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Short communication

Congenital co-infection with different *Trypanosoma cruzi* lineages

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ABSTRACT

Variability of mixed *Trypanosoma cruzi* congenital infection in Chile in twenty one congenital samples of Chagas disease is reported. Recognition of infecting strains was performed by minicircle hybridization tests. Seven newborns with double infection were found. *Trypanosoma cruzi* TcII and TcV lineages were the most frequent in single and mixed infections. With these results we pretend to understand the epidemiological significance of the *T. cruzi* lineages for which the placenta does not seem to represent an actual barrier in congenital infections.

The agent of Chagas disease infects 16–18 million people in Latin America. In endemic countries where national programs of vectorial control and selection of blood donors have been developed, maternal–fetal transmission of parasites has evolved as the most important route of *T. cruzi* infection, making possible transmission from one generation to another [1]. Such transmission occurs in 1–12% of infected mothers, and morbidity and mortality of congenital infection vary from asymptomatic to severe and mortal clinical forms of disease [2]. Prompt diagnosis (in and timely) of infected newborns is necessary because, the earlier specific antiparasitic treatment starts, the greater the chance of cure. Congenital *T. cruzi* infections can lead to chronic chagasic myocardiopathy and/or digestive megaviscera 25–35 years later [3]. *T. cruzi* infective parasites are heterogeneous complexes of genetic lineages. Such phylogenetic differences might have relevant consequences on parasitic virulence, congenital transmission and pathology [4,5]. The *T. cruzi* intra-specific nomenclature considers six main lineages [6]. Genotypes TcI, TcII, TcV and TcVI have been identified in congenital Chagas disease [7–12]. The TcV genotype predominates in congenital infections of Bolivia and Argentina, with a distribution of frequencies similar to that observed in the infected local population.

Few congenital cases of co-infection with lineages of *T. cruzi* have been reported and the genotypes detected in mothers are generally found in infected newborns [7,10,13]. *T. cruzi* belongs to the Kinetoplastida order, known by the mitochondrial or kinetoplast DNA (kDNA) composed of maxicircles and minicircles. A highly polymorphic sequence present in different minicircle classes is useful for direct *T. cruzi* genotyping by means of hybridization tests with a panel of well characterized kDNA probes. In Chile, using PCR test, the figures of vertical transmission ranged between 3 and 8.4% [14,15]. Many diagnostic methods have been implemented but its limitations need to be considered for example with a gold standard method as microhematocrit the detection limit of such parasitological detection was estimated to be 40 parasites/ml. [11,12]. Nowadays 3.7% of pregnant mothers are infected with *T. cruzi*, representing a threat to the newborns estimated in 445 new cases/year of congenital infection in the most endemic areas of Chile [14]. In this work we present information about *T. cruzi* found in congenital infections of Chile in an area with interrupted vector transmission.

All the samples were detected from pregnant mothers. Chagas disease diagnosis was performed in the mothers by serology (ELISA and IFI Ig G) as described [14]. Congenital infection was diagnosed by microscopic examination of the buffy coat from blood collected at delivery in four microhematocrit heparinized tubes (each containing 50 μl of blood), as described, [16] or hemoculture of 2 ml of blood for 2–8 weeks and/or after birth by serial serological determinations until the diagnosis titer of IFI IgG was over (1/40) and that state remained constant over eight months. The blood samples were collected systematically as part of a longitudinal chagasic infection screening program of the Ministry of Health of Chile. Samples were taken under the guidance of the ethics committee of the Faculty of Medicine, University of Chile. The PCR test was used for genotyping assay on DNA blood samples at delivery. All cases seropositive confirmed as infected at eight months (twenty one children) received specific treatment with Nifurtimox for 60 days at a dose of 7–10 mg/kg.

Later on to assess the presence of the parasite post-therapy blood samples were obtained at 2, 6, 12, and 24 months. These blood samples were tested by serology and PCR.

The PCR assay is directed to amplify minicircle DNA as described [17]. The products which represent a pool of amplified variable region of representative *T.cruzi* lineages minicircles were analyzed on agarose gels, taking into account only the presence of fragments obtained, in

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at least three different assays. The PCR amplicons were subjected to genotyping with the panel P^{32} kDNA probes of the four more frequent *T. cruzi* lineages in Chile (TCI, TCII, TCV and TCVI) cloned stocks analyzed with a Bio-Rad Molecular Imager [18,19]. The probes were prepared as described [13]. There are numerous assays for the validation of this technique with clones of *T.cruzi* [20,21]. We present results of 21 congenital samples. Fig. 1 shows a representative result of this kind of analysis with DNA probes. Both single and mixed infections were found. Of the 21 samples with congenital infection 47.6% were infected with a single type of *T.cruzi* lineage. The single infections were: TcI (2 samples), TcII (2 samples), TcV (5 samples), TcVI (1 sample), and 19% could not be identified (4 samples). The unsuccessful identification of genotype of these 4 samples would probably be due to low yields in the amplification of the parasite DNA by the PCR assays, which resulted in unsuccessful hybridization. The rest of the samples correspond to 33.3% mixed infections with two strains of *T. cruzi*. These seven double infections were: TcII + TcV (3 samples), TcI + TcV (2 samples), TcI + TcII and TcII + TcVI (one sample each). The most frequently found *T.cruzi* lineage was TcV and the less is TcVI, same as described before in 1–10 year-old Chilean children and adults, including *T.cruzi* mixtures [17,18]. TcV was also the lineage most represented singly or mixed with TcI, in Bolivian patients, with a tendency to found mixed infections in acute rather than chronic patients [21]. TcIII and TcIV never have been found in Chile using a high sample size [19]. All the newborn were treated with specific chemotherapy (Nifurtimox) and they become seronegative and PCR negative after two months treatment, except for two cases with discontinued treatment which continued seropositive and PCR positive. Later on these two cases were treated again and became seronegative at one and a half year old. Treatment success was previously demonstrated in 1–10 year old children [22]. This work with 21 congenital cases represents the largest study on this issue performed in Chile. Considering that the vector transmission is already controlled, same other routes, the congenital transmission represents the most important in Chile. Nowadays a programme to detect congenital transmission is desirable since chemotherapeutical treatment is highly successful. The present study reports evidence of single and mixed congenital infections same as detected in chronic patient of Chile. We were able to detect in newborns the most frequent and prevalent lineages circulating in adults, same as described in other Latin American countries where Chagas disease is endemic. The results suggest a limited selection in the transmission of the different lineages of *T. cruzi*. The placenta apparently did not represent a real selective barrier to the transmission of these parasites. It may mean a potential uncontrolled transmissibility of the parasite for long periods of time in endemic areas free of vector transmission.

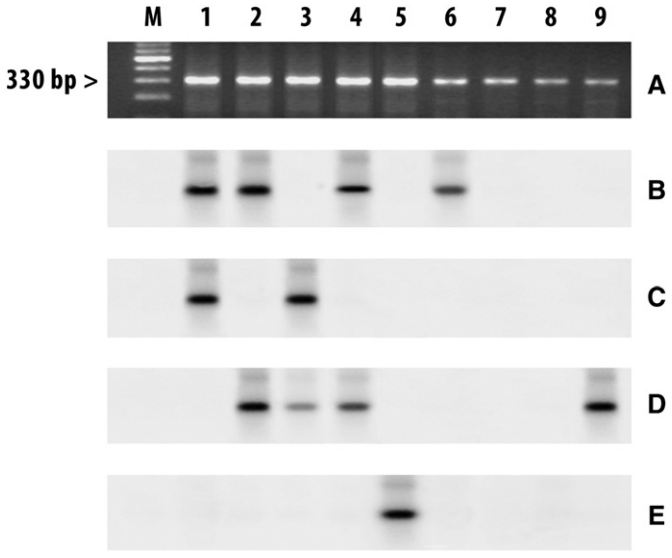

Fig. 1. *Trypanosoma cruzi* amplicons stained with ethidium bromide (A). Hybridization profiles obtained with genotype specific probes corresponding to TcI (B), TcII (C), TcV (D), and TcVI (E). 330 base pairs (bp) product represents a positive assay. Lanes 1, 2, 3, and 4 show double infections. Lanes 5, 6 and 9 show single infections. Lanes 7 and 8 show unknown infections.

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References

[1] Carlier Y, Torrico F, Sosa-Estani S, Russomando G, Luquetti A, Freilij H, et al. Congenital Chagas disease: recommendations for diagnosis, treatment and control of newborns, siblings and pregnant women. PLoS Negl Trop Dis 2011;5(10):e1250.
[2] Torrico F, Alonso-Vega C, Suarez E, Rodriguez P, Torrico MC, Dramaix M, et al. Maternal *Trypanosoma cruzi* infection, pregnancy outcome, morbidity, and mortality of congenitally infected and non-infected newborns in Bolivia. Am J Trop Med Hyg 2004;70:201–9.
[3] Rassi Jr A, Rassi A, Marin-Neto JA. Chagas disease. Lancet 2010;375:1388–402.
[4] Conway DJ. Molecular epidemiology of malaria. Clin Microbiol Rev 2007;20:188–204.
[5] Macedo A, Segatto M. Implications of *Trypanosoma cruzi* intraspecific diversity in the pathogenesis of Chagas disease. In: Telleria J, Tibayrenc M, editors. American Trypanosomiasis-Chagas Disease. One Hundred Years of Research. 1st ed. London, UK/Burlington, MA, USA: Elsevier; 2010. p. 489–522.
[6] Zingales B, Miles M, Campbell D, Tibayrenc M, Macedo A, Teixeira M, et al. The revised *Trypanosoma cruzi* subspecific nomenclature: rationale, epidemiological relevance and research applications. Infect Genet Evol 2012;12(2):240–53.
[7] Burgos JM, Altcheh J, Bisio M, Duffy T, Valadares HM, Seidenstein ME, et al. Direct molecular profiling of minicircle signatures and lineages of *Trypanosoma cruzi* bloodstream populations causing congenital Chagas disease. Int J Parasitol 2007;37:1319–27.
[8] Corrales RM, Mora MC, Negrette OS, Diosque P, Lacunza D, Virreira M, et al. Congenital Chagas disease involves *Trypanosoma cruzi* sub-lineage IId in the northwestern province of Salta, Argentina. Infect Genet Evol 2009;9:278–82.
[9] Falla A, Herera C, Fajardo A, Montilla M, Adolfo G, Guhl F. Haplotype identification within *Trypanosoma cruzi* I in Colombian isolates from several reservoirs, vector and humans. Acta Trop 2009;110:15–21.
[10] Pavia PX, Montilla M, Florez C, Herrera G, Ospina JM, Manrique F, et al. The first case of congenital Chagas' disease analyzed by AP-PCR in Colombia. Biomedica 2009;29:513–22.
[11] Virreira M, Alonso-Vega C, Solano M, Jijena J, Brutus L, Bustamante Z, et al. Congenital Chagas disease in Bolivia is not associated with DNA polymorphism of *Trypanosoma cruzi*. Am J Trop Med Hyg 2006;75:871–9.
[12] Virreira M, Truyens C, Alonso-Vega C, Brutus L, Jijena J, Torrico F, et al. Comparison of *Trypanosoma cruzi* lineages and levels of parasitic DNA in infected mothers and their newborns. Am J Trop Med Hyg 2007;77:102–6.
[13] Ortiz S, Zulantay I, Solari A, Bisio M, Schijman A, Carlier Y, et al. Presence of *Trypanosoma cruzi* in pregnant women and typing of lineages in congenital cases. Acta Trop 2012;124(3):243–6.
[14] Apt W, Zulantay I, Solari A, Ortiz S, Oddo D, Corral G, et al. Vertical transmission of *Trypanosoma cruzi* in the Province of Choapa, IV Región, Chile. Preliminary Report (2005–2008). Biol Res 2010;43(3):269–74.
[15] Jercic MI, Mercado R, Villarroel R. Congenital *Trypanosoma cruzi* infection in neonates and infants from two regions of Chile where Chagas' disease is endemic. J Clin Microbiol 2010;48(10):3824–6.
[16] Freilij H, Muller L, Gonzalez Cappa SM. Direct micro method for diagnosis of acute and congenital Chagas' disease. J Clin Microbiol 1983;18:327–30.
[17] Solari A, Campillay R, Ortíz S, Wallace A. Identification of *Trypanosoma cruzi* genotypes circulating in Chilean chagasic patients. Exp Parasitol 2001;97:226–33.
[18] Torres JP, Ortiz S, Muñoz S, Solari A. *Trypanosoma cruzi* isolates from Chile are heterogeneous and composed of mixed populations when characterized by schizodeme and Southern analyses. Parasitology 2004;128(Pt 2):161–8.
[19] Barnabé C, Neubauer K, Solari A, Tibayrenc M. *Trypanosoma cruzi*: presence of the two major phylogenetic lineages and of several lesser discrete typing units (DTUs) in Chile and Paraguay. Acta Trop 2001;78(2):27–37.
[20] Veas F, Breniere SF, Cuny G, Brengues C, Solari A, Tibayrenc M. General procedure to construct highly specific kDNA probes for clones of *Trypanosoma cruzi* for sensitive detection by polymerase chain reaction. Cell Mol Biol 1991;37:73–84.
[21] Brenière SF, Bosseno MF, Telleria J, Bastrenta B, Yacsik N, Noireau F, et al. Different behavior of two *Trypanosoma cruzi* major clones: transmission and circulation in young Bolivian patients. Exp Parasitol 1998;89(3):285–95.
[22] Solari A, Ortíz S, Soto A, Arancibia C, Campillay R, Contreras M, et al. Treatment of *Trypanosoma cruzi*-infected children with nifurtimox: a 3 year follow-up by PCR. J Antimicrob Chemother 2001;48(4):515–9.