Short Report: Use of a Rapid Test on Umbilical Cord Blood to Screen for Trypanosoma cruzi Infection in Pregnant Women in Argentina, Bolivia, Honduras, and México

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Abstract. We conducted a cross-sectional study of Chagas disease in five endemic areas in Argentina, Bolivia, Honduras, and México to estimate the prevalence of Trypanosoma cruzi–specific antibodies in pregnant women, and to assess the use of a rapid test (Chagas Stat-Pak) to screen for T. cruzi infection at the time of delivery. The prevalence of antibodies to T. cruzi measured by enzyme-linked immunosorbent assay (ELISA) in maternal blood was 5.5% (a range of 0.8–28.8% among the countries) in 2,495 women enrolled. Compared with ELISA in maternal blood samples, the Chagas Stat-Pak rapid test sensitivity and specificity in umbilical cord blood were 94.6% and 99.0%, respectively. These results show the ability for a rapid determination of the presence of T. cruzi–specific antibodies in umbilical cord blood as a pragmatic strategy to screen for infection in pregnant women.

Chagas disease, or American Trypanosomiasis, is caused by the protozoan parasite Trypanosoma cruzi. It is a major cause of morbidity and mortality in Latin America. Infection is transmitted mainly by vectors, but also by transfusion of infected blood. A strategy to identify maternal infection at delivery will be an asset to select children needing care. Umbilical cord blood is readily available for testing among women delivering in health facilities, and avoids additional venipuncture of the mother. However, the placental transfer of maternal antibodies to T. cruzi measured by enzyme-linked immunosorbent assay (ELISA) in maternal blood was 5.5% (a range of 0.8–28.8% among the countries) in 2,495 women enrolled. Compared with ELISA in maternal blood samples, the Chagas Stat-Pak rapid test sensitivity and specificity in umbilical cord blood were 94.6% and 99.0%, respectively.

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with maternal blood using ELISA as the gold standard, with their respective exact binomial 95% CI. A sample size of 500 women per site provided a 95% precision rate of ± 2% if the prevalence was 6%. All tests and procedures were standardized in all sites. The analyses were done using SPSS version 14.0 (SPSS, Chicago, IL) and Epi Info version 3.4 (CDC).

The study was approved by the Institutional Review Board of Tulane University and the ethics committees of the respective participating institutions. Newborns from mothers confirmed positive for \textit{T. cruzi} infection were referred for appropriate clinical follow-up and treatment if needed, according to the established local practices at each site.

Among 2,495 female participants (Figure 1), the distribution of population by site was 518 women in Tucumán, 488 in Santa Cruz, 500 in Intibucá, and 988 in Celaya and Mérida. The mean age was 25.3 ± 6.0 years. Among total women enrolled in the study, 205 volunteers were positive by ELISA for antibodies to \textit{T. cruzi} infection, yielding a median of prevalence of 5.5%. The seroprevalence rate was heterogeneous between countries; the highest rate was observed in Santa Cruz (Bolivia) (28.8%), followed by Tucumán (Argentina) (6.6%), Intibucá (Honduras) (4.4%), and Celaya and Mérida (México) (0.8%) (Table 1). The absorbance of \textit{T. cruzi}-specific antibodies in maternal samples assayed by ELISA showed a strong correlation with that of umbilical cord samples ($r = 0.986$). Concordance among ELISA and Chagas Stat-Pak for maternal and umbilical cord samples were 98% (CI = 97.5–96.6) and 98.6% (CI = 98.1–99.1), respectively. At most sites, the prevalence from samples tested by Chagas Stat-Pak in cord blood were in close agreement with the rates obtained with the gold standard (ELISA in maternal blood). Only results from Intibucá (Honduras) showed a significant difference between maternal rates using ELISA and umbilical cord samples using Stat-Pak ($P < 0.01$), (Table 1). Overall, the performance of the Chagas Stat-Pak for detection of anti-\textit{T. cruzi} antibodies in umbilical cord samples in comparison with the gold standard showed values of 94.6% Se; 99% Sp; 98.6% Ac; 89% PPV; and 99.5% PNV. Test performance varied among countries (Table 2).

All sites used a standardized methodology to collect data and samples, which allowed us to reduce internal bias. However, the results presented in this study have some limitations,
TABLE 2

Detection of Trypanosoma cruzi-specific antibodies in cord blood samples tested by a Chagas Stat-Pak rapid test in comparison with the maternal blood samples tested by ELISA, among 2,495 pregnant women from Argentina, Bolivia, Honduras, and México, 2006–2007

| Umbilical cord samples tested by Stat-Pak in comparison with maternal blood samples tested by ELISA | Tucumán (Argentina) (N = 518) | Santa Cruz (Bolivia) (N = 488) | Intibucá (Honduras) (N = 500) | Celaya and Mérida (México) (N = 988) | Total (N = 2,495) |
|---|---|---|---|---|---|
| % | [95% CI] | % | [95% CI] | % | [95% CI] | % | [95% CI] | % | [95% CI] |
| Sensitivity | 85.3 | [71.9; 98.7] | 98.6 | [96.3; 100.0] | 95.4 | [84.5; 100.0] | 62.5 | [22.7; 100.0] | 94.6 | [91.3; 98.0] |
| Specificity | 99.4 | [98.6; 100.0] | 98.6 | [97.2; 100.0] | 97.5 | [96.0; 99.0] | 99.6 | [99.2; 100.0] | 99.0 | [98.5; 99.4] |
| Accuracy | 98.5 | [97.3; 99.6] | 98.6 | [97.4; 99.7] | 97.4 | [95.9; 98.9] | 99.3 | [98.7; 99.9] | 98.6 | [98.1; 99.1] |
| Positive predictive value | 90.6 | [79.0; 100.0] | 96.5 | [93.2; 99.9] | 63.6 | [45.7; 81.6] | 55.6 | [17.5; 99.5] | 89.0 | [84.6; 93.4] |
| Negative Predictive value | 99.0 | [98.0; 100.0] | 99.4 | [98.5; 100.0] | 99.8 | [99.3; 100.0] | 99.7 | [99.3; 100.0] | 99.5 | [99.2; 99.8] |

such as the pregnant women who participated may not be representative of pregnant women among the general population. Furthermore, participating hospitals were not randomly selected from among other hospitals in the country or study area, and only women older than 17 years of age were enrolled. For this reason, our results are restricted to women older than 17 years of age that sought care in the study sites selected for convenience. We also lost precision for the estimation of prevalence in Mexico and Honduras, because the measured prevalence was unexpectedly lower than that used for the sample size calculation, which was based on previous estimates of seroprevalence. The external quality control of ELISA in maternal blood samples showed Kappa indexes of 0.83, 0.97, and 0.93 in Tucumán, Intibucá, and Celaya and Mérida, respectively (P < 0.05).

Our study recruited women from five sites with some differences in socio-demographic characteristics, such as age, formal education level, and residence in urban or rural areas (data not shown). Differences in the characteristics of the study area, the dynamics of vectorial transmission, the history of programs for the control and prevention of vectorial and non-vectorial transmission in each country, and inter-strain variability of T. cruzi may be additional factors to consider when interpreting differences in prevalence.

The Chagas Stat-Pak has been found to be appropriate for screening asymptomatic T. cruzi infection in a rapid assessment of schoolchildren. However, a field evaluation in children and adolescents from Bolivia has shown lower sensitivity. Our results have shown that the Chagas Stat-Pak was able to detect maternal antibodies in umbilical blood samples. However, its sensitivity was heterogeneous among countries.

Some of the potential explanations for this finding include: 1) Differences in levels of T. cruzi–specific antibodies in maternal and umbilical cord blood samples were 0.98, 0.54, and 0.05 for concordant samples, ELISA reactive and Stat-Pak not reactive, and ELISA not reactive and Stat-Pak reactive, respectively. These results suggest that in some cases, differences in antibody concentrations in umbilical cord can affect the ability of Stat-Pak to detect it. 2) A different type of antigen used in the assays; however, our data do not permit us to clarify this issue. The lower sensitivity of the rapid test in Mexico could be the result of poor detection by the antigen used in the test to the specific immune response induced for the autochthonous strain of parasites. 3) Technical errors while performing or reading the tests. Although the Chagas Stat-Pak test is easy to perform, it uses a visual inspection of bands, which may depend on the subjectivity of the operator. The results in the external quality control show that the validation of the Stat-Pak assay can be affected by the variable performance of the ELISA gold standard at the different study sites.

We have shown in this pilot study a pragmatic strategy using umbilical cord samples and a rapid test to screen T. cruzi in pregnant women, which permits assessment in results in minutes and would be convenient in primary health care settings. Other large-scale studies would be necessary to confirm the viability of the strategy and to assess cost-effectiveness and acceptability. The potential use of this strategy is based on the fact that the collection of cord blood samples is done routinely in several countries for perinatal screening, including all participating sites, as part of perinatal care, making this proposed strategy even more viable.

Research about the risk of congenital T. cruzi infection and the effective detection of infected newborns is essential, because it is recognized as a major cause of transmission in non-endemic countries and endemic countries with relatively successful vector control and blood screening programs. The elimination of congenital T. cruzi infection will be a critical final step toward the elimination of Chagas disease, after elimination of transmission by vectors and blood transfusion.

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REFERENCES

1. World Health Organization. 2002. Report of the Expert Committee on the Control of Chagas Disease. Geneva: World Health Organization, 115.

2. Cumberland P, Shulman CE, Maple PA, Bulmer JN, Dorman EK, Kwuondo K, Marsh K, Cutts FT. 2007. Maternal HIV infection and placental malaria reduce transplacental antibody transfer and tetanus antibody levels in newborns in Kenya. J Infect Dis 196; 550–557.

3. Torrico F, Carlier Y, 2005. Immune responses of non-infected neonates of mothers infected with Trypanosoma cruzi. Rev Soc Bras Med Trop 38 (Suppl 2); 96–100.

4. Lin S, Sartori MJ, Mezzano L, de Fabro SP, 2005. Placental alkaline phosphatase (PLAP) enzyme activity and binding to IgG in Chagas' disease. Placenta 26: 789–795.

5. Caballero ZC, Sousa OE, Marques WP, Saez-Alquezar A, Umezawa ES, 2007. Evaluation of serological tests to identify Trypanosoma cruzi infection in humans and determine cross-reactivity with Trypanosoma rangeli and Leishmania spp. Clin Vaccine Immunol 14: 1045–1049.

6. Luquetti AO, Ponce C, Ponce E, Esfandiari J, Schijman A, Revollo S, Anez N, Zingales B, Ramgel-Aldao R, Gonzalez A, Levin MJ, Umezawa ES, Franço da Silveira J, 2003. Chagas' disease diagnosis: an interlaboratory evaluation of Chagas Stat-Pak, a rapid immunochromatographic assay with recombinant proteins of Trypanosoma cruzi. Diagn Microbiol Infect Dis 46: 265–271.

7. Ponce C, Ponce E, Vinelli E, Montoya A, de Aguilar V, Gonzalez A, Zingales B, Ramgel-Aldao R, Levin MJ, Esfandiari J, Umezawa ES, Luquetti AO, da Silveira JF, 2005. Validation of a rapid and reliable test for diagnosis of Chagas’ disease by detection of Trypanosoma cruzi-specific antibodies in blood of donors and patients in Central America. J Clin Microbiol 43: 5085–5088.

8. Cruz-Reyes and Pickering-Jones L. 2006. Chagas disease in Mexico: an analysis of geographical distribution during the past 76 years—a review. Mem Inst Oswaldo Cruz 101: 345–354.

9. Guzman-Bracho C. 2001. Epidemiology of Chagas disease in Mexico: an update. Trends Parasitol 17: 372–376.

10. Lopez-Cardenas J, Gonzalez Bravo FE, Salazar Schettino PM, Galagaga Solorzano JC, Ramirez J, Martinez Mendez J, Sanchez-Cordero V, Peterson AT, Ramsey J, 2005. Fine-scale predictions of distributions of Chagas disease vectors in the state of Guanajuato, Mexico. J Med Entomol 42; 1088–1081.

11. Dumontel E, 1999. Update on Chagas’ disease in Mexico. Salud Pública Mex 41: 322–327.

12. Cortez MR, Emperaire L, Piccinini RV, Gurtler RE, Torrico F, Jansen AM, Noireau F, 2007. Sylvatic Triatoma infestans (Reduviidae, Triatominae) in the Andean valleys of Bolivia. Acta Trop 102: 47–54.

13. Pizarro JC, Lucero DE, Stevens L, 2007. PCR reveals significantly higher rates of Trypanosoma cruzi infection than microscopy in the Chagas vector, Triatoma infestans: high rates found in Chiquisica, Bolivia. BMC Infect Dis 7: 66.

14. Blanco SB, Segura EL, Cura EN, Chuit R, Tulian L, Flores I, Garbarino G, Villalonga JF, Gurtler RE, 2000. Congenital transmission of Trypanosoma cruzi: an operational outline for detecting and treating infected infants in north-western Argentina. Trop Med Int Health 5: 293–301.

15. Coll-Cardenas R, Espinoza-Gomez F, Maldonado-Rodriguez A, Reyes-Lopez PA, Huerta-Viera M, Rojas-Larios F, 2004. Active transmission of human Chagas disease in Colima Mexico. Mem Inst Oswaldo Cruz 99: 363–368.

16. Guzman-Bracho C, Lahuruta S, Velasco-Castrejon O, 1998. Chagas disease. First congenital case report. Arch Med Res 29: 195–196.

17. Sosa-Estani S, 2005. Congenital transmission of Trypanosoma cruzi infection in Argentina. Rev Soc Bras Med Trop 38 (Suppl 2): 29–32.

18. Moncayo A, 2003. Chagas disease: current epidemiological trends after the interruption of vectorial and transfusional transmission in the southern cone countries. Mem Inst Oswaldo Cruz 98: 577–591.

19. Velasco-Castrejon O, Valdespino JL, Tapia-Conyer R, Salaverry B, Guzman-Bracho C, Magos C, Lauvas A, Gutierrez G, Sepulveda J, 1992. Seropidemiology of Chagas disease in Mexico. Salud Publica Mex 34: 186–196.

20. Pan American Health Organization. 2006. Acuerdos y recomendaciones de la IXa reunión anual de la OPA (Guatemala, 11–13 September 2006), 2007: 5.

21. Pan American Health Organization. 2004. XIIa reunión de la comisión intergubernamental del cono sur para la eliminación de triatominae instellas y la interrupción de la transmisión transfusional de la tripanosomiasis americana (INCOUS/Chagas, March 26–28, 2003), 2007: 24. Available at: http://www.paho.org/SPAN/AD/DPC/CD/ch-dh-XII-INCOUS-INF-final-arg.pdf. Accessed December 3, 2007.

22. Zaidenberg M, Spellman C, Carrizo Pérez R, 2004. Control de...
Chagas en la Argentina. su evolución. Rev Arg de Cardiol 72: 375–380.

24. Roddy P, Goiri J, Flevaud L, Palma PP, Morote S, Lima N, Villa L, Torrico F, Albajar-Vinas P, 2008. Field evaluation of a rapid immunochromatographic assay for detection of Trypanosoma cruzi infection by use of whole blood. J Clin Microbiol 46: 2022–2027.

25. Russomando G, de Tomassone MM, de Guillen I, Acosta N, Vera N, Almiron M, Candia N, Calcena MF, Figueredo A, 1998. Treatment of congenital Chagas’ disease diagnosed and followed up by the polymerase chain reaction. Am J Trop Med Hyg 59: 487–491.

26. Garcia A, Bahamonde M, Verdugo S, Correa J, Pastene C, Solari A, Tassara R, Lorca M, 2001. Trypanosoma cruzi transplacental infection: situation in Chile. Rev Med Chil 129: 330–332.

27. Torrico F, Alonso-Vega C, Suarez E, Rodriguez P, Torrico MC, Dramaix M, Truyens C, Carlier Y, 2004. Maternal Trypanosoma cruzi infection, pregnancy outcome, morbidity, and mortality of congenitally infected and non-infected newborns in Bolivia. Am J Trop Med Hyg 70: 201–209.

28. Brutus L, Schneider D, Postigo J, Delgado W, Mollinedo S, Chipaux JP, 2007. Evidence of congenital transmission of Trypanosoma cruzi in a vector-free area of Bolivia. Trans R Soc Trop Med Hyg 101: 1159–1160.