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Benznidazole treatment in chronic children infected with *Trypanosoma cruzi*: Serological and molecular follow-up of patients and identification of Discrete Typing Units

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ABSTRACT

A total of 221 children from two rural settlements in Northeast Argentina were examined for *T. cruzi* infection. Blood samples were taken for serology tests and PCR assays. In addition, *T. cruzi* Discrete Typing Units (DTUs) were determined by hybridization with specific DNA probes of the minicircle hypervariable regions (mHVR). Serological results indicated that 26% (57/215) were reactive against *T. cruzi* antigens. PCR analyses were performed on seropositive samples showing presence of parasite DNA in 31 out of 53 samples (58.5%). All seropositive children underwent specific chemotherapy with Benznidazole (5 mg/kg/day) for a period of two months and were monitored two and five years after treatment. Overall the treatment was well tolerated and low side effects were observed. Serological conversion was observed at two years post -treatment in one child form Pampa Ávila and at five years in two children from Tres Estacas. However, at the end of the follow-up period, *T. cruzi* DNA could not be detected by PCR in samples from treated children, except in two cases. In addition, the results of hybridizations with specific DNA probes showed that DTU TcV was detected in 68% (21/31), TcVI in 7% (2/31) and TcV/VI in 3% (1/31) of the samples. Altogether, results of the follow-up of treated children showed a low rate of seroconversion; however trend toward seroconversion was evident at five years post-treatment. On the other hand, detection of *T. cruzi* DNA by PCR significantly decreased after Benznidazole treatment. The existence of data regarding serological and molecular follow-ups from controlled studies in the Chaco Region will be important for future treatment efforts against *T. cruzi* infection in this region. The results obtained in the present study represent a contribution in this regard.

1. Introduction

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is the most important neglected tropical disease and represents a major public health problem in Latin America (WHO, 2007). The illness is characterized by two phases: an acute phase which appears just after infection, and a chronic phase which may have no obvious pathology or evolves to irreversible lesions mainly in the heart and/or digestive system (Mitelman et al., 2011). In addition, the disease cannot be prevented by vaccination nor, in many situations, be reliably cured by anti-parasitic drugs (Marin-Neto et al., 2009).

The Chagas disease specific treatment has been recommended for people with the acute and chronic phase (Rassi et al., 2010). Two drugs known as Nifurtimox (a nitrofuran derivative) and Benznidazole (a nitroimidazole) have been widely used for the treatment of Chagas disease (Guedes et al., 2011). The goal of specific treatment for *T. cruzi* is to eliminate the parasite from the infected individual, to decrease the probability of developing illness and to break the chain of disease (Sosa-Estani et al., 2009).

Several serological and parasitological techniques to evaluate therapeutic efficacy in chagasic chronic children after Nifurtimox or Benznidazole treatment have been used (de Andrade et al., 1996;

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Escriba et al., 2009; Flores-Chavez et al., 2006; Galvao et al., 2003; Schijman et al., 2003; Solari et al., 2001; Sosa Estani et al., 1998). While a success rate of cure of more than 70% during the specific treatment of infected individuals has been shown with serological tests, only a few of these clinical trials have evaluated the performance of the PCR in post-treatment follow-ups (Flores-Chavez et al., 2006; Galvao et al., 2003; Schijman et al., 2003; Solari et al., 2001).

A major difficulty and controversy in accurate evaluation of therapeutic efficacy depends on a reliable cure criterion when blood samples are assessed by serological and parasitological techniques after drug treatment. Changes in serology, parasite load and clinic evaluation have been used as criterion of cure in clinical trials of Chagas disease (Guedes et al., 2011). The serological techniques most currently used are Enzyme-Linked Immunosorbent Assay (ELISA), Indirect Haemagglutination (IHAs) and Indirect Immunofluorescence (IIF) (Rassi et al., 2010). Due to the low sensitivity display by the parasitological tests traditionally used (xenodiagnoses and hemoculture), detection of *T. cruzi* DNA by Polymerase Chain Reaction (PCR) technique is at present the most frequently used tool for direct parasite detection in blood samples (Schijman et al., 2011). The kinetoplastid minicircle DNA (kDNA) is one of the most used targets for *T. cruzi* DNA detection by PCR because of the high sensitivity given by the large copy number per cell (Avila et al., 1990). This PCR amplifies a hypervariable region of minicircles which has been successfully used for strain identification from biological samples by Southern-blot assays (Coronado et al., 2006; Flores-Chavez et al., 2006; Solari et al., 2001b; Veas et al., 1991).

In Argentina some clinical studies were carried out to assess the efficacy of treatment against *T. cruzi* infection in the chronic phase (Schijman et al., 2003; Sosa Estani et al., 1998; Streiger et al., 2004). In these studies the success of treatment was determined by the disappearance of antibodies using serological testing, while therapeutic failure is determined by the persistence of the parasite using parasitological testing. In the present study we report the serological and parasitological results obtained from the follow-up of a group of children younger than 16 years-old after Benznidazole treatment, who were screened in an epidemiological survey previously carried out in rural areas of the Chaco Province (Diosque et al., 2004). In addition, we attempted to evaluate the presence of the most prevalent *T. cruzi* genotypes circulating in the area using hybridization tests with specific DNA probe.

2. Materials and methods

2.1. Study area and population

The study was carried out in Pampa Avila and Tres Estacas, two settlements situated in Chacabuco department, in the Chaco Province, Argentina. Most of the samples were obtained within an area of 322 km^2 from a central point in the Tres Estacas settlement (S 26°55′27″; W 61°37′36″). As part of the investigation, all houses were sprayed by the National Vector Control Agency with pyrethroid insecticide before starting drug treatment. An entomological surveillance system was started, including periodical control of houses to prevent reinfestation. Before pyrethroid spraying, households were examined for triatomine infestation as described in a previous report (Diosque et al., 2004).

All children under 16 years old were diagnosed for *T. cruzi* infection in both rural areas. All seropositive children for two serological techniques were treated with Benznidazole. The exclusion criteria were: (1) positive pregnancy test in females of childbearing age, (2) presence of any acute infectious disease, (3) kidney and liver failure, (4) hematological disorders and (5) malnutrition. No child was excluded from the study.

The profits and health hazards were informed to all parents or guardians before drugs treatment; thereafter they signed the informed-consent form. The study was approved by the Bioethics Committee of the Faculty of Health Sciences of the National University of Salta, Argentina.

2.2. Benznidazole treatment and patient follow-up

Clinical examinations (anamnesis, physical examination and electrocardiograms [ECGs]) and biochemical studies (hemogram including red blood, hematocrit, hemoglobin, neutrophils, monocytes, lymphocytes, basophils, eosinophils and erythrocyte sedimentation rate and platelet counts) were assessed before starting treatment and at the end of the follow-up period. Benznidazole was given twice a day at a total daily dose of 5 mg per kg of weight for a period of 60 days (WHO, 2005). Blood samples from patients from Pampa Ávila were collected before treatment in 2007 and two years after treatment in 2009. In Tres Estacas, blood samples were taken before treatment in 2004 and two and five years after treatment (2006 and 2009, respectively). Pre- and post-treatment samples were studied by serological tests and PCR assays. Negativization by two serological tests at the end of a follow-up period was considered the cure criterion.

2.3. Serological test

Two milliliters of peripheral blood were centrifuged (3000 rpm for 15 min) and sera was recovered and stored at −20 °C. The serum samples were analyzed by Indirect Hemagglutination test (IHA,Chagatest HAI, Wiener Laboratory, Rosario, Argentina) and by Enzyme-Linked Immunosorbent Assay (ELISA,Chagatest ELISA recombinant, Wiener Laboratory, Rosario, Argentina) according to manufacturer's instruction. An indirect immunofluorescence assays (IIF) was performed as a third diagnostic test when discordant results were obtained between ELISA and IHA. IIF was performed as previously described (Palacios et al., 2000). Reactive samples at dilutions ≥1:16 were considered positive in the IHA assays. In the ELISA assay a cut-off value was established, based on the average optic density (OD) of the negative control, plus 0.300 OD units, according to the manufacturer's instructions. All samples were processed simultaneously to avoid technical variations.

2.4. Polymerase chain reaction

Five milliliters of blood were mixed with an equal volume of 6 M guanidine hydrochloride-200 mM EDTA solution, boiled for 15 min and stored at 4 °C until used. DNA was extracted from 200 μl of the mixture using the phenol-chloroform method and precipitated using ethanol as described (Wincker et al., 1994). Appropriate controls were used to rule out possible DNA contamination. Each sample was tested in duplicate. DNA was resuspended in 50 μl of distilled water. A hot-start PCR procedure, targeted to the 330-bp minicircle hypervariable regions (mHVR) of the *T. cruzi* kinetoplastid minicircles DNA (kDNA), was carried out as follows: 7.5 μl of extracted DNA were added to 50 μl final volume of PCR reaction mix. Final concentrations were: buffer 5×, 3 mM MgCl2, 0.2 mM of each deoxyribonucleotide triphosphate (dNTP), 0.5 μM of primer 121 (5′AAATAATGTACGGG(T/G)GAGATGCATGA-3′) and 0.5 μM of primer 122 (5′-GGTTCGATTGGGGTTGGTGTAATATA-3′) and 1.25 IU of Taq DNA polymerase (Promega). Amplification was performed in a MJR PTC-100 thermocycler (MJ Research, Watertown, MA, USA). Cycling parameters were: one step of 3 min at 94 °C; two cycles at 97.5 °C for 1 min and 64 °C for 3 min, followed by 33 cycles of 1 min at 94 °C followed by 1 min at 64 °C with a final extension at 72 °C

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for 10 min. 15 μl of the reaction product were visualized in a 2% agarose gel stained with ethidium bromide.

2.5. DTUs analysis: Southern blot and DNA probe construction

Detection of the Discrete Typing Units of *T. cruzi* in blood samples was carried out by hybridization with specific mHVR-kDNA non-radioactive probes from *T. cruzi* clones: X10cl1 (TcI), Tu18cl2 (TcII), M5631cl5 (TcIII), LL055R3cl2 (TcV) and CL-Brener (TcVI). Briefly, Southern blot analysis was performed with 10 μl of each PCR product. Samples were subjected to electrophoresis, transferred to Hybond N + nylon membranes (Roche Diagnostics) and cross-linked with u.v. light to fix the DNA. The membranes were pre-hybridized for at least 30 min at 42 °C and hybridized with a panel of five genotype-specific probes labeled by the random priming method with digoxigenin-dUTP (Roche Diagnostics). After hybridization, the membranes were submitted to low and high stringency washings according to the manufacturer's instructions (Roche Diagnostics). Construction of specific probes was performed by amplification of the variable region of the *T. cruzi* minicircles. The primers for probe generation were CV1 (5′-GATTGGGGTTGGAGTACTAT-3′) and CV2 (5′-TTGAACGGCCCTCCGAAAAC-3′) which produced a 290-bp fragment. Restriction sites for Sau96I and ScaI which allow elimination of the minicircle constant region of these PCR fragments were included in the sequence of these primers (Veas et al., 1991). The CV1-CV2 PCR fragments were further digested with the restriction endonucleases obtaining a 250-bp final product. For validating our experimental conditions, each generated DNA probe was evaluated by Southern blot analysis against different 330-bp mHVR PCR products of several *T. cruzi* stocks. All of the probes were specific to their corresponding DTU as they cross-hybridized only with the homologous samples (not shown).

2.6. Data analysis

The mean absorbance of ELISA and the inverse of titers values (Log_{10}) obtained by IHA were compared between the serum samples before and after treatment. The information obtained was analyzed by the Wilcoxon test and Friedman test. PCR results were analyzed for the samples available before and after treatment and the rate of negativization of parasite detection was compared. All analyses were performed using the software Infostast (Di Rienzo et al., 2009) and GraphPad (Prism version 5.0, GraphPad Software, San Diego California USA). A p-value < 0.05 was considered significant.

3. Results

3.1. Pre-treatment detection of T. cruzi infection by specific antibodies and PCR

Blood samples from children from two endemic rural areas (Pampa Avila y Tres Estacas) were analyzed by serology. Seventy three samples were obtained from Pampa Avila settlement and 148 from Tres Estacas. When Pampa Avila samples were analyzed, 34.2% (25/73) of seropositivity was detected. In this case, 4 samples showed discordant results between ELISA and IHA while positive by IIF, therefore those samples were considered positive for *T. cruzi* infection. In Tres Estacas, a seroprevalence of 22% (32/148) was observed with concordant results between ELISA and IHA. When PCR assays from seropositive children were performed, *T. cruzi* DNA was detected in 16 out of 23 (70%) subjects from Pampa Ávila and in 15 out of 30 (50%) subjects from Tres Estacas, given a global 58.5% (31/53) of positive PCR from seropositive individuals. Only four samples could not be analyzed by PCR.

3.2. Benznidazole treatment regimen and side effects

All seropositive children (aged 3–15 years) from both rural areas were submitted to drug treatment (25 from Pampa Avila and 32 from Tres Estacas). The treatment was generally well tolerated. About 30% (9 of 32) of the children from Tres Estacas presented side effects such as allergic dermatitis (44%; 4/9), headache (33%; 3/9), intestinal colic (22%; 2/9), queasiness (11%; 1/9), vomiting (11%; 1/9), diarrhea (11%; 1/9) and paraesthesia (11%; 1/9). The appearance of more than one side effect was only observed in 4 of 9 (44%) patients. Nevertheless, the adverse effects observed were generally mild and allowed completion of the drug regimen. When the dose was halved or temporarily suspended for not longer than seven days all side effects disappeared. Symptomatic medication (antihistamines, ranitidine and metoclopramide) was administered in some cases. Clinical records from Pampa Avila children during treatment monitoring could not be recovered. Finally, at the end of the follow-up period, normal ECGs studies from children from both settlements were obtained.

3.3. Serological follow-up after treatment

Post-treatment monitoring was conducted in 23 from 25 treated children from Pampa Avila and in 22 from 32 from Tres Estacas. In the settlement Pampa Ávila, serological evaluation by ELISA after 2 years of the drug treatment showed that just one child seroconverted, while the 96% (22/23) of children had positive results. Fourteen percent of the 22 seropositive children showed a reduction in antibody titers (Fig. 1A). However, by IHA tests no seroconversion was observed. Results obtained from IHA showed to be more disperse than the ones obtained from ELISA regarding concentration of antibodies titers after treatment (Fig. 1B). Reduction in antibodies titers was detected in 74% (17/23) of the treated children while increase concentrations were detected in 43% (10/23). The ELISA and IHA results from samples obtained after treatment showed no significant differences when compared to the results obtained from samples before treatment ($p > 0.05$).

In the second settlement (Tres Estacas), all samples taken two years after treatment remained seropositive for ELISA and IHA; while from the 22 samples taken five years after treatment, 2 cases of seroconversion were detected by ELISA (2/22; 9%). From the remaining 20 seropositive samples, 4 of them (20%) showed a considerable decrease in the concentration of antibody titers (Fig. 2A), with significant differences in ODs values ($p < 0.05$) when comparing to samples before treatment. Serological evaluation by IHA showed a reduction in concentration of antibodies titers in 45% (5/22) and an increase in 54% (10/22) of the treated children (Fig. 2B). Antibody titer variations observed by IHA were not statistically significant.

3.4. Post-treatment PCR follow-up

In Pampa Ávila, 23 from 25 treated individuals were analyzed by PCR before and after treatment. When post treatment samples were analyzed by PCR all but one sample was negative (the positive sample came from a patient with positive PCR before treatment). In Tres Estacas, we detected by PCR 50% of infection (15/30) before treatment. From these individuals, and 5 years after treatment, only 21 children could be monitored by PCR (12 positive and 9 negative pre-treatment). In this case, also in one child *T. cruzi* DNA was still detected.

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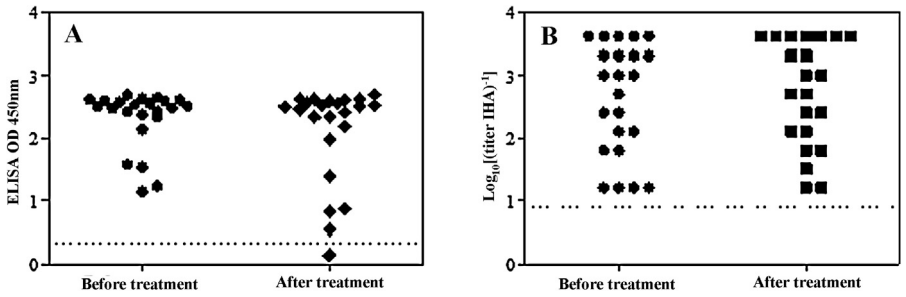

Fig. 1. Comparison of antibodies titer before and after treatment in children from Pampa Ávila. Each point represent individual child. (A) ELISA, concentration of antibodies measured in optical density (OD) a 450mn. (B) IHA, concentration of antibodies measured as the inverse of titer values (Log_{10}). The horizontal dotted lines represent the cut off value used to discriminate negative and positive results (0.33 and 0.9 for ELISA and IHA, respectively).

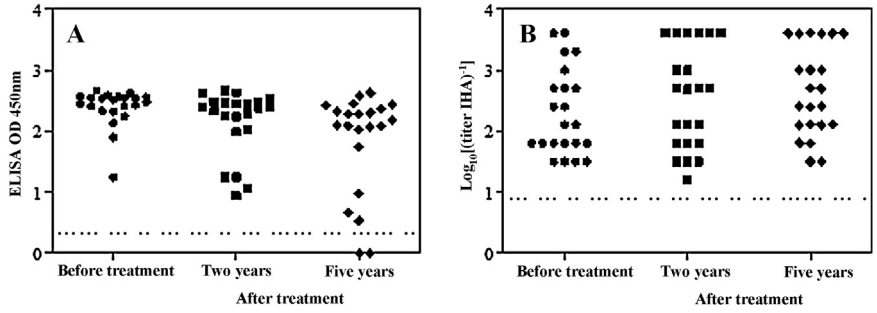

Fig. 2. Comparison of antibodies titer before and after treatment in children from Tres Estacas. Each point represent individual child. (A) ELISA, concentration of antibodies measured in optical density (OD) a 450mn. (B) IHA, concentration of antibodies measured as the inverse of titer values (Log_{10}). The horizontal dotted lines represent the cut off value used to discriminate negative and positive results (0.33 and 0.9 for ELISA and IHA, respectively).

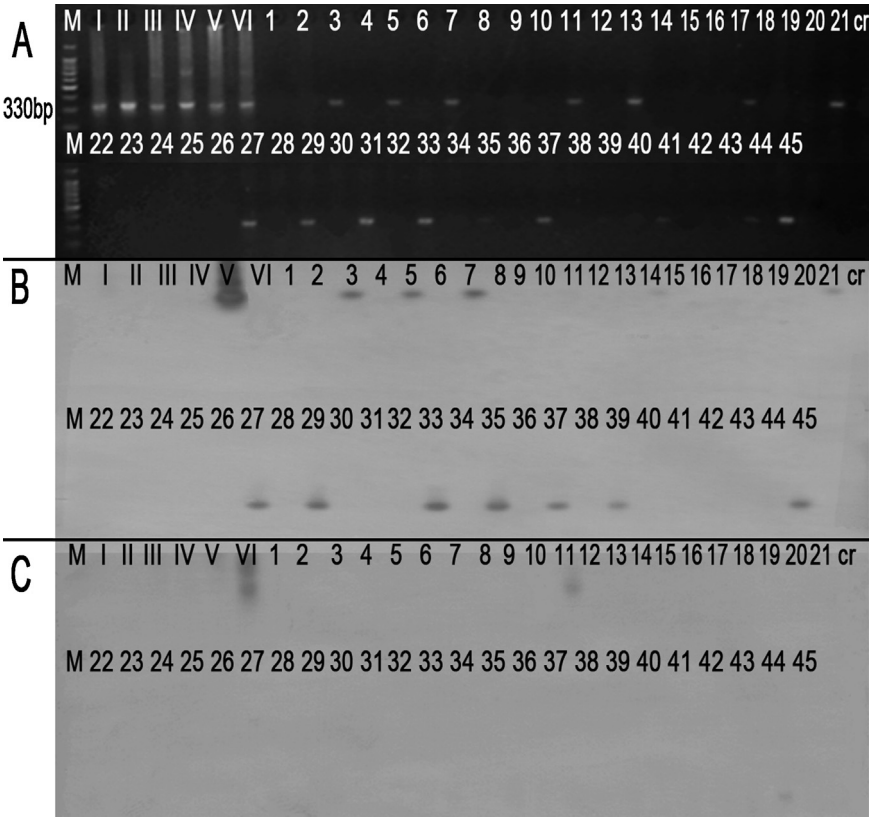

Fig. 3. (A) Electrophoretic pattern of minicircle regions of 300-bp amplified PCR for the blood samples of children from Pampa Ávila. M, molecular weight marker (100-bp ladder; Promega); Lane I -VI: positive control probe generated: TcI (X10cl1), TcII (Tu18cl2), TcIII (M5631cl5), TcV (LL055R3cl2) and TcVI (CL-Brener). Lane 1–45: DNA from the blood samples of children: odd numbers correspond to samples before treatment and even numbers correspond to samples after treatment; Cr: reaction control. (B) kDNA of (A) transferred to a membrane nylon, labeled probe hybridized with LL055R3cl2 (TcV). (C) kDNA of (A) transferred to a membrane nylon, labeled probe hybridized with CL-Brener (TcVI).

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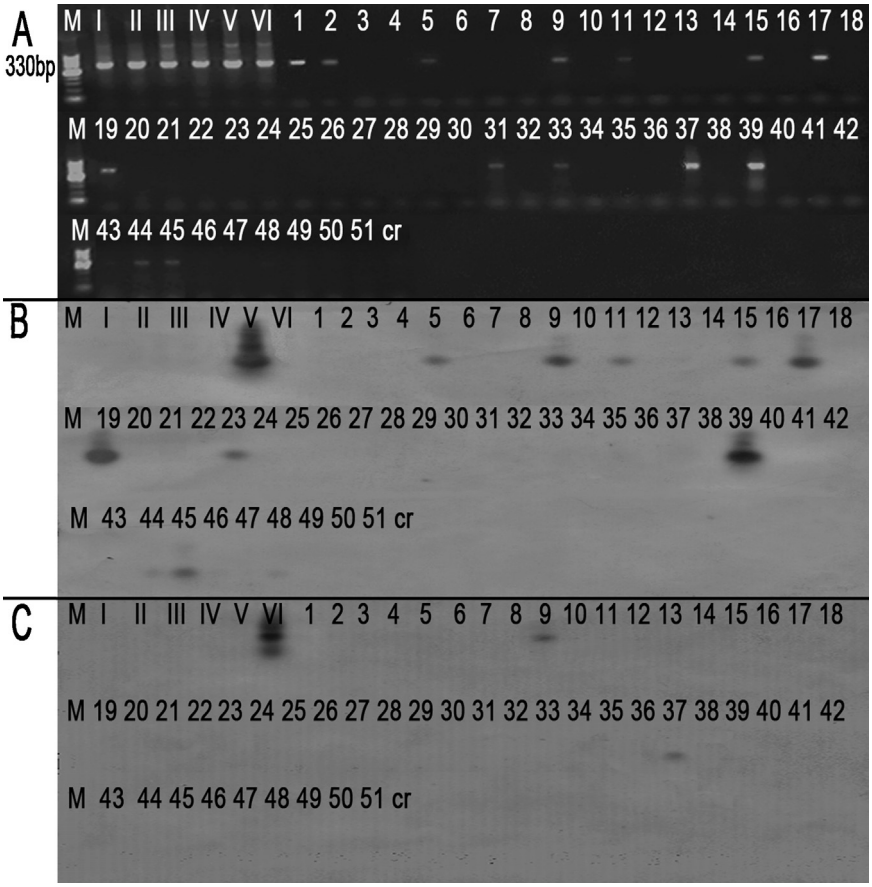

Fig. 4. (A) Electrophoretic pattern of minicircle regions of 300-bp amplified PCR for the blood samples of children from Tres Estacas. M, molecular weight marker (100-bp ladder; Promega); Lane I–VI: positive control probe generated: TcI (X10cl1), TcII (Tu18cl2), TcIII (M5631cl5), TcV (LL055R3cl2) and TcVI (CL-Brener). Lane 1–51: DNA from the blood samples of children: odd numbers correspond to samples before treatment and even numbers correspond to samples after treatment; Cr: reaction control. (B) kDNA of (A) transferred to a membrane nylon, labeled probe hybridized with LL055R3cl2 (TcV). (C) kDNA of (A) transferred to a membrane nylon, labeled probe hybridized with CL-Brener (TcVI).

3.5. DTUs identification by hybridization with specific DNA probes

In order to characterize the *T. cruzi* DTUs infecting children in both settlements, blood samples from seropositive children were analyzed by Southern blot before and after treatment. Specific kDNA label probes corresponding to five from the six *T. cruzi* DTUs were used. Probe corresponding to TcIV was not employed since this DTU was never detected in this region. We were able to identify the infecting DTU in 13 out of the 16 samples from Pampa Avila which were positive by PCR before treatment DTU TcV was detected in 12 samples (70%) while TcVI was only detected in one child. The remaining 3 samples showed no hibridization with our probes (Fig. 3). No DTU mixed infection was detected in children from this settlement. The only positive sample by PCR after treatment from this settlement hybridized with TcV probe. Surprisingly, for this same child we did not have hybridization whit any probe when pre-treatment samples were examined.

Thirty pre-treatment samples corresponding to seropositive children from Tres Estacas were analyzed for DTU determination. Fifteen out of these 30 samples were PCR positive. DTU TcV was detected in 60% (9/15), TcVI in 7% (1/15) and TcV/TcVI in one case (1/15). In the remaining 4 PCR positive samples no DTU could be identified with our probes. After treatment, only 21 samples could be obtained and analyzed. From these samples, only one was still PCR positive but no hybridization was obtained with our probes (sample taken before treatment from this patient showed no hybridization with any probe neither) (Fig. 4). These results are summarized in Table 1.

4. Discussion

In this study a group of children undergoing the chronic phase of Chagas disease was treated with Benznidazole and monitored by serological and PCR techniques throughout two and five years after treatment. Furthermore, the DTU involved in children's infections was determined. Along the follow-up period we observed in general a low seroconversion rate detected by ELISA in children from both rural areas. TcV was the most prevalent DTU (68%) detected in children from these settlements.

In general, drug tolerance was good for most treated children, adverse effects were mild and similar to those recorded in other studies (Sosa Estani et al., 1998; Streiger et al., 2004) and the electrocardiograms were normal throughout and after treatment. These results indicate that there was no progression of signs or symptoms associated with the disease.

Variations in seroconversion rates, due to the effectiveness of anti-parasitic treatment, have been reported by other authors (Guedes et al., 2011; Sosa-Estani et al., 2009). The low rates of seroconversion observed in our study was strikingly lower than those reported by other studies that showed rates of seroconversion of more than 60% (de Andrade et al., 1996; Escriba et al., 2009; Sosa Estani et al., 1998; Streiger et al., 2004). However, they were similar to those reported by others authors (Schijman et al., 2003; Silveira et al., 2000; Solari et al., 2001). The low seroconversion we are referring to was detected only by ELISA, while for IHA the antibody profiles showed a wide variation and there were no seroconverted children. This incongruence may be due to that each of these

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Table 1
Detection of *T. cruzi* genotypes TcV and TcVI by hybridization with DNA specific probes in chagasic children before Benznidazole treatment.

Settlements	Samples PCR		PCR-hibridizations % (No/PCR positive)			
	Total	Positive	TcV	TcVI	TcV/TcVI	Others
Pampa Ávila	23	16	75 (12/16)	6.3 (1/16)	–	18.7 (3/16)
Tres Estacas	30	15	60 (9/15)	7 (1/15)	7 (1/15)	26 (4/15)
Total	53	31	68 (21/31)	7 (2/31)	3 (1/31)	22 (7/31)

serological techniques operates in different specificity systems (Belluzo et al., 2011; Souza and Amato Neto, 2012). Furthermore, it should be considered that antibodies demand different times to disappear according to the phase of the disease, the age of patients and the strain of *T. cruzi* (Coura and Borges-Pereira, 2012).

The PCR results were negative for almost all children after treatment and they were similar to those reported in other studies (Flores-Chavez et al., 2006; Solari et al., 2001). However, these results should be carefully interpreted because a negative PCR result does not necessarily indicate absolute absence of the parasite (Rassi et al., 2010). Accordingly, the implementation of quantitative PCR assay to determine the bloodstream parasitic load and follow their evolution throughout treatment could be particularly useful as an indicator of response in prolonged therapeutic regimens (Marin-Neto et al., 2009; Schijman et al., 2003). Treatment failure was only detected in two children, since positive PCR results were obtained after treatment. It should be noted that the two rural areas were sprayed with insecticide before and throughout the follow-up period to warrant the absence of triatomine vectors and hence to discard any possibility of reinfection. Therefore, possible explanations for those children which showed a parasite presence after treatment could be attributed to a poor effectiveness of the Benznidazole to penetrate the infected tissues (Schijman et al., 2003) or to the existence of *T. cruzi* strains naturally resistant to drugs currently used, as previously described in experimental studies (Mejia-Jaramillo et al., 2012; Moreno et al., 2010; Murta et al., 1998). However, Benznidazole susceptibility of *T. cruzi* strains circulating in the study area has not yet been evaluated.

The lack of correlation between negative PCR results and still positive serology after treatment may have possible explanations: (i) that an small numbers of parasites (not detected by qualitative PCR from peripheral blood) could be sequestered for body tissues and be able to sustain a persistent immune response (Tarleton and Zhang, 1999); or (ii) the persistence of positive results by serology for long periods could be due to immunological memory (Ahmed and Gray, 1996). Therefore the use of complementary technical approaches such as multiplex serological assays or specific T cell response and a quantitative PCR might better reflect the impact of treatment (Laucella et al., 2009; Marin-Neto et al., 2009; Viotti et al., 2011).

In a previous work conducted in the same endemic area in the Chaco Province, we had reported the presence of TcV and TcVI associated with humans and dogs, respectively, whereas TcI was more closely associated with sylvatic mammals (Diosque et al., 2003) In order to identify which DTUs could be involved in infection of children from the study area, we characterized the blood samples before and after treatment by means of hybridization with five specific DNA probe. We have demonstrated the presence of TcV and TcVI in children who received treatment. The most prevalent *T. cruzi* genotype was TcV, and in a minor proportion TcVI, which was also detected in a mixed infection with TcV. Other different genotypes not detected by our probes may be involved in infections of children in the study area. In one case we were not able to detect the infecting DTU before treatment; however, after it, TcV was detected in this child. These could be a case of a mixed infection in which one genotype could not be detected by our probes. Our findings are consistent with the results of epidemiological studies in other areas of Argentina which showed that simple infections by TcV are the most prevalent in humans (Burgos et al., 2007; Cardinal et al., 2008; Corrales et al., 2009; Diez et al., 2010; Diosque et al., 2003) and although simple infections due to TcVI are less frequent, these are present at higher frequencies in mixed infections with TcV (Cura et al., 2012).

To summarize, a low seroconversion by ELISA, negativization of PCR and clinical stability suggest that the treatment may have caused a positive impact on the natural history of the disease, even though it will be necessary longer follow-up periods in order to define if real cure does occur in this children cohort.

To the extent that control of *T. cruzi* transmission progresses in the Chaco Region, an increasing number of people will be treated. In this framework, the existence of data regarding serological and molecular follow-ups from controlled studies will be important for future treatment efforts against *T. cruzi* infection in this region. The results obtained in the present study represent a contribution in this regard.

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