Spatial Patterns in Discordant Diagnostic Test Results for Chagas Disease: Links to Transmission Hotspots

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Diagnosis of Chagas disease is hindered by discordance between screening and confirmatory test results for Trypanosoma cruzi infection. In periurban Arequipa, Peru, spatial analysis revealed that individuals with discordant test results are spatially clustered in hotspots of T. cruzi transmission, suggesting that discordant results likely represent true infections in this setting.

Trypanosoma cruzi, the etiologic agent of Chagas disease, causes more morbidity and mortality in the Americas than any other parasite [1]. An estimated 20%–30% of persons infected with T. cruzi develop cardiac or digestive disease years to decades after the initial infection [1]. Drug treatment can prevent disease progression, but efficacy is thought to decrease with increasing duration of infection [2]. Population screening is therefore recommended for the early detection and treatment of T. cruzi infection [3]. Screening, however, is hindered by the lack of a gold standard test for diagnosis of chronic T. cruzi infection. Diagnosis currently requires positive results on ≥2 serological tests based on different principles or antigens [1]. Discordance between test results occurs in a proportion of specimens in nearly all comparative studies and is often difficult to resolve [4]. In some cases, discordance is attributable to infection by cross-reacting organisms, such as Leishmania species or Trypanosoma rangeli [5]. In large-scale population surveys, participants are usually tested first by a sensitive screening assay, such as an ELISA, followed by a more specific confirmatory test. Participants with positive screening results but negative confirmatory test results are not generally offered etiological treatment, on the basis of the assumption that discordant results represent false-positive results due to inadequate screening test specificity.

In a community-based study in the periurban community of Arequipa in southern Peru, we demonstrated marked spatial aggregation of confirmed cases of T. cruzi infection in transmission “hotspots” [6], and others have noted high degrees of household clustering elsewhere [7, 8]. When cases of T. cruzi infection are clustered, spatial information can serve as a proxy measure for parasite exposure, and spatial analysis provides an independent basis to evaluate assumptions about discordance between screening and confirmatory test results. Our objective was to use spatial analysis to test whether discordant results in the periurban community survey data were due to lack of screening test specificity or lack of confirmatory test sensitivity. If discordant results were due to low screening test specificity, whether ultimately caused by an intrinsic error of the test or by a cross-reaction to another independently transmitted parasite, they should be randomly distributed relative to individuals with confirmed diagnosis in the community. If, on the other hand, discordant results represented true infections and were due to low sensitivity of confirmatory tests, they would be more likely to occur among individuals residing in spatial hotspots of T. cruzi transmission.

Methods. Arequipa is a city of ∼860,000 inhabitants in the southern Peruvian highlands. Vector-borne T. cruzi transmission has become established, especially in periurban pueblos jovenes (poorer recent settlements) [9]. A vector control campaign initiated in 2002 interrupted transmission in 5 periurban districts, but many individuals were infected before their houses were sprayed with insecticide. The present study was conducted in 1 pueblo joven, Guadalupe, on the southwestern margin of the city. No instances of Leishmania transmission have been
reported in the area, and insects of the genus *Rhodnius* [10], which serve as vectors of *T. rangeli*, are not present in southern Peru [6].

Serologic testing was conducted from August through October 2005; methods were described previously [9]. In brief, a 5-mL peripheral blood specimen (3 mL for children aged <5 years) was collected from each participant. Specimens were stored at 4°C until blood was separated by centrifugation; serum specimens were then stored at −20°C in aliquots.

Serum specimens were screened for antibodies to *T. cruzi* by means of a commercial ELISA kit with an epimastigote lysate antigen (Chagatek; bioMérieux); the threshold for positive was 0.100 units above the mean optical density of 2 negative control specimens on each plate, according to the manufacturer’s instructions.

Two confirmatory tests, the immunofluorescent antibody assay (IFA) and the radioimmunoprecipitation assay (RIPA), were performed on all ELISA-positive samples and 12% of the ELISA-negative samples. The IFA was performed at the Centers for Disease Control and Prevention (Atlanta, GA), according to standard methods; a titer of 1:32 was considered a positive result. The RIPA test was performed in the laboratory of Dr. L. V. Kirchhoff, at the University of Iowa (Iowa City), according to methods published elsewhere [4]. The study protocol was approved by the institutional review boards of the Johns Hopkins Bloomberg School of Public Health and 3 additional institutions. Confirmed seropositive children (ELISA+/IFA+) were offered treatment by the Ministry of Health. Confirmed seropositive adults were referred for evaluation of symptoms or signs of chronic Chagas disease.

We conducted a spatial analysis of discordant results. The geographic position of each household was determined with a handheld global positioning system unit with an accuracy of 10 m (Garmin). We tested the null hypothesis underlying current diagnostic algorithms—namely, that discordant results are random events arising from the lack of screening test specificity. The residences of confirmed positive (ELISA+/IFA+) individuals were known to be spatially clustered [6]. However, under the null hypothesis, the house locations of individuals with discordant results should be distributed at random and no closer to houses of ELISA+/IFA+ individuals than expected by chance. We calculated the average distance between the residence of individuals with discordant test results and that of the closest ELISA+/IFA+ individual. We used a random-labeling simulation [11] to determine the Monte Carlo significance level of the observed statistic under the null hypothesis. Analyses were performed using the R statistical package, version 2.6 (http://www.r-project.org).

In each simulation, we randomly selected a group of individuals equal in number to the population of discordant (ELISA+/IFA−) individuals (13 individuals), excluding ELISA+/IFA+ individuals. We calculated the average distance between these randomly selected individuals’ houses and the nearest observed ELISA+/IFA+ individual’s house. We repeated the simulation 10,000 times to create a histogram describing the null distribution of this test statistic [11]. The *P* value for our observed data was estimated as the percentile of the observed test statistic in this histogram of simulated test statistics. In the same manner, we also tested whether the ELISA+/IFA−/RIPA− test pattern (6 individuals) occurred due to a lack of specificity of the ELISA screening test.

**Results.** Of 1053 participants, 60 (5.7%) had positive ELISA results. Forty-seven (78.3%) ELISA+ specimens were positive by IFA and RIPA, while 13 (21.7%) had discordant results (7 ELISA+/IFA−/RIPA+ and 6 ELISA+/IFA+/RIPA−). Spatial data were linked to serological results for 994 participants (94.4%), including 43 of 47 confirmed positive and all 13 with discordant results (figure 1). The 13 participants with discordant results lived a mean distance of 21.36 m from a household with an individual with confirmed positive results, similar to the distance between households with an individual with confirmed positive results in each (21.13 m). The mean distance between households of 13 randomly selected individuals and households with confirmed cases was 51.5 m (95% CI, 31–74 m). In only 13 of 10,000 random-labeling simulations was the average distance to a confirmed case <21.36 m (*P* = .001 against the null hypothesis). The observed mean distance of the 6 ELISA+/IFA−/RIPA− individuals to a confirmed case was 18.47 m, compared with a mean distance of 51.5 m (95% CI, 21.6–85.58 m) from random-label simulations. The *P* value testing the null hypothesis that the 6 ELISA+/IFA−/RIPA− were randomly distributed in the community was *P* = .013. The 7 ELISA+/IFA+/RIPA+ individuals lived an average of 23.84 m from confirmed positive cases.

**Discussion.** Available antitrypanosomal drugs have frequent, occasionally severe, adverse effects [3], and Chagas disease can be fatal. Precautions to avoid unnecessary drug treatment must be balanced against the consequences of withholding potentially beneficial therapy. In periurban Areequipa, we found that individuals with discordant test results lived significantly closer to those with confirmed infection than expected by chance, suggesting that these participants likely had true *T. cruzi* infection. We cannot exclude the possibility that a cross-reacting organism was present in our study site. However, individuals with discordant results associated with a cross-reacting organism would likely be spatially clustered around each other, rather than around hotspots of confirmed *T. cruzi* infection at opposite ends of the community, as we observed. In areas with transmission of *Leishmania* species or *T. rangeli*, more involved spatial analyses may be necessary to determine whether one of these agents could be responsible for discordant serologic tests for *T. cruzi*. 
and with consideration of the specifics of each area of endem-icity [12]. Targeted screening and available diagnostic tests are imperfect, and current drugs lack optimal efficacy and have significant adverse effect profiles. Nevertheless, if these partial solutions are combined wisely, many more patients could access appropriate treatment while minimizing unnecessary risk.

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References


Figure 1. Locations of individuals with confirmed positive test results and with discordant test results for Chagas disease in Guadalupe, Arequipa, Peru. Filled circles, 43 confirmed positive case patients residing in 31 households; empty squares, the 7 houses in which ELISA+/IFA—/RIPA— individuals lived; empty triangles, the 6 households in which ELISA+/IFA—/RIPA— individuals lived; empty circles, households with no confirmed cases (locations of the last have been randomly perturbed to protect the anonymity of research subjects).

Consideration of spatial and epidemiologic evidence along with diagnostic test results could improve screening for Chagas disease. In periurban Arequipa and possibly other areas affected by T. cruzi, the spatial location of an individual’s place of residence is a key component of his or her medical history and should be taken into account by clinicians if diagnostic test results are inconclusive. Spatial and epidemiologic information may also be used to target screening to high-risk subpopulations, further increasing the positive predictive value of diagnostic tests. A recent commentary emphasized that the partial solutions currently available to control T. cruzi infection could have a substantial impact if applied in a coordinated manner.