Some Limitations for Early Diagnosis of Congenital Chagas Infection by PCR

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Trypanosoma cruzi, the causing agent of Chagas disease, can be transmitted to the offspring of infected pregnant women, thus being an epidemiologically important way of parasite transmission in humans. In addition, the migration of infected women from endemic areas to nonendemic countries may export this parasite infection. The diagnosis of congenital Chagas disease relies on the detection of the parasite because maternal antibodies are passively transferred to infants during pregnancy. The diagnosis of congenital infection can also be confirmed by detection of infant-specific anti–*T cruzi* antibodies at 10 months after delivery. Because early detection of *T cruzi* infection in newborns allows an efficient trypanocidal treatment and cure, more sensitive molecular techniques such as DNA amplification are being used for a prompt parasitological diagnosis of children born to seropositive mothers. In this report, we describe a diagnosis case of a child congenitally infected with *T cruzi* who tested negative for parasite detection both by microscopic observation and DNA amplification at 20 days and 6 months after delivery. However, at 7 months of age, a hemoculture was made from the infant's blood, and the infective parasite was finally isolated and classified as *T cruzi* discrete typing unit I. In a retrospective study, real-time polymerase chain reaction also allowed detecting the parasite but failed to detect any parasite load in earlier control samples. This case report stresses that even when molecular techniques are negative, a long-term follow-up is necessary for the diagnosis of infants congenitally infected with *T cruzi*.

Trypanosoma cruzi is the etiological agent of Chagas disease. This hemoflagellate protozoan parasite is genetically diverse and classified into 6 discrete typing units (DTUs), *T cruzi* discrete typing unit I (TcI) through *T cruzi* discrete typing unit VI (TcVI). Regarding the *T cruzi* DTUs infecting humans, TcI is found mainly in the north of the Amazon basin but is usually associated with the sylvatic cycle throughout the American continent. *T cruzi* DTU II is found mainly in the Atlantic and central Brazil, whereas *T cruzi* discrete typing unit V (TcV) and TcVI are found in the American Southern Cone, in the Gran Chaco area.[1,2]

Congenital infection with *T cruzi*, also called vertical transmission, occurs in ∼5% of the children born to chronically infected mothers in endemic areas and can be repeated in each pregnancy (family clustering of congenital cases).[3] In areas in which the insect vector is controlled, congenital infection has become the most important epidemiologic route of transmission.[4] In the last few decades, increasing international migration and travel from Latin America to nonendemic countries have favored the emergence of Chagas disease outside its "historical" boundaries,[3,5] and the infection has been increasingly

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TABLE 1 Serological and Parasitological Tests Performed in Mother and Child During the 7-Month Follow-up

Sample	Age	Serology			Parasitology				
		ELISA[a]	IHA[b]	IIF[c]	INPmm	cPCR[14]	qPCR[d]	HC[10]	DTU
Mother	23 y	0.281	128	256	Not tested	Undetectable	Undetectable	Not tested	—
Child	20 d	0.288	64	128	Undetectable	Undetectable	Undetectable	Undetectable	—
Child	6 mo	0.283	Neg	128	Undetectable	Undetectable	Undetectable	Undetectable	—
Child	7 mo	0.369	128	128	Not tested	Pos	47 Pe/mL	Pos	TcI

Mother and child antibody titers and parasitological studies performed in 3 control visits at 1, 6, and 7 mo after delivery. ELISA, enzyme-linked immunosorbent assay titers; IHA, indirect hemagglutination; IIF, indirect immunofluorescence; INPmm, Instituto Nacional de Parasitología "Dr Mario Fatala Chaben" micromethod[8]; Neg, negative; Pe/mL, parasite equivalents per milliliter; Pos, positive; —, not applicable.

[a] Expressed as the optical density at 490 nm (considered reactive when >0.200).

[b] Expressed as the inverse of the reactive serum dilution (considered reactive when ≥32).

[c] Expressed as the inverse of the reactive serum dilution[8] (considered reactive when ≥32).

[d] With Sybr Green and TaqMan protocols.[9–11]

detected in the United States, Canada, Europe, and Western Pacific countries, with >300 000 cases of *T cruzi* infection only in the United States[6] and >60 000 cases in Spain.[7]

T cruzi congenital infection is often asymptomatic at birth and frequently remains undetected, so undiagnosed children might develop a serious chronic infection later in their adult life.[3,8]

According to our Chagas National Program, an early diagnosis of infants born to pregnant women infected with *T cruzi* relies on the direct examination of fresh blood samples by microscopic observation of the blood buffy coat (Instituto Nacional de Parasitología "Dr Mario Fatala Chaben" micromethod),[8] with a limited analytical sensitivity (~40–50 parasites per mL), when maternal transferred antibodies could still be present. If parasites are not detected, infants have to be followed-up and return for a second parasitological and serological control at ~6 months of age, and when the result for parasite detection is still negative, a third control visit is pursued ~10 months of age for detection of parasite-specific antibodies through serological assays, which will confirm the positive or negative diagnosis for the *T cruzi* infection. These diagnostic tests are available at a low cost in primary health care facilities. Nowadays, several specialized and equipped laboratories can perform molecular techniques to detect parasite DNA (conventional polymerase chain reaction [cPCR] or quantitative polymerase chain reaction [qPCR]), which have proved to have substantially higher sensitivity for parasite detection, ~0.1 to 1 parasite equivalents per milliliter,[9–11] but are rarely performed in rural endemic areas.

An early diagnosis of congenital *T cruzi* transmission in newborns is important because it assures an effective trypanocidal treatment that is well-tolerated and results in the cure of *T cruzi* infection.[12] In this article, we report an infrequent diagnosis of *T cruzi* congenital infection in which molecular tools such as cPCR or qPCR did not detect any parasite load until 7 months of age, in a retrospective study.

CASE REPORT

This infant was born by caesarean delivery in the city of Buenos Aires, Argentina, a nonendemic area, and was the first child of his mother. His mother, a 23-year-old asymptomatic woman infected with *T cruzi*, was also born in Buenos Aires, but his grandmother had been born in an endemic area in the northwest of Argentina. Because this infant's mother resided in a nonendemic area and had not returned to any endemic area even after her infant was born, the parasite might have infected this infant only through congenital transmission.[13]

This infant, who was asymptomatic for congenital Chagas disease, has been breastfed and was first brought to the Instituto Nacional de Parasitología "Dr Mario Fatala Chaben" (Buenos Aires) at 20 days after delivery. His serum showed reactivity to anti–*T cruzi* antibodies, but the results for parasite detection were negative when searched by microscopy, by cPCR,[14] and by hemoculture ([HC] a parasitological diagnosis method not currently used in the Chagas Diagnosis Reference Guidelines because it is labor- and time-consuming, but it was performed in this case for research purposes).[10] Because no infecting parasites were found in the newborn's first control, his mother was then cited for a second visit when the infant was 6 months of age for another parasitological control. Table 1 describes the titers for the 3 serological techniques[8] in which stable titers can be observed for enzyme-linked immunosorbent and indirect immunofluorescence assays comparing the child's antibody levels in the first and second control performed at 20 days and 6 months of age, respectively.

Because the parasitological techniques detected no parasites at 6 months of age and the clearance of the mother's transferred antibodies could not be observed, the infant's mother was then invited to come back to our institution and bring her child for a third control at 7 months

of age. The serological assays in this control, out of our standard follow-up congenital *T cruzi* diagnosis schedule, still showed reactive antibody titers, and the blood HC allowed the observation of living parasites that could be isolated (Table 1). This was the first evidence that the child was *T cruzi* congenitally infected.

Retrospective quantification of parasite load by qPCR with all available blood samples from this child was performed by targeting both satellite and kinetoplast DNA, controlling DNA preservation and recovery of isolated DNA, as it has been previously described.[9–11] The blood sample collected at 7 months of age was positive for *T cruzi* amplification by cPCR and qPCR, whereas those collected at 20 days and 6 months of age tested negative for all DNA amplification methods performed. In addition, we could not find any parasite load by all parasitological methods in the blood sample collected from his mother during her third month of pregnancy (Table 1).

An interesting feature was that the isolated *T cruzi* parasite from this infant at 7 months after delivery was identified as TcI because an amplified product of 350 bp was obtained with primers TCC, TC1, and TC2.[15] This was the only TcI out of 57 isolated *T cruzi* parasites characterized as TcV in which 38 samples were from pregnant women and 19 samples were from children who were congenitally infected recruited from January 2008 to December 2011[10] (Table 1). The finding of an infecting *T cruzi* parasite when this infant was 7 months of age allowed the prescription of a trypanocidal treatment performed at the Hospital de Niños "Ricardo Gutiérrez," Buenos Aires, Argentina. Treatment with Benznidazole (7.4 mg/kg per day, 2 daily doses for 2 months) was efficient and with excellent tolerance; his serological titers clearly decreased and no detectable parasite load was found in his posttreatment samples (J. Altcheh, PhD, and M. Bisio, PhD, personal communication, 2017).

DISCUSSION

Parasite DNA amplification has been proven to be a more sensitive detection method than other parasitological diagnostic methods, such as microscopic observation of the blood buffy coat (40 parasites per mL),[16] or other methods not currently used, such as HC and xenodiagnosis.[17] At present, qPCR targeting satellite and kinetoplast DNA is performed for the detection of congenital *T cruzi* infection at our institution.[11] This method has exhibited 100% sensitivity in the detection of *T cruzi* in infected offspring born to seropositive mothers within the first 3 months of age (C. Albizu, K.S., unpublished observations). However, we show that *T cruzi* quantitative DNA amplification could not detect any parasitic load in the first 2 control samples in an infant that was congenitally infected.

Regarding the TcI isolated from this child's blood, it is interesting to remark that this DTU does not frequently circulate in humans in this South American endemic region.[2]

Another research group has isolated parasites from infected patients living in the Gran Chaco ecoregion (Argentina) and found mainly TcV or TcVI parasites, whereas TcI isolates were isolated mainly from sylvatic mammals like opossums.[18,19] Nevertheless, the Discrete Typing Unit TcI was found infecting humans mainly in organ explants such as human cardiac or brain tissues[20–23] and in patients who had undergone immunosuppressive therapy, suggesting that TcI parasites circulate at low parasitemia levels, which makes it difficult to detect this DTU in peripheral blood.[22] In congenital human infections, in our endemic region, no TcI parasites have been detected,[23,24] except for the case report presented in this article, which was first described in a previous study[10] and in a few more cases.[21,25] This case report poses 2 main differences with previous studies from our laboratory. First, we had demonstrated that infants infected with *T cruzi* whose results were negative for microscopic search of the parasite early after birth increased their parasitemia when quantified at ~6 months of age.[10] Also, the only infant found infected with a TcI showed no parasitemia at 6 months after delivery even when searched by real-time polymerase chain reaction. Another striking difference found is that although we have previously demonstrated that the parasite burden is an important factor related to *T cruzi* congenital transmission (a sevenfold increase in parasite load in mothers who gave birth to infected children),[13] in the pregnant woman studied in this case report, we detected no infecting parasites in her blood sample.

The trypanocidal treatment of this infant infected with TcI was successful, taking into account the decreased antibody titers observed and the lack of evidence of parasite load. The success of treating a patient from Central America infected with *T cruzi* TcI, whose infection was reactivated after immunosuppression, has been reported.[26] Moreover, no resistance to Benznidazole treatment has been found in mice experimentally infected with 6 different TcI strains, as well as for other infecting parasite DTUs.[27]

CONCLUSIONS

In this case report, we suggest that, even by using molecular tools such as conventional or real-time DNA amplification, a child infected with *T cruzi* can return negative results for parasite search during the first 6 months of age, and it is important to assure 1-year follow-up to test serological titers or make additional efforts to detect the

infective parasite in children born to mothers infected with *T cruzi* for an efficient congenital Chagas disease detection. A successful diagnosis will allow the prescribing of a trypanocidal treatment, which has been proven to be efficient and well-tolerated.

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ABBREVIATIONS

cPCR: conventional polymerase chain reaction
DTU: discrete typing unit
HC: hemoculture
qPCR: quantitative polymerase chain reaction
TcI: *Trypanosoma cruzi* discrete typing unit I
TcV: *Trypanosoma cruzi* discrete typing unit V
TcVI: *Trypanosoma cruzi* discrete typing unit VI

REFERENCES

1. Zingales B, Miles MA, Campbell DA, et al. The revised *Trypanosoma cruzi* subspecific nomenclature: rationale, epidemiological relevance and research applications. *Infect Genet Evol*. 2012;12(2):240–253
2. Brenière SF, Waleckx E, Barnabé C. Over six thousand *Trypanosoma cruzi* strains classified into discrete typing units (DTUs): attempt at an inventory. *PLoS Negl Trop Dis*. 2016;10(8):e0004792
3. Carlier Y, Truyens C. Congenital Chagas disease as an ecological model of interactions between *Trypanosoma cruzi* parasites, pregnant women, placenta and fetuses. *Acta Trop*. 2015;151:103–115
4. Carlier Y, Torrico F. Congenital infection with *Trypanosoma cruzi*: from mechanisms of transmission to strategies for diagnosis and control. *Rev Soc Bras Med Trop*. 2003;36(6):767–771
5. Angheben A, Boix L, Buonfrate D, et al. Chagas disease and transfusion medicine: a perspective from non-endemic countries. *Blood Transfus*. 2015;13(4):540–550
6. Hsu RC, Burak J, Tiwari S, Chakraborti C, Sander GE. Chagas cardiomyopathy in New Orleans and the Southeastern United States. *Ochsner J*. 2016;16(3):304–308
7. Rodriguez-Guardado A, González ML, Rodriguez M, Flores-Chavez M, Boga JA, Gascon J. *Trypanosoma cruzi* infection in a Spanish liver transplant recipient. *Clin Microbiol Infect*. 2015;21(7):687.e1–687.e3
8. De Rissio AM, Riarte AR, García MM, Esteva MI, Quaglino M, Ruiz AM. Congenital *Trypanosoma cruzi* infection. Efficacy of its monitoring in an urban reference health center in a non-endemic area of Argentina. *Am J Trop Med Hyg*. 2010;82(5):838–845
9. Duffy T, Bisio M, Altcheh J, et al. Accurate real-time PCR strategy for monitoring bloodstream parasitic loads in Chagas disease patients. *PLoS Negl Trop Dis*. 2009;3(4):e419
10. Bua J, Volta BJ, Perrone AE, et al. How to improve the early diagnosis of *Trypanosoma cruzi* infection: relationship between validated conventional diagnosis and quantitative DNA amplification in congenitally infected children. *PLoS Negl Trop Dis*. 2013;7(10):e2476
11. Ramírez JC, Cura CI, da Cruz Moreira O, et al. Analytical validation of quantitative real-time PCR methods for quantification of *Trypanosoma cruzi* DNA in blood samples from Chagas disease patients. *J Mol Diagn*. 2015;17(5):605–615
12. Altcheh J, Moscatelli G, Mastrantonio G, et al. Population pharmacokinetic study of benznidazole in pediatric Chagas disease suggests efficacy despite lower plasma concentrations than in adults. *PLoS Negl Trop Dis*. 2014;8(5):e2907
13. Bua J, Volta BJ, Velazquez EB, Ruiz AM, Rissio AM, Cardoni RL. Vertical transmission of *Trypanosoma cruzi* infection: quantification of parasite burden in mothers and their children by parasite DNA amplification. *Trans R Soc Trop Med Hyg*. 2012;106(10):623–628
14. Velázquez EB, Rivero R, De Rissio AM, et al. Predictive role of polymerase chain reaction in the early diagnosis of congenital *Trypanosoma cruzi* infection. *Acta Trop*. 2014;137:195–200
15. Souto RP, Fernandes O, Macedo AM, Campbell DA, Zingales B. DNA markers define two major phylogenetic lineages of *Trypanosoma cruzi*. *Mol Biochem Parasitol*. 1996;83(2):141–152
16. Torrico MC, Solano M, Guzmán JM, et al. Estimation of the parasitemia in *Trypanosoma cruzi* human infection: high parasitemias are associated with severe and fatal congenital Chagas disease. *Rev Soc Bras Med Trop*. 2005;38(suppl 2):58–61
17. Britto C, Silveira C, Cardoso MA, et al. Parasite persistence in treated Chagasic patients revealed by xenodiagnosis and polymerase chain reaction. *Mem Inst Oswaldo Cruz*. 2001;96(6):823–826
18. Cardinal MV, Lauricella MA, Ceballos LA, et al. Molecular epidemiology of domestic and sylvatic *Trypanosoma cruzi* infection in rural northwestern Argentina. *Int J Parasitol*. 2008;38(13):1533–1543
19. Diosque P, Barnabé C, Padilla AM, et al. Multilocus enzyme electrophoresis

analysis of *Trypanosoma cruzi* isolates from a geographically restricted endemic area for Chagas' disease in Argentina. *Int J Parasitol*. 2003;33(10):997–1003

20. Burgos JM, Diez M, Vigliano C, et al. Molecular identification of *Trypanosoma cruzi* discrete typing units in end-stage chronic Chagas heart disease and reactivation after heart transplantation. *Clin Infect Dis*. 2010;51(5):485–495
21. Cura CI, Duffy T, Lucero RH, et al. Multiplex real-time PCR assay using TaqMan probes for the identification of *Trypanosoma cruzi* DTUs in biological and clinical samples. *PLoS Negl Trop Dis*. 2015;9(5):e0003765
22. Lucero RH, Brusés BL, Cura CI, et al. Chagas' disease in Aboriginal and Creole communities from the Gran Chaco region of Argentina: seroprevalence and molecular parasitological characterization. *Infect Genet Evol*. 2016;41:84–92
23. Corrales RM, Mora MC, Negrette OS, et al. Congenital Chagas disease involves *Trypanosoma cruzi* sub-lineage IId in the northwestern province of Salta, Argentina. *Infect Genet Evol*. 2009;9(2):278–282
24. Rumi MM, Pérez Brandán C, Gil JF, et al. Benznidazole treatment in chronic children infected with *Trypanosoma cruzi*: serological and molecular follow-up of patients and identification of discrete typing units. *Acta Trop*. 2013;128(1):130–136
25. Ortiz S, Zulantay I, Solari A, et al. Presence of *Trypanosoma cruzi* in pregnant women and typing of lineages in congenital cases. *Acta Trop*. 2012;124(3):243–246
26. Costales JA, Kotton CN, Zurita-Leal AC, et al. Chagas disease reactivation in a heart transplant patient infected by domestic *Trypanosoma cruzi* discrete typing unit I. *Parasit Vectors*. 2015;8:435
27. Gruendling AP, Massago M, Teston AP, et al. Impact of benznidazole on infection course in mice experimentally infected with *Trypanosoma cruzi* I, II, and IV. *Am J Trop Med Hyg*. 2015;92(6):1178–1189

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