

Seroprevalence of *Trypanosoma cruzi* in Rural Ecuador and Clustering of Seropositivity within Households

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Abstract. We performed a cross-sectional study of *Trypanosoma cruzi* seroprevalence in 14 communities in three provinces of Ecuador and estimated the magnitude of the association of seropositive individuals within households. A total of 3,286 subjects from 997 households were included. Seroprevalence was 5.7%, 1.0%, and 3.6% in subjects in the Manabí, Guayas, and Loja provinces, respectively. Seroprevalence increased with increasing age in Manabí and Guayas, whereas in Loja, the highest prevalence occurred in children ≤ 10 years of age. In the coastal provinces, clustering of seropositives within households was not observed after adjustment for other household factors. However, in the Andean province of Loja, the odds of seropositivity were more than two times greater for an individual living in a household with another seropositive person. Our results indicate that transmission of *T. cruzi* is ongoing in Ecuador, although intensity of transmission and mechanisms of interaction between humans and the insect vectors of disease vary between geographic regions.

INTRODUCTION

The presence of human infection with *Trypanosoma cruzi*, the causative agent of Chagas disease, has been documented in Ecuador as early as 1927. During the 1940s and 1950s, new disease foci were reported from the coastal provinces of Guayas, Manabí, and Los Ríos and the Andean provinces of Loja, Azuay, and Bolívar. Today, Guayas and Manabí, along with the coastal province of El Oro, are thought to be the main endemic areas of the country.¹ However, there are no recent reports of population-based studies assessing the current status of *T. cruzi* transmission in these areas. We report the prevalence of *T. cruzi* seropositivity in population-based samples of communities in rural areas of the Manabí, Guayas, and Loja provinces of Ecuador.

Another objective of this study was to estimate the extent to which *T. cruzi* infections cluster within households. In a paper published in 1976, Mott and others² stated “Since transmission of Chagas’ disease largely occurs within households, description and analysis of the characteristics of household clustering of seropositivity to *T. cruzi* are of particular interest.” Knowledge of the extent to which *T. cruzi* clusters within households can provide information about the extent to which an individual’s risk of infection is affected by living in close proximity to another infected person who can serve as a reservoir of *T. cruzi*. Additionally, the persistence of clustering within households after adjustment for other known household risk factors for *T. cruzi* infection may indicate that other as yet unknown factors are involved in the transmission of *T. cruzi* within households. Mott and others² reported significant clustering of *T. cruzi* seropositivity within households but were unable to quantify the degree of clustering or control for the effect of other household factors.² A later analysis by Gurtler and others³ found no effect of clustering after

adjustment for other covariates using a random effects model. Although a random effects model can determine whether the parameter associated with clustering is statistically significant, the parameter itself does not have an easily understandable interpretation in terms of the magnitude of the clustering. We use the technique of alternating logistic regressions (ALRs) introduced by Carey and others⁴ to quantify the degree of clustering of *T. cruzi* infections within households. ALR provides pairwise odds ratios (PORs) of association of the outcome, in this case *T. cruzi* seropositivity, within clusters while also taking into account the dependence of the outcome on individual and cluster-specific covariates. The PORs obtained from ALR are interpreted similarly to conventional ORs, with a POR > 1 indicating an association of seropositivity between individuals within a household. We also show how the POR can be used in the calculation of design effects, which is useful for planning the sample size of future studies in which subjects are sampled in households or other related units.^{4–6}

MATERIALS AND METHODS

Study population. The subjects included in this study were residents of 14 rural communities in the Manabí, Guayas, and Loja provinces of Ecuador (Figure 1). Data were collected between June 2001 and August 2003. Manabí and Guayas are coastal provinces with a tropical climate, whereas Loja is located in the Andean highlands and has a temperate climate. Study personnel visited all households in the selected communities as part of a study of household risk factors for *T. cruzi* transmission. All members of each household were invited to attend study-sponsored medical clinics. All subjects that presented to the medical clinic and donated a blood sample to be tested for serologic evidence of *T. cruzi* infection were included in this study. Informed consent was obtained from all adult participants and parents of minor children. All study procedures were approved by the Institutional Review Boards of Ohio University and Catholic University of Ecuador.

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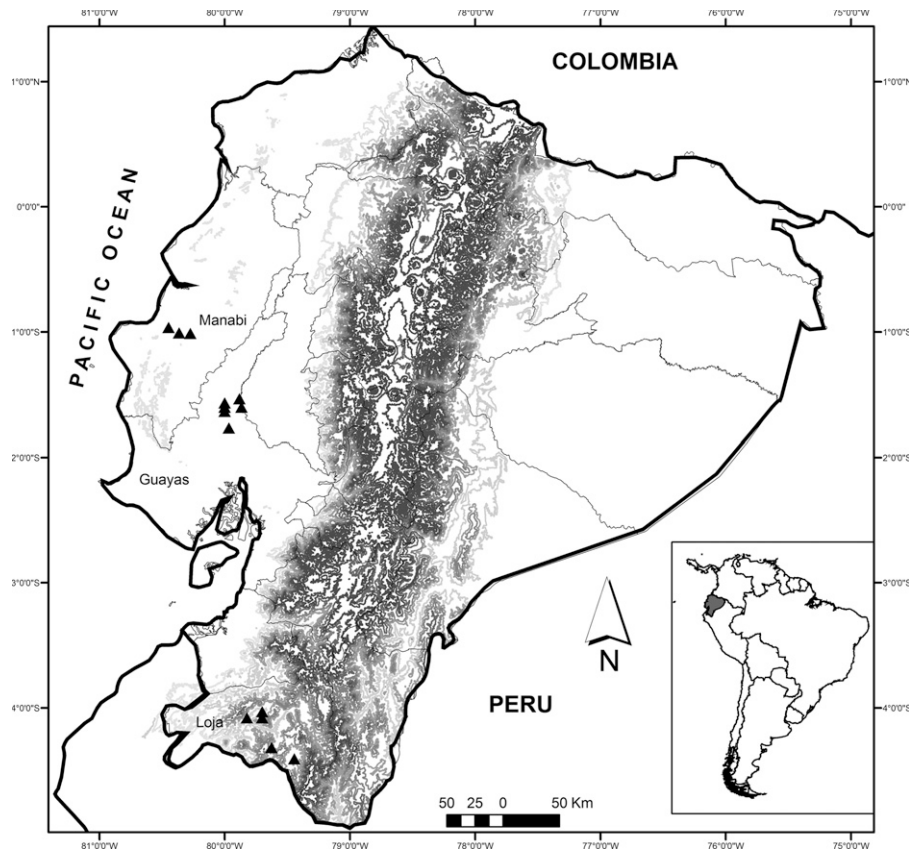


FIGURE 1. Map of Ecuador showing the elevation gradient as a gray scale, the provincial boundaries (solid lines), and the location of the studied communities (triangles).

A total of 4,530 subjects presented to the medical clinics, representing 80% of the eligible households in the study communities; 1,244 subjects were excluded from the analysis because they did not consent to serologic testing, leaving a final sample of 3,286 subjects.

Serologic testing. Blood was collected by venous puncture from all participants. A different serologic testing scheme was used in each of the three study sites. Initial screening in all three sites was performed by enzyme-linked immunosorbent assay (ELISA) using detergent-extracted *T. cruzi* epimastigote antigens as previously described.⁷ The optical density (OD) values of previously confirmed positive and negative controls were analyzed and used to define the limits for seropositivity and seronegativity of the assay. OD values within 2.5 SD of the OD average for the positive controls were considered seropositive, and all OD values within 2.5 SD of the OD average for negative controls were considered seronegative. Samples with OD values outside of the range of positive or negative were classified as borderline. Positive and borderline samples were assayed at least two more times. Samples that were positive at least three times were considered positive. Samples with repeated borderline results were considered negative.

For samples collected in the Manabí province, all samples positive by the initial ELISA were confirmed by immunofluorescence at the Centers for Disease Control and Prevention (Atlanta, GA), with a titer of 1:32 used as the positive cut-off. All samples collected in Guayas province, including those positive and negative by the initial ELISA, were also tested

using a commercially available ELISA kit (Chagatest ELISA recombinante V 3.0; Wiener Laboratories, Rosario, Argentina) and a commercial indirect hemagglutination test (Chagatest HAI; Wiener Laboratories). All samples collected in the Loja province that were positive by the initial ELISA and ~50% of the negative samples were subsequently tested with two commercial ELISA kits (Chagatest ELISA recombinante V 3.0; Wiener Laboratories, and Chagas Serum Antibody Detection Assay Microwell ELISA; IVD Research, Carlsbad, CA) and the Chagatest HAI (Wiener Laboratories). These tests were performed according to the manufacturer's instructions. Samples from Loja and Guayas that were positive by the recombinant ELISA or by at least two of the other tests were considered positive.

Analysis of prevalence. Poisson regression was used to estimate age-specific prevalences and corresponding 95% confidence intervals (CIs) for each province.^{8,9} The log-linear regression model included a linear and a squared variable for age, two indicator variables corresponding to the three provinces, and terms for linear and squared age by province interactions. Age was coded as an ordinal variable ranging from 0 to 7 representing 10-year age categories. Variables for age squared and squared age by province interactions were included to improve model fit. The natural logarithm of the number of subjects in each 10-year age category in each province was included in the model as an offset term to provide a model for the rate of infections as opposed to the count. The fully specified model is shown in supplemental Appendix 1 (available online at www.ajtmh.org).

The variance estimates used in the calculation of CIs were multiplied by the design effect to adjust for possible non-independence of *T. cruzi* infections within households. The design effect is the amount by which the variance of the prevalence estimated under the assumption of simple random sampling must be inflated to account for the clustering of disease. The design effect (D) caused by the correlation of *T. cruzi* seropositivity within households was calculated from the PORs according to the following formula outlined by Katz and Zeger¹⁰:

$$D = 1 + \left(\frac{p_{11} - p^2}{p(1-p)} \right) \left(\left[\frac{m-1}{N} \right] s^2 + \bar{u} - 1 \right)$$

where *p* = prevalence of infection, *m* = the number of households, *N* = the total sample size, *u* = the mean of the household sizes, *s*² = the variance of the household sizes, and *p*₁₁ = the probability that two subjects chosen at random both have disease

$$= \frac{1 - 2p(1-\alpha) - \sqrt{[1 - 2p(1-\alpha)]^2 - 4ap^2(\alpha - 1)}}{2(\alpha - 1)}$$

where α = within-household POR.

Estimation of the PORs is described below. The unadjusted PORs for each region were used in the calculation of the design effects.

Alternating logistic regressions. The magnitude of household clustering of *T. cruzi* seropositivity was estimated in the form of PORs of the association of seropositivity within households. The POR is interpreted as the increased odds in favor of seropositivity for an individual from a household where another individual chosen at random from that household is seropositive relative to the odds in favor of seropositivity if that randomly chosen individual is seronegative.⁵ PORs were estimated with the use of ALRs, as described by Carey and others.⁴ ALRs fit a model for the within-household OR while simultaneously adjusting for the effect of other covariates on the risk of seropositivity. A description of the ALR algorithm is given in supplemental Appendix 2 (available online at www.ajtmh.org).

Separate ALR models were constructed for the coastal provinces, which include Manabí and Guayas, and the Loja province, which is located in the Andean highlands. These two regions are geographically distinct with different insect vectors of *T. cruzi*, and a previous analysis of these data showed that household risk factors for *T. cruzi* differed between the two regions.¹¹ In the previous analysis, type of materials used in the construction of roofs (palm or tile versus metal) and walls (cane or adobe versus cement) of houses were identified as risk factors for *T. cruzi* seropositivity. The presence of firewood and trash in the peridomicile area were additionally associated with seropositivity in the coastal provinces but not in the highlands. These factors were included as adjustment variables in the β model of the ALRs. The resulting final models are shown in supplemental Appendix 2.

ALR analyses were performed with the GENMOD procedure in SAS version 8.2 (SAS, Cary, NC). An exchangeable structure was specified for the POR, assuming constancy across all households.

RESULTS

Prevalence of *T. cruzi* seropositivity. The prevalence of *T. cruzi* seropositivity was 5.7% (59/1041), 1.0% (14/1343), and 3.6% (32/902) in the studied communities of the Manabí, Guayas, and Loja provinces, respectively. Prevalence varied by community within each province (Table 1), ranging from 2.1% to 7.9% in Manabí, 0.6% to 2.0% in Guayas, and 1.2% to 7.2% in Loja.

Model-based age-specific prevalence estimates for each province are shown in Table 2 and Figure 2. In the Manabí province, prevalence increased with increasing age, ranging from 1.5% among children < 10 years of age to a peak of 11.4% in persons 50–59 years of age. Prevalence also increased with age in the Guayas province, from 0.3% among 0–9 year olds to 2.4% in persons \geq 70 years of age. In the province of Loja, the highest prevalence of 7.1% occurred in children < 10 years of age. Prevalence decreased with age until age 30, after which it remained steady at ~2%.

Clustering of *T. cruzi* seropositivity within households. In the coastal provinces of Manabí and Guayas, 73 seropositive subjects were identified from 693 households. Seven of these households had two cases living in the same house. The unadjusted POR for seropositivity within households was 1.42 (95% CI, 0.76, 2.65). The design effect based on this estimate was 1.1. After adjustment for type of roof and the presence of firewood and trash in the peridomicile area, the POR was reduced to 0.97 (0.54, 1.74). A similar estimate of 0.96 (0.52, 1.77) was obtained in a model adjusted for wall type, firewood, and trash. Roof type and wall type were not included in the same model because of co-linearity between the two variables.

In the Loja province, 32 cases were identified in 304 households, with 5 households having 2 cases each. The unadjusted POR was 2.72 (1.18, 6.29), with a corresponding design effect of 1.8. Although housing construction materials were associated with *T. cruzi* seropositivity in the Loja province as well, these variables could not be included as covariates in the ALR model because no seropositive subjects lived in households

TABLE 1
Prevalence of *T. cruzi* seropositivity by community, Ecuador, 2001–2003

Province and community	Number of cases/total sampled	Prevalence (%)
Manabí		
Cruz Alta	42/534	7.87
Pasaje	6/284	2.11
Pimpiguasí	11/223	4.93
Total	59/1,041	5.67
Guayas		
La Alegría	2/289	0.69
Lomas de Colimes	2/220	0.91
Los Angeles	3/175	1.71
Macul	2/337	0.59
Puerto Rico	3/222	1.35
San Antonio	2/100	2.00
Total	14/1,343	1.04
Loja		
Bramaderos	6/173	3.47
Jacapo	9/125	7.20
Naranjo Dulce	9/179	5.03
Pindo Alto	5/173	2.89
Playas	3/252	1.19
Total	32/902	3.55

TABLE 2

Estimated prevalence of *T. cruzi* seropositivity and 95% CIs by geographic region and age category, Ecuador, 2000–2002

Region and age (years)	Number of cases/total sampled	Estimated prevalence* (%) (95% CI)
Manabí		
0–9	3/184	1.45 (0.64, 3.44)
10–19	5/276	2.96 (1.82, 4.81)
20–29	13/159	5.11 (3.58, 7.29)
30–39	9/127	7.67 (5.32, 11.07)
40–49	10/103	10.01 (6.92, 14.48)
50–59	8/83	11.35 (7.96, 16.17)
60–69	6/58	11.18 (7.04, 17.75)
≥ 70	5/42	9.56 (4.32, 21.15)
Guayas		
0–9	1/265	0.33 (0.07, 1.68)
10–19	1/302	0.54 (0.20, 1.45)
20–29	1/214	0.84 (0.40, 1.77)
30–39	1/162	1.20 (0.56, 2.58)
40–49	3/149	1.59 (0.74, 3.44)
50–59	1/118	1.96 (0.96, 4.01)
60–69	3/69	2.24 (0.96, 5.20)
≥ 70	1/64	2.37 (0.59, 9.59)
Loja		
0–9	7/99	7.40 (3.21, 17.09)
10–19	10/243	4.20 (2.42, 7.29)
20–29	4/111	2.77 (1.36, 5.66)
30–39	3/99	2.13 (0.90, 5.09)
40–49	0/84	1.91 (0.78, 4.68)
50–59	2/91	2.00 (0.87, 4.61)
60–69	2/90	2.44 (0.98, 6.04)
≥ 70	3/77	3.45 (0.88, 13.56)

*Prevalence estimated from Poisson loglinear regression.

constructed of cement walls or metal roofs, the referent categories for these variables. All seropositives lived in houses with adobe walls and tile roofs, with the exception of two cases living in a house without walls. An analysis restricted only to subjects from households with tile roofs and adobe or no walls reduced the within household association of *T. cruzi* seropositivity only slightly (POR = 2.39; 1.05, 5.44), indicating that factors other than shared exposure to substandard housing conditions are contributing to the clustering of infections within households. Although the presence of firewood and trash in the peridomicile was not identified as risk factors for seropositivity in the highlands region, a model containing these two variables was run to produce an estimate directly comparable to that from the coastal region. The POR result-

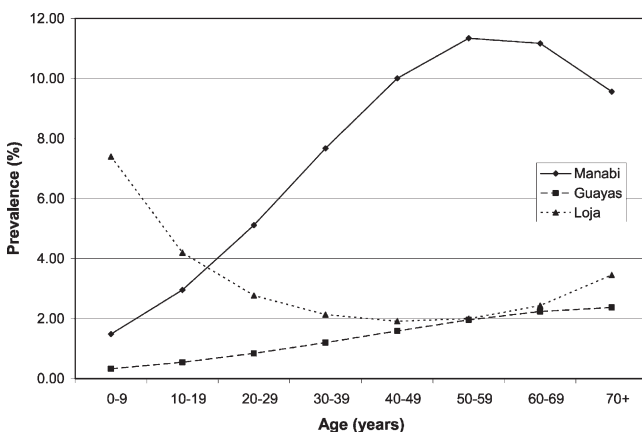


FIGURE 2. Prevalence of *T. cruzi* seropositivity by province and age category, Ecuador, 2001–2003.

ing from this model was 2.51 (1.08, 5.81), also similar to the unadjusted estimate.

DISCUSSION

Our finding of 5.7% overall prevalence of *T. cruzi* seropositivity in the Manabí province is consistent with previous studies, which report seropositivity in various locations in Manabí ranging from 1% to 17%.¹ The pattern of age-specific prevalence is lowest in the youngest ages and steadily increases with increasing age, a pattern that is indicative of a chronic infection that has been endemic in the population for a long period of time. Many previous studies in areas of ongoing endemic *T. cruzi* transmission have shown a proportional increase in prevalence according to age.^{3,12–14} Our results indicate that *T. cruzi* infection remains a significant problem in this region, and there is no evidence of a reduction in transmission over the past 50 years. The continued transmission of *T. cruzi* is also supported by a recent entomologic survey from the same areas of Manabí, which found 21% of households to be infested with triatomine insects capable of serving as vectors of *T. cruzi* (MJ Grijalva, unpublished data).

Like Manabí, the province of Guayas is historically considered to be endemic for *T. cruzi*, with previous estimates of prevalence as high as 24% reported in 1959.¹ We found an overall prevalence in Guayas of 1.0%, an estimate lower than those from previous studies. A trend for increased prevalence with increasing age was observed, suggesting that *T. cruzi* was once endemic in this area. However, the prevalence was very low among those in the youngest age categories (< 1% in all age groups < 30). These results are indicative of a reduction or possible recent interruption of *T. cruzi* transmission in this area. An entomologic survey of 476 households in Guayas conducted in conjunction with this study found evidence of triatomine vectors in only one domicile (MJ Grijalva, unpublished data).

The prevalence of *T. cruzi* infection in the Loja province has not been widely studied. A prevalence rate of 2% from a 1955 survey of schoolchildren is the only previous report from this area that could be found in the medical literature.¹ In this study, we found a prevalence of 7.1% among children < 10 years of age, suggesting that active transmission of *T. cruzi* is occurring in this area. Prevalence did not increase with increasing age as would be expected if *T. cruzi* has been endemic in this area for many years. Conversely, prevalence actually decreased with age in the youngest age groups and remained steady after age 30. This pattern suggests possible recent introduction or re-introduction of *T. cruzi* into this area. A similar result was seen in a recent study by Bowman and others,¹⁵ where prevalence rates were similar in adults and teenagers in an area of Peru where onset of transmission was estimated to have begun 11–12 years before the time of the survey. An entomologic survey conducted concurrently with this study in the same communities of Loja found a household infestation rate by triatomine insects of 35%. The study also found evidence to suggest that the triatomine vectors in this area can colonize human dwellings in a relatively short period of time.¹⁶ This further supports the notion that humans have recently been exposed to triatomine vectors that previously circulated *T. cruzi* in wild ecotopes and have presently become domiciliated.

Because different protocols for serologic testing were used in each province, the actual prevalence estimates are not directly comparable between provinces, although trends across age categories within provinces should be comparable. In the Manabí province, only those samples that were positive by the initial screening test were tested again, and only one additional test was performed on these samples. As such, this was the least sensitive and most specific testing scheme of the three sites, and the reported prevalence from Manabí likely underestimates the true prevalence. The testing scheme used in Guayas was the most sensitive and least specific, because every sample was tested with three separate tests. The true prevalence in the Guayas province was possibly even lower than what we reported.

Our prevalence estimates are also limited by a high non-participation rate. Although persons from 80% of the total households in the study communities reported to the clinics and were thus eligible for inclusion in the survey, we do not have data on the total number of people living in the non-participating households nor the number of the people in the participating households that did not report to the clinics. However, we do know that 75% of the 1,244 subjects that came to the clinics but were excluded from the analysis because of lack of serology were children < 10 years of age. Had these subjects been included in the analysis, the resulting overall prevalence would likely be lower than the reported prevalence in Manabí and Guayas and higher than the reported prevalence in Loja.

In the coastal provinces, the degree of clustering of seropositives within households was small and not statistically significant. The value of the POR became very close to one after adjustment for shared household risk factors for *T. cruzi* infection, indicating that living in the same household as an infected person is not an independent risk factor for *T. cruzi* seropositivity. However, in the Loja province, the risk of seropositivity was more than two times greater if another randomly selected person from the same household was seropositive than if that randomly selected person was seronegative. The difference in household clustering might be explained by different behavior of the triatomine vectors in the two regions. As previously stated, household infestation with triatomine vectors of *T. cruzi* was found in both the Manabí and Loja provinces. In Loja, almost all captured insects were of the species *Rhodnius ecuadoriensis* (98%), with small populations of *Triatoma carrioni* (2%), *Panstrongylus rufotuberculatus* and *Panstrongylus chinai* (one bug each).¹⁶ In Manabí, the majority of captured insects were also *R. ecuadoriensis* (77%), but a sizable population of *Panstrongylus howardi* (22%) and a small number of *P. rufotuberculatus* (0.6%) were also present (MJ Grijalva, unpublished data). However, even though the main vector of *T. cruzi* is the same in both regions, the entomologic situation is quite different. In Loja, bugs were almost exclusively intradomiciliary. Vectors were found in peridomicile areas only in occupied chicken nests.¹⁶ In Manabí, vectors were mainly peridomiciliary. In 64% of infested houses, vectors were found solely in peridomicile areas such as piles of wood and brick and pens of domestic animals, with no bugs present in the intradomicile (MJ Grijalva, unpublished data). Evidence of colonization, indicated by the presence of triatomine eggs and or nymphs, was found in 85% of infested houses in Loja¹⁶ compared with 49% in Manabí (MJ Grijalva, unpublished data).

An analysis of risk factors for *T. cruzi* seropositivity conducted in this same population also supports the notion that differing behavior of triatomine vectors impacts where and how humans become infected. Although construction materials of houses were associated with seropositivity in both regions, peridomicile factors were not associated with seropositivity in Loja, which was expected as triatomine insects were found mainly inside houses. However, in the coastal region, the presence of firewood in the peridomicile increased the risk of seropositivity, possibly because vectors are passively transported into homes along with the firewood or because people are bitten as they carry the wood into their homes. Accumulation of trash and organic matter outside the home was protective against *T. cruzi* seropositivity. While a surprising finding, this could be an indication that vectors have colonized these areas rather than inside the domicile.¹¹

In Manabí, where vectors are more likely peridomiciliary, if humans are being infected outside of the house or by vectors that only sporadically enter the house, an infected person within the house is not likely to serve as a reservoir of *T. cruzi* by which other triatomine insects can become infected. In Loja, where triatomines were found mostly in the intradomicile, humans are probably infected by vectors that have colonized their homes. Once infected, a person serves as a reservoir capable of infecting other insects inside the house, which in turn infect other persons sleeping in close proximity to the index case. Also, the percentage of seropositive individuals with detectable parasitemia has been reported to decline with age.^{17,18} Given the high prevalence of seropositivity among children in Loja, seropositive persons here likely have higher levels of parasitemia than seropositive persons in Manabí and Guayas, who are on average older and may have been infected for several decades. Thus, persons in Loja could be more infective to the triatomine vectors when bitten, and prevalence of *T. cruzi* infection in the vector population is more likely to be associated with human infections here than in the coastal regions, where rodents and domestic animals are probably more important sources of infection for the vectors. Another possible explanation for the observed clustering of seropositivity within households in Loja is that other unmeasured household characteristics are associated with *T. cruzi* transmission in this area.

Based on the PORs, we calculated design effects of 1.1 for the coastal region and 1.8 for Loja province. An estimation of design effects is often important when conducting population-based surveys in developing countries where random sampling is not always feasible and subjects are routinely sampled by households, villages, or other related units. Although the design effect for the coastal region is small because of the low degree of household clustering in this region, a study of *T. cruzi* prevalence in Loja using a household sampling scheme would require a sample almost twice as large as a study design in which subjects were randomly sampled to produce prevalence estimates with equivalent precision. Because design effects are a function of within-cluster correlation, prevalence of disease, and cluster sizes, design effects can vary across studies. Relying on design effects reported from previous studies is not always useful for the planning of future studies. Because PORs are consistent across studies, PORs can be used to calculate design effects and estimate sample sizes for future studies when cluster sizes can be predetermined or predicted by the investigator and expected prevalence of the outcome is known.^{5,10} It should

be noted that unadjusted PORs were used in the calculation of design effects. Even if household clustering of *T. cruzi* cases could be explained by shared household factors or other variables, the phenomenon that cases of *T. cruzi* are more likely to occur in the same household still exists and must be accounted for to produce accurate estimates of prevalence when a study design using household sampling is used.

In conclusion, we found evidence of *T. cruzi* infection in all three studied provinces of Ecuador. Prevalence patterns were suggestive of endemic infection in the Manabí province, reduction in transmission over time in the Guayas province, and recent introduction or re-introduction of transmission in the Loja province. *T. cruzi* infections clustered within households in the Loja province, whereas significant clustering was not observed in the coastal provinces. Differences in clustering between the regions might be caused by differing behavior of the triatomine vectors in each region or might be an indication that other unknown household factors play a role in the transmission of *T. cruzi* in the highlands region.

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Note: Supplemental Appendices 1 and 2 can be found online at www.ajtmh.org.

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