# Seroprevalence of *Trypanosoma cruzi* infection in three rural communities in Guatemala

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**ABSTRACT**    *A systematic, house-based serological survey for* Trypanosoma cruzi *seroreactivity was conducted in three contiguous communities in Olopa municipality, Chiquimula Department, Guatemala. Blood samples from a total of 292 individuals in 63 households were examined by enzyme-linked immunosorbent assay. The seropositive rate ranged from 0% to 20.8% for the three communities, with a mean of 15.1%. Log-linear models showed that seroprevalence was significantly related to age (P < 0.005) but not to sex. However, when the age group with the lowest prevalence (1–9 years) was excluded from the analysis, age was not a significant factor (P = 0.55). Data from a stratified sample collected at the same time were combined with those of the systematic sample to analyze the relationship between seropositivity and possible explanatory variables. Log-linear models, based on 586 individuals in 129 households from the two surveys, revealed a significant positive association between seropositivity and thatched roofs (P = 0.01).*

Guatemala is located in the endemic zone of *Trypanosoma cruzi*, which extends from the southern United States to southern Argentina and Chile (1). Humans are rarely infected in the northern extreme of this range, but seroprevalence of 51% to 76% was reported among adults in three villages in Oaxaca State, in the southern part of Mexico on the country's Pacific coast (2). Reports from Guatemala indicate that seroprevalence is highest among the departments that border El Salvador, Honduras, or the Pacific Ocean (3).

This is the first report of a systematic, community-based serological study of *T. cruzi* infections in Guatemala. The study was conducted in three communities in Chiquimula Department, the region of the country with the highest reported *T. cruzi* seropositive rate (3). We present prevalence rates and demographics of persons testing seropositive and examine possible associations between seropositivity and potential contributory factors.
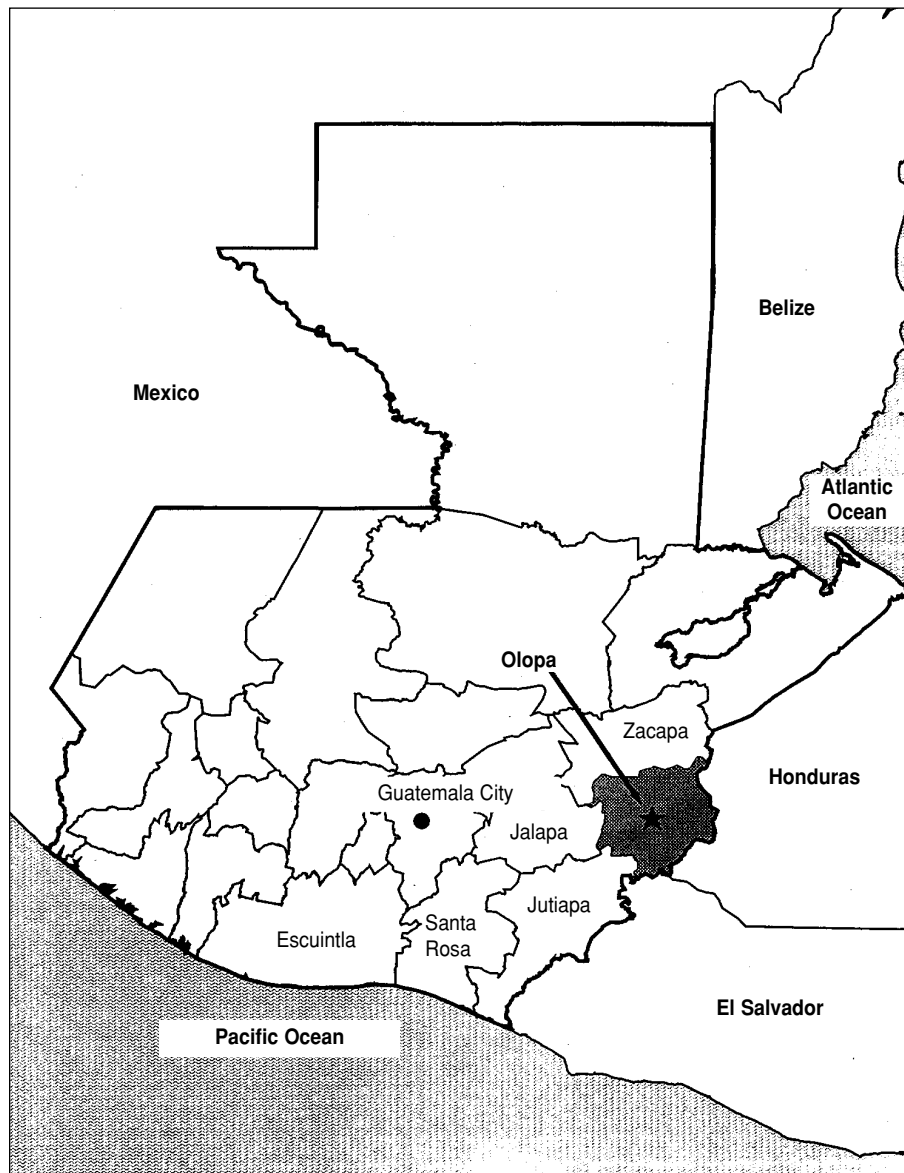
## MATERIALS AND METHODS

### Study area and study sample

The study was carried out in 1995 and 1996 in three contiguous communities in the municipality of Olopa, Chiquimula Department, Guatemala: Tituque, Tuticopote, and Talquezal. This is an impoverished area near the border with Honduras, about 125 km due east of Guatemala City and 10 km from the town of Olopa (Fig. 1). The terrain is mountainous and rugged, and the three communities are at altitudes ranging from about 1 000 to 1 300 meters above sea level. A distinct rainy season occurs from April to November, and the average

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**FIGURE 1. Map of Guatemala showing the location of Olopa in Chiquimula Department**

Mexico

Belize

Atlantic Ocean

Olopa

Zacapa

Guatemala City

Jalapa

Honduras

Jutiapa

Escuintla

Santa Rosa

El Salvador

Pacific Ocean

gia. During the house-to-house visits, information was recorded pertaining to house construction and the presence or absence of dogs, since these features may affect vector populations and/or the prevalence of *T. cruzi* infections in humans (4–10).

With rare exceptions, houses were simple one-room structures with dirt floors. Walls were either earthen, plant material, mixed earthen/plant material, or cement block. Earthen walls were made of mud-brick (adobe) or mud-stick (*bajareque*), but were not finished with plaster, as is common in other regions. Walls of plant material consisted of two basic types. One was a tightly spaced lattice of narrow sticks or strips of bamboo. The other was a similar lattice covered with banana leaves or straw. There were two categories of roofing materials, synthetic and plant. Synthetic roofing materials included corrugated metal, corrugated cement, tile, and formed cement. Plant roofs were either thatched straw or thatched palm.

Serological examinations were performed on participants from two sampling frames. One was a systematic sample that included every seventh household. The other was a stratified sample, taken in conjunction with an entomological study. Houses were stratified according to type of wall (earthen or plant) and type of roof (synthetic or thatched). A representative sample of houses constructed with these materials was selected systematically using house numbers.

## Serological methods

We collected finger prick blood samples on individually labeled strips of Whatman No. 1 filter paper (Fisher Scientific, Pittsburgh, PA, United States of America), which were air dried and returned to the main laboratory in Guatemala City. Dried samples were maintained at 4 °C until processed. A modification of Voller et al. (11) was used to perform the enzyme-linked immunosorbent assay (ELISA) (12). The antigen used was a crude extract of *T. cruzi* (Brazil strain) culture forms

annual rainfall is between 1 000 and 2 000 mm.

Farming is the principal occupation in the area. Corn and beans are the main staple crops, and coffee is the main cash crop. At the time of the study, neither electrical nor telephone service was available in the communities. We conducted a house-to-house census and recorded the name, age, and sex of all individuals. Each individual was assigned an identification number based on the community, household, and order in the family.

The purpose of the serological study was explained during the house-to-house visits, including the requirements and benefits of participation. All occupants, except for infants less than 1 year old, were invited to participate. A consent form was read aloud and participants were asked to sign or fingerprint individual forms, with parents or guardians providing consent for those below 10 years of age. The project was approved by the Institutional Review Board of the U.S. Centers for Disease Control and Prevention, Atlanta, Geor-

prepared as described by McCormick and Rowland (13). Antiparasite antibodies were detected using peroxidase-conjugated goat antihuman immunoglobulins (gamma and light chains) (Biosource International, Camarillo, California, United States of America) followed by 3,3′,5,5′-tetramethylbenzidine with 0.01% $H_2O_2$ (Kirkland & Perry Laboratories, Gaithersburg, Maryland, United States). The enzyme reaction was developed in the dark at ambient temperature and was stopped using a 2M solution of $H_2SO_4$. The optical density (OD) of each well was determined at 450 nm using an automated ELISA reader (Titertek Multiskan, Flow Laboratories, McLean, Virginia, United States).

A 6-mm-diameter circle, made with an ordinary paper punch, was taken from a completely saturated area of each dried blood sample. The punches were eluted for at least 1 hour in 0.25 mL of phosphate buffered saline containing 0.5% Tween 20 (Sigma Chemical Co., St. Louis, MO, United States). Eluates were tested in duplicate on 96-well PVC microtiter plates. Positive and negative controls were included in duplicate on each plate. The OD values obtained for positive and negative controls were analyzed to define the limits of seropositivity and seronegativity of the assay. Individuals were considered positive if the OD value was greater than 2.5 times the average of the seronegatives. Positive samples were retested using a second eluate. Those negative on the repeat assay were tested again. All samples with positive results on two assays were considered positive.

### Data analysis

Data analysis was performed using SPSS 6.0.1 software for Windows (SPSS, Chicago, Illinois, United States). Because they provided a more representative sample of the population, only results from the systematic sample were used for demographic analysis of the data. Heterogeneity chi-square analysis was used to determine whether results from the systematic and stratified samples could be combined (14). Hierarchical log-linear models were used to test for relationships between seroreactivity (dependent variable) and factors believed to be associated with *T. cruzi* infections (explanatory variables). In this type of analysis, chi-square contingency tables test the null hypotheses of independence between the dependent and explanatory variables. Significant effects (nonindependence) arise when the observed frequency of seropositive persons is significantly lower or higher than expected from the null model for a particular combination of the explanatory variables (15). In those cases, the null hypothesis of independence is rejected. Partial chi-square associations generated by log-linear analysis were used to determine which explanatory variables had significant effects. Materials used for house construction and the presence of dogs were tested as explanatory variables in our log-linear model.

## RESULTS

At the time of our census in 1995, the combined population of the three communities was 2 890. There were 552 houses in total, for an average of 5.2 inhabitants per household. Tituque and Tuticopote were virtually equal in population (1 337 and 1 317 inhabitants, respectively), but Talquezal was smaller (236 inhabitants). Of the population, 46.5% were below the age of 15, which is typical of rural populations in developing countries (16). Sex distribution was essentially equal, 50.6% male and 49.4% female.

Table 1 gives a breakdown, by sampling frame and community, of the participation and seroprevalence rates. Although every seventh house was targeted, only 63 (11%) of the households were included in the systematic sample. Houses were excluded because the inhabitants refused to participate, the residents were not home during the survey, or the house was unoccupied. Seventy-five houses participated in the stratified sample. The participation rate among households included in the study was 76% (292 of 382 persons) for the systematic sample and 78% (349 of 445 persons) for the stratified sample. The overall seroprevalence rate was 12.3%. Seroprevalence differed significantly between the two sampling frames ($\chi^2$ = 3.74, df = 1, $P$ = 0.05) and among the three communities ($\chi^2$ = 11.3, df = 2, $P$ = 0.004). Even when Talquezal, which had an overall seroprevalence of 2.4%, was excluded, the difference between Tituque and Tuticopote was significant ($\chi^2$ = 7.02, df = 1, $P$ = 0.008).

Log-linear analysis showed that age was a significant factor related to seropositivity ($\chi^2$ = 20.3, df = 7, $P$ <

**TABLE 1. Participation and number of *T. cruzi*-seropositive persons by sampling frame in three communities in Olopa municipality, Chiquimula Department, Guatemala, 1995–1996**

| Sampling frame/ Community | No. of houses | No. of persons | No. tested by ELISA | No. seropositive | % seropositive |
|---|---|---|---|---|---|
| Systematic sample | | | | | |
| Tituque | 29 | 179 | 149 | 31 | 20.8 |
| Tuticopote | 27 | 150 | 111 | 13 | 11.7 |
| Talquezal | 7 | 53 | 32 | 0 | 0 |
| Subtotal | 63 | 382 | 292 | 44 | 15.1 |
| Stratified sample | | | | | |
| Tituque | 35 | 216 | 175 | 22 | 12.6 |
| Tuticopote | 38 | 220 | 165 | 12 | 7.3 |
| Talquezal | 2 | 9 | 9 | 1 | 11.1 |
| Subtotal | 75 | 445 | 349 | 35 | 10.0 |
| Total | 138 | 827 | 641 | 79 | 12.3 |

0.005) but that sex was not ($\chi^2 = 0.01$, df = 1, $P = 0.91$). The difference by age was not significant when the youngest age group (1–9), with the lowest seroprevalence, was excluded from the analysis ($\chi^2 = 4.95$, df = 6, $P = 0.55$).

Heterogeneity chi square was performed with data from the systematic and stratified samples (14). The null hypothesis of homogeneity was not rejected for seroreactivity vs. wall type ($\chi^2 = 0.54$, df = 1, $P > 0.50$) or seroreactivity vs. roof type ($\chi^2 = 0.39$, df = 1, $P > 0.75$). On this basis, data from the two samples were combined for analysis. Seropositive individuals resided in 48 (35%) of the total of 138 households. Among those 48 households with seropositive persons, 15 (31%) had two or more seropositive persons and together accounted for 46 (58%) of the 79 cases.

Cement floors or a combination of cement and dirt floors were present in only 3 of the 138 houses. Six houses had mud-brick walls and 80 had mud-stick walls. We found no significant difference for seroreactivity of individuals living in mud-brick versus mud-stick houses ($\chi^2 = 1.118$, df = 1, $P = 0.290$), and so those categories were combined under the general category of "earthen." Table 2 shows the frequency of the generalized housing types, based on construction materials used for the wall and the roof. Also shown in Table 2 is the total number of seropositive persons and the average number of persons seropositive per household for each category. Dogs were present in 90% of houses and were generally allowed to roam freely outside and inside the house.

To keep the number of cells with low expected values at an acceptable minimum, characteristics that occurred with low frequency were excluded from the log-linear model (14). For that reason, houses were omitted that had mixed earthen/plant walls, cement block walls, or mixed synthetic/thatched roofs. Because only three houses did not have dirt floors, floor type was also excluded from our analysis. The final log-linear model analyzed the association of seroreactivity with wall type (earthen or plant material), roof type (synthetic or thatched), and presence of dogs. A total of 586 participants in 129 households were included in the final analysis. Even with this limited data set, 38% of the cells had an expected value of less than 5.

Log-linear analysis revealed that four-way effects were not significant but three-way effects were. Table 3 presents results for the partial chi-square three-way and two-way associations. Among the three-way associations, only the combination of roof and wall type had a significant effect ($P < 0.05$) on seroreactivity. Two-way associations showed that only roof type had a significant effect on seroreactivity ($P < 0.05$). These data indicate that the wall type affected the association between roof type and seroreactivity, as seen in the three-way association, but did not have an independent significant association with seroreactivity. The frequency of seropositive individuals (Table 2) demonstrates how wall type affected the association of seropositivity and roof types in the three-way associations. The difference between the average number of seropositive individuals living in houses with plant walls and roofs of synthetic material (0.0) or thatched roofs (0.64) is significantly greater than the difference between the figures for earthen wall with

**TABLE 2. The frequency of wall and roof types among houses in three communities in Guatemala, with total number and average number of _T. cruzi_-seropositive individuals per household, 1995–1996**

| Wall | Roof | | | |
|---|---|---|---|---|
| | Synthetic | Thatched | Mixed | Total |
| Earthen | 51 (24; 0.47)[a] | 32 (24; 0.75) | 3 (2; 0.67) | 86 (50; 0.58) |
| Plant | 4 (0; 0.0) | 45 (29; 0.64) | 0 | 49 (29; 0.59) |
| Mixed: Earthen/plant | 0 | 2 (0; 0.0) | 0 | 2 (0; 0.0) |
| Cement block | 1 (0; 0.0) | 0 | 0 | 1 (0; 0.0) |
| Total | 56 (24; 0.41) | 79 (53; 0.67) | 3 (2; 0.67) | 138 (79; 0.57) |

[a] = (total number of _T. cruzi_-seropositive individuals; average number per household).

**TABLE 3. Partial three-way and two-way chi-square associations for log-linear analysis of _T. cruzi_ seroreactivity among individuals in three communities in Guatemala by house characteristics (wall and roof type) and presence of dogs, 1995–1996**

| Effect | df | $\chi^2$ | $P$ |
|---|---|---|---|
| Seroreactivity × dog × wall | 1 | 0.480 | 0.488 |
| Seroreactivity × dog × roof | 1 | 0.163 | 0.687 |
| Seroreactivity × roof × wall | 1 | 4.383 | 0.036 |
| Seroreactivity × roof | 1 | 6.017 | 0.014 |
| Seroreactivity × wall | 1 | 0.001 | 0.980 |
| Seroreactivity × dog | 1 | 2.872 | 0.090 |

roofs of man-made material (0.47) or thatched roofs (0.75). Although statistically significant, that difference is not robust because of the low occurrence of individuals living in houses with plant walls and roofs of man-made material.

The significant two-way association for roof type is also demonstrated by the difference among the number of seropositives compared to the total number of individuals living under each roof and wall type (Table 2). The difference is also evident in comparing the seroprevalence rates among individuals living in houses with thatched roofs (18.5%) versus those living in houses with roofs of synthetic material (9.1%) (Table 4). The presence of dogs had a near-significant effect on seroreactivity ($P = 0.09$), and the seroprevalence among individuals living in houses where dogs were present (15.6%) was more than double the prevalence in houses where dogs were absent (6.3%).

The frequency distribution of individuals by roof type differed significantly between the three communities ($P < 0.01$). The percentage of participants living in houses with thatched roofs was 65.9% in Tuticopote, 44.4% in Tituque, and 34.1% in Talquezal.

## DISCUSSION

Results from the systematic sample in the three communities showed a 15.1% seroprevalence for *T. cruzi*, which is similar to the prevalence previously reported from Chiquimula Department. Matta (3) summarized findings from studies using an indirect hemagglutination assay (IHA) to detect seroreactivity among individuals from eight departments in Guatemala. Those surveys, conducted between 1982 and 1986, revealed a seroprevalence of 18.2% for Chiquimula Department. The next highest rates were in adjacent departments located to the north and the west and that formed a northeast-southwest line: Escuintla, 13.9%; Jutiapa, 12.8%; Santa Rosa, 7.6%; and Zacapa, 6.6% (Figure 1). These seroprevalence rates are somewhat lower than in other nearby coun-

tries. In Honduras, surveys conducted in 15 villages in the department of Francisco Morazán revealed a range in seroprevalence of 27.3% to 67.0% (mean 40.2%) in seven communities where *Rhodnius prolixus* was the predominant vector and of 12.0% to 21.0% (mean 15.2%) in eight communities where *Triatoma dimidiata* was the only vector found (17). A review of serological surveys conducted in El Salvador between 1958 and 1972 showed that in four departments outside the major urban areas the seroprevalence ranged from 9.0% to 46.7% (mean 22.7%) (18).

Previous studies have established an association between materials used in house construction and vector density and, in some cases, with *T. cruzi* infections. In communities in northeastern Brazil, where a primary vector is *Panstrongylus megistus*, a significant association was reported between mud-stick walls and vector infestation, as well as seropositivity (10). In Costa Rica, *T. dimidiata* infestation showed a positive significant association with dirt floors, but not with earthen or wood walls (6, 7). In northern South America and much of Central America, *R. prolixus* is an important vector and is associated with thatched palm walls and roofs (8, 9). Our results revealed a significant three-way asso-

ciation between roof type, wall type, and seroreactivity. However, only roof type had a significant two-way association; that was the result of a positive association between seropositivity and thatched roofs of palm or straw. Other researchers (4) have demonstrated a significant positive association in households for the presence of infected dogs, vectors, and humans. In our study, the presence of dogs in households had a near-significant association ($P = 0.09$) with seropositivity.

Except for the relatively large disparity in rates seen for the very youngest and very oldest age groups, our study shows a slow, steady increase in the *T. cruzi*-seropositive rate with age (Figure 2). This gradual acquisition of *T. cruzi* infections is in sharp contrast with some but not all areas of South America. Some of those regions have seropositive rates of 30% or higher among children under the age of 10 and of 60% or greater among persons 10–19 years old (19–21).

The significant difference we saw in seropositive rates by age group was not evident when the youngest age group was excluded from the analysis. This suggests there has been a noticeable reduction in *T. cruzi* transmission in our study area over the past 10 years. Inhabitants and local officials indicated

**TABLE 4. Distribution of *T. cruzi*-seropositive individuals in three communities in Guatemala by housing type and presence of dogs, 1995–1996**

| Character/Condition | No. of individuals | No. seropositive | % seropositive |
|---|---|---|---|
| Roof type | | | |
| Thatched | 297 | 55 | 18.5 |
| Synthetic | 265 | 24 | 9.1 |
| Wall type | | | |
| Earthen | 382 | 50 | 13.1 |
| Plant | 155 | 29 | 18.7 |
| Mixed (Earthen/plant) | 14 | 0 | 0 |
| Cement | 11 | 0 | 0 |
| Floor type | | | |
| Dirt | 546 | 79 | 14.5 |
| Cement | 13 | 0 | 0.0 |
| Mixed | 3 | 0 | |
| Dogs | | | |
| Present | 481 | 75 | 15.6 |
| Absent | 63 | 4 | 6.3 |

**FIGURE 2. Age-specific prevalence of *T. cruzi* seropositive persons in three communities in Olopa municipality, Chiquimula Department, Guatemala**
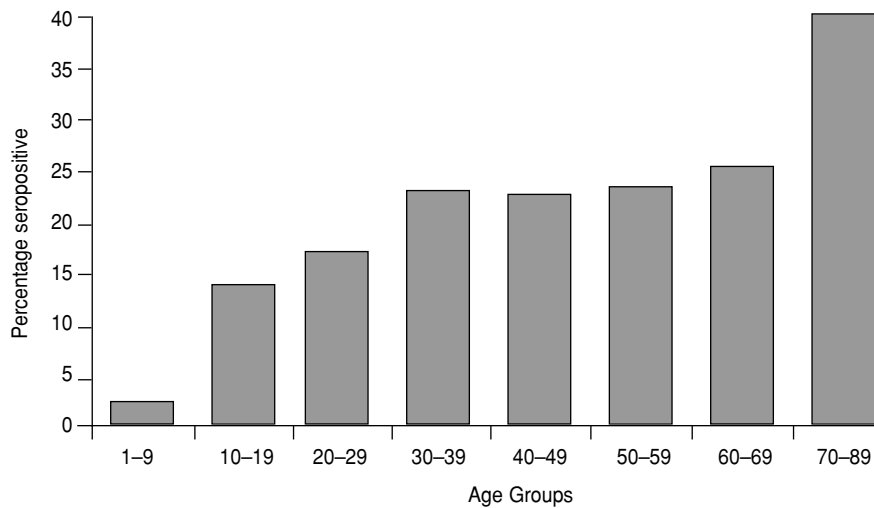


that there had been no vector control programs for Chagas' disease or malaria in the area during this time. The decrease may be related to an increase in the number of homes with roofs of synthetic material or an increased use of insecticides in the homes. While we do not have data to support these hypotheses, both corrugated roofing material and insecticides have become more affordable and accessible to rural populations in Guatemala over the past decade. It is also possible that the vector populations and/or vector contact with humans have been reduced through unknown natural phenomena or as a result of human activity, such as increased destruction of the vectors' sylvatic habitats.

There are puzzling significant differences in seropositive rates among the three contiguous communities, where both *T. dimidiata* and *R. prolixus* are present. One possible explanation is that the seroprevalence rate is related to differences in the frequency distribution of individuals living in houses with thatched roofs. For Talquezal, the relatively low percentage of individuals living in houses with thatched roofs correlates well with a low seroprevalence. However, for Tituque, with the highest seroprevalence, the percentage of individuals living in houses with thatched roofs was significantly lower than in Tuticopote. Another possible factor is altitude. In El Salvador, Wilton and Cedillos (22) found that, up to 1 000 m in altitude, the percentage of houses infested and vector density varied directly with altitude for *T. dimidiata* and varied inversely for *R. prolixus.* No data were presented for houses between 1 000 m to 1 400 m, and no triatomines were found in houses above 1 400 m. In our study, the seropositive rate varied directly with altitude, even though the differences in elevation were small (Talquezal, 1 040 m; Tuticopote, 1 220 m; Tituque, 1 280 m). It is important to keep in mind that the gradual acquisition of *T. cruzi* infections means that recently collected information on risk factors may not reflect conditions that existed at the time when most of the individuals became infected.

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## RESUMEN

### Seroprevalencia de infección por *Trypanosoma cruzi* en tres comunidades rurales de Guatemala

Una encuesta serológica sistemática y domiciliaria para detectar serorreactividad a *Trypanosoma cruzi* se llevó a cabo en tres comunidades contiguas de la municipalidad de Olapa, en el departamento de Chiquimula, Guatemala. Se examinaron mediante inmunoadsorción enzimática muestras de sangre obtenidas de un total de 292 habitantes que residían en 63 viviendas. La tasa de seropositividad varió de 0 a 20,8% en las tres comunidades y tuvo una media de 15,1%. Los modelos logarítmico-lineales mostraron una asociación significativa entre la seroprevalencia y la edad ($P < 0,05$), pero no entre aquella y el sexo. No obstante, cuando el grupo de edad que tuvo la seroprevalencia más baja (el de 1 a 9 años) se excluyó del análisis, la edad dejó de ser un factor significativo ($P = 0,55$). Datos obtenidos de una muestra estratificada recogida simultáneamente se combinaron con los de la muestra sistemática con el fin de analizar la relación entre la seropositividad y algunas variables que podrían ser explicativas. Los modelos logarítmico-lineales, aplicados en 586 habitantes de 129 viviendas incluidas en ambas encuestas, revelaron una asociación positiva significativa entre la seropositividad y la presencia de techo de paja ($P = 0,01$).