

Congenital *Trypanosoma cruzi* Infection in Neonates and Infants from Two Regions of Chile Where Chagas' Disease Is Endemic[▼]

Chagas' disease, or American trypanosomiasis, whose etiological agent is the protozoan parasite *Trypanosoma cruzi*, is an important public health concern in Latin America. At least 8 million people acquired the infection via the arthropod vector or another mechanism of transmission, such as blood transfusion or vertical transmission from mother to child (9). In Chile (11), the parasitose is endemic and enzootic in the northern and central biogeographical regions (18°30'S to 34°36'S).

Three species of Reduviidae insect are transmitters of Chagas' disease to mammals, including humans, in Chile: *Triatoma infestans*, *Triatoma spinolai*, and *Mepraia gajardoi* (3, 13). Modes of transmission of Chagas' disease other than infections through insect vectors have been documented to occur in Chile, including accidental laboratory infections, infections caused by blood transfusion, and transplacental infections. Infections by the oral route in humans have not been described to occur in Chile (12).

Epidemiological studies conducted in previous decades, mainly with blood banks in areas where Chagas' disease is endemic, with hospital maternity wards, and with specific age groups, especially children, as well as an intensive campaign of *T. infestans* disinfestations promoted by the Chilean health authority, have led to accurate knowledge of the epidemiological aspects of Chagas' disease and the control of transmission by those insect vectors and in blood banks in areas of the country where Chagas' disease is endemic and enzootic (4, 8, 11). In 1999, this led to the declaration of the interruption of transmission of Chagas' disease via the *T. infestans* vector in Chile (1). However, the emergence of new cases of *T. cruzi* infections in Chile both in blood banks and via transplacental infection maintains the importance of systematic studies on the epidemiological and epizootic aspects of this parasitic disease in Chile (4). Congenital Chagas' disease is considered the principal mode of *T. cruzi* infection in geographical areas where transmissions by insect vectors and blood transfusion are controlled (15).

PCR used in laboratory diagnosis of Chagas' disease is considered a sensitive and specific test and additionally a useful probe for evaluating the efficacy of treatment of infected patients (14, 15). Since there have been few studies of Chagas' disease in recent years in Chile, in the present research we describe the use of kinetoplast KNA (kDNA) PCR to detect *T. cruzi* infections in neonates and infants born from mothers with Chagas' disease in two regions of Chile where Chagas' disease is endemic. The purpose of this research was to determine the frequency of congenital infections in children delivered from chagasic mothers by using PCR for kDNA as a laboratory diagnostic tool.

During the years 2007 and 2008 at the Reference Laboratory of Parasitology of the Institute of Public Health of Chile (ISP), blood samples from 179 children less than 2 years of age from regions IV and V of Chile were received. In those regions, samples were collected in maternity wards of hospitals in the

cities of Ovalle (region IV) and La Serena (region V) and in the hospitals of Los Andes, San Camilo, and Gustavo Fricke, located in region V. The blood samples were collected systematically as part of a chagasic infection screening program launched by the Health Ministry of Chile (MINSAL) in these geopolitical regions of the country. Samples were taken under the guidance of the ethics committee for scientific research of the ISP-MINSAL. The ages of the children ranged from 1 day to 2 years 8 months. Eighty-two (45.8%) children were less than 3 days of age, 15.1% (27/179) were between 4 and 15 days old, 30.2% (54/179) were more than 15 days and less 1 year old, and 8.9% (16/179) were 1 year to 2 years 8 months old; 98 were female (54.7%), and 81 were male (45.3%). Samples were taken from peripheral blood and stored until shipment to the laboratory where they were processed. With each sample, we performed an indirect immunofluorescence assay (IF) and an enzyme-linked immunosorbent assay (ELISA) for detecting serum-specific antibodies to *T. cruzi* (2, 14). PCR was performed as described by Wincker et al. (16), with the following modifications. DNA was extracted with a commercial kit (Favor-Prep blood genomic DNA extraction minikit) in accordance with the instructions of the supplier. With this extracted DNA, PCR was performed using 2.5 μ l of each sample. We used primer 121 (5' TAA TGT AAA ACG GGG GAG ATG CAT GA 3') (10 μ mol/liter) and primer 122 (5' GGT TCG ATT GGG GTT GGT GTA ATA TA 3') (10 μ mol/liter) to amplify a region of the kinetoplast DNA of *T. cruzi*. As a control for the integrity of the extracted DNA and inhibition of PCR, we amplified a DNA segment of the human β -globin gene by use of the following primers: b_glo1 (5' CCT CCT TTG AAG TTC TCC AA 3') and b_glo 2 (5' CCT CTT CAC TCA TGG CTT AG 3'). In each run, a negative (no-DNA) sample and positive controls (*T. cruzi* DNA, Tulahuen strain) were included. Amplification products were visualized by agarose gel electrophoresis in 2% ethidium bromide, using a running time of 55 min at 107 mV. Samples were considered positive when a 330-bp product was amplified by primers 121/122. Primers for b_glo1/b_glo2 amplified a 239-bp product (10).

In region IV of Chile, most of the samples studied were from the Ovalle hospital. In region V, most samples were derived from the G. Fricke and Los Andes hospitals. All serum samples were IF and ELISA positive ($\geq 1:20$ serum dilution). A positive PCR result, detecting kDNA of *T. cruzi* in blood samples of the 179 neonates and infants, was found in 15 cases (8.4%). Of the 15 PCR-positive samples, 9 were from region V. The frequencies of *T. cruzi* infections observed in the two biogeographical regions were not significant by a chi-square test (Table 1). We also observed no significant difference in the percentages of transplacental *T. cruzi* infection by sex: females, 9.2% (9/98), and males, 7.4% (6/81) ($\chi^2 = 0.18$; $P = 0.67$). Figure 1 shows the results for the 15 positive PCR results, with a 330-bp band of kDNA of *T. cruzi*. The β -globin control amplification showed a band of 239 bp.

The 8.4% rate of positive PCR results for kDNA of *T. cruzi*

TABLE 1. PCR for *Trypanosoma cruzi* kDNA for 179 chagasic children <2 years old from biogeographical regions IV and V of Chile (2007 and 2008)

Region	Hospital	No. of chagasic children	No. of positive PCR results (%)
IV	Ovalle	91	
IV	La Serena	2	
Total (IV)		93	6 (6.5) ^a
V	G. Fricke	34	
V	Los Andes	36	
V	San Camilo	16	
Total (V)		86	9 (10.5) ^a
Total		179	15 (8.4)

^a Chi-square result, 0.94; *P* value, 0.34 (no statistical difference).

observed in children most likely indicates the occurrence of *T. cruzi* transplacental infection in these two geopolitical regions of Chile. The majority of the positive cases were detected in children who were only a few days old and whose mothers had given birth in the maternity wards of hospitals in these regions and not in their homes, where they might have been exposed to the insect vector by being bitten (12). Moreover, transmission of Chagas' disease by *T. infestans* vectors inside dwellings is considered virtually nonexistent in Chile, reaffirming that children with positive PCR results had an *in utero* infection transmitted by their mothers (1). PCR was run only with venous blood samples of the children, avoiding the use of umbilical cord blood samples, where contamination with *T. cruzi* DNA from the mother could be possible. Few studies of transplacental transmission caused by *T. cruzi* have been done in Chile. In 1989, Schenone et al. (11) found that 7.1% (24/336) of the newborn of mothers with Chagas' disease showed a positive xenodiagnosis test result, a figure that rose to 14.5% (53/364) when they studied the persistence of specific anti-*T. cruzi* antibodies up to 18 months after birth, as determined by an indirect hemagglutination reaction in the blood samples of the children (10). In another study, Mercado et al. used xenodiagnosis to detect the parasite in 297 newborn delivered in the maternity ward of the hospital of Salamanca in region IV of Chile; they observed that 29 (9.8%) had serum-specific antibodies against *T. cruzi* but described no transplacental *T. cruzi* infections (7). In the only other published study on congenital Chagas' disease in Chile using PCR, Garcia et al. reported 21.2% (32/151) cases as positive. This frequency is significantly higher than and not consistent with that determined by us (5). In other South American countries (Argentina, Bolivia, Brazil, and Paraguay) and in a country where Chagas' disease is not endemic, rates of transplacental infection by *T.*

cruzi ranged from 1 to 12% (6, 15). Our results are in concordance with this reported frequency of transmission.

Our research provides valuable evidence that PCR for *T. cruzi* kDNA is an appropriate tool for diagnosis of transplacental infections and confirms the frequencies reported for other countries where Chagas' disease is endemic or not endemic (6, 15). In a comparative study done with chagasic blood donors in Chile, it was observed that some patients with positive xenodiagnoses presented negative PCR results for kDNA (4). Thus, negative PCR results for children delivered from chagasic mothers will not disconfirm the presence of *T. cruzi* infection (6); consequently, an underestimation of the transmission frequency is possible when PCR is used exclusively. Further studies on the use of PCR as a diagnostic test for transplacental Chagas' infection are required to determine with certainty the performance of this test, especially in combination with other direct or indirect parasitological diagnostic tests.

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FIG. 1. Positive amplification of *T. cruzi* kDNA extracted from blood samples of 15 chagasic children. Columns: 1, molecular weight standard; 2, *T. cruzi* kDNA amplification positive control (330 bp); 3, no-*T. cruzi* DNA control; 4, β -globin control amplified band (239 bp); 5 to 19; positive cases of chagasic children.

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