the intervention procedures of Chagas' disease control programs. Ecoepidemiologic and environmental changes, in addition to continuous insecticide spraying, were responsible for the low prevalence and density of triatomines inside the houses in our study area. As result, no clear association could be shown between the presence of *Triatoma infestans* and *Triatoma sordida* in the dwellings and children's seropositivity. Also, low percentages of infected bugs were found in the present study. The high sensitivity (98%) of residents' reports on vector infestation in detecting the house of an infected child emphasizes the opportunity of exploring rapid assessment procedures for Chagas' disease control based on the community knowledge.

Epidemiologic studies related to vector colonization and Chagas' disease transmission may yield quite different results even within the same ecogeographic region. For example, in the municipality of Mambai, a neighboring locality to the study area, vector control has been successfully achieved, where house infestation by *Triatoma infestans* has decreased from almost 30% to zero from 1980 to 1988,[15] and zero incidence of infection among young children has been found.[16] In contrast, a completely different epidemiologic scenario was found in our study region, with prevalence infection rates of approximately 4% among seven-year-old children.[7]

One of the limitations of case-control studies for identifying risk factors related to infectious diseases is that the assumption of independence of outcome at the individual level may not be entirely appropriate, especially when the source of infection is inside the house.[17,18] In the present study, to control for the dependence of outcome (risk of getting infection) among siblings, the analysis was also car-

ried out using a reduced data set that included only one subject for dwelling and excluded cases and controls living in the same house. No material difference in the results was observed with this approach. An additional restriction in the interpretation of epidemiologic studies on Chagas' disease is that the serologic antibody marker represents an a past infection and the current evaluation of environmental and behavioral exposures does not necessarily correspond to the conditions at the time of infection. Matching cases and controls by community resulted in an apparent overmatching of some exposure variables, particularly those related to the socioeconomic and environmental conditions. For example, when controls were chosen from the same community as the cases, house-building characteristics tended to have greater similarity in both groups. Therefore, overmatching may be considered as a possible explanation for the absence of association of cases (seropositive children) with socioeconomic and environmental variables when compared with controls (seronegative children). Both groups presented similar socioeconomic situations and environmental exposures. Therefore, it was not possible to discriminate these variables of seropositivity in relation to seronegative children.

One of the advantages of population-based case-control methodology is the possibility of using the exposure level among the control group as representative of the population as a whole. In this sense, according to our findings, the prevalence of infection among adult females was approximately 60% in the study area. With a total population of 7,500 women of reproductive age and assuming a 10% fertility rate, at least 400 infected pregnant women per year could be estimated. The probability of children being born to chronically infected mothers is high, and the possibility of persistence of infection deserves attention. The results from both matched and unmatched analyses confirm the strong association between seropositive mothers and seropositive offspring, which was not observed in relation to the father's serology. According to our results, there was a two-fold excess risk of mother's versus father's seropositivity in relation to the child being seropositive. Taking the risk estimate of the father's seropositivity as being representative of vector-human exposure, this risk could be explained by 1) possible transplacental transmission, 2) increased susceptibility of offspring to vector infection from the infected mother, or 3) increased exposure to triatomines because of maternal behavioral patterns.

Congenital Chagas' disease has been reported in endemic South American countries, with an incidence up to 10.5% in Brazil.[19,20] These high rates may be due to a study bias that included a large number of abortions, stillbirths, and/or severe neonatal disease. Most information available on congenital transmission has been gathered from descriptive and case reports that indicate that congenital infection may result in abortions, premature births, and fetal growth retardation.[20] An impairment of placental immunophagocytosis and different behaviors of *T. cruzi* strains in placental infection have been described.[21,22] The mechanisms related to maternally induced impairment of the immune response of the offspring and the duration of *T. cruzi* infectiousness in the mother with regard to transplacental transmission still need to be determined in order to understand the efficiency of congenital transmission. Experimentally, a decreased resistance to *T. cruzi* acquired infection in offspring of infected mice has been demonstrated; however, these findings may not be directly extrapolated to humans.[23] In a population-based study conducted in Bahia State, seropositive children of seropositive mothers were younger than seropositive children of seronegative mothers and a possible different immune response of children of mothers seropositive for *T. cruzi* infection was suggested.[24]

We have shown in a population-based case-control study that besides the vector-to-human epidemiologic link, the mother-to-offspring route may have been responsible for part of the burden of *T. cruzi* infection. In addition to improving vector surveillance in areas of low bug density, the implementation of a prenatal serologic screening should be considered. The detection of *T. cruzi* parasite antigens in newborns acquired from infected mothers through sensitive techniques[25] would be an important complementary public health measure for the treatment of acute infection.[26] Further studies on cohorts of seronegative newborns, either from seropositive and seronegative mothers, should be encouraged to assess the possible impact of an infected mother on the risk of vectorial transmission.

Acknowledgments: We express deep appreciation to Dr. A. Luquetti for performing the serologic tests, Dr. R. M. Oliveira for the field work coordination, and Dr. S. A. Silva for helping with data processing. Health personnel from the Fundacao Nacional de Saude were extremely helpful in the field activities. We also thank V. Bosnyak for reviewing the manuscript and D. H. Resende, M. A. Chaves, and J. B. Peres, Jr. for secretarial support.

Financial support: This investigation received support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Disease (TDR) and by the Brazilian Research Council-CNPq.

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