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EARLY DIAGNOSIS OF CONGENITAL *TRYPANOSOMA CRUZI* INFECTION USING PCR, HEMOCULTURE, AND CAPILLARY CONCENTRATION, AS COMPARED WITH DELAYED SEROLOGY

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ABSTRACT: Congenital *Trypanosoma cruzi* infection is a highly pathogenic and underreported condition. Early recognition is essential for effective treatment. Umbilical chord blood from newborns ($n = 302$) to infected mothers was analyzed with microhematocrit, hemoculture, and PCR methods. Each subject was then followed serologically. In calibrated suspensions of *T. cruzi* in blood, the sensitivity of PCR was 27-fold higher than hemoculture. However, this advantage was not reflected during routine testing of samples from maternities, partly because of the uneven distribution of few parasites in small samples. Levels of detection of congenital infection were 2.9% (8/272) for microhematocrit, 6.3% (18/287) for hemoculture, 6.4% (15/235) for PCR, and 8.9% (27/302) for cumulated results. Evaluation against the standard of delayed serology indicates that the regular application of PCR, hemoculture, and microhematocrit to blood samples allows the rapid detection of about 90% of the congenitally infected newborns, in samples that can be obtained before the mother and child leave the maternity ward.

Chagas disease is highly prevalent in Central and South America. The estimated number of infected humans exceeds 16 million (Moncayo, 1999). A variable proportion of newborns to *Trypanosoma cruzi*-infected mothers acquire the infection congenitally. In different reports, this proportion has ranged in Argentina from 2.6% to 8.8%, and some reviews indicate that close surveillance usually reveals more than 5% congenital cases (Freilij et al., 1994; Freilij and Altcheh, 1995; Streiger et al., 1995; Contreras et al., 1999).

Congenital infection with *T. cruzi* is presently a serious public health problem because of peculiar epidemiologic, pathologic, and diagnostic features. In contrast with vector-delivered infection, congenital cases are not restricted to countries where vectors proliferate; they may be frequent in any country with a high proportion of Latin-American immigrants, such as the United States. Successful vector control in some areas has led to predictions that insect transmission may soon be halted (Moncayo, 1999). In these areas, the risk of congenital transmission will still persist for the average fertile lifespan of infected women. The pathogenic effects of *T. cruzi*, which are characteristically disclosed many years after infection, may affect congenital patients during their youth. However, clinical discovery of infection is not easy during the first years of life because clinical signs are scarce (Yaeger and Klein, 1983; Freilij and Altcheh 1995; Zaidenberg, 1999).

Early detection and treatment of congenital infection usually leads to the successful elimination of the parasite and is highly desirable because it has few side effects (Moya et al., 1985; Streiger et al., 1995). However, diagnosis is hampered by the lack of specificity of the serological reactions and the low sensitivity of parasitological methods. In most cases, treatment has to be delayed for about 6 mo to allow the detection of the baby's own antibodies, after the antibodies transferred by the mother have been cleared. *Trypanosoma cruzi*-specific IgGs are readily transmitted from mother to newborn via placenta and milk, so all babies of infected mothers remain seropositive for several months, regardless of their infection status (Kolodny, 1939; Miles, 1972; Carlier and Truyens, 1995). Parasitological methods, on the other hand, are highly specific, but lack sensitivity because parasitemias are most often too low for direct detection.

The high sensitivity achieved with the polymerase chain reaction (PCR) for detection of *T. cruzi* in blood has stimulated trials of this method for the diagnosis of congenital infection (Russomando et al., 1998; Schijman et al., 2003; Virreyra et al., 2003). Although PCR results have been compared with a few alternative diagnostic methods, some problems remain to be elucidated, i.e., the relationship between early PCR results and late serological confirmation of infection. The question whether a routine PCR assay, adapted for hospital diagnosis, can improve detection over hemoculture (HC) or microhematocrit capillary concentration (MH), also demands a quantitative comparison of sensitivity levels. These considerations are relevant to the cost-effectiveness of installing relatively expensive molecular biology assays in endemic countries where even microscopy and culture methods are still on their way toward full routine application.

Working with umbilical chords and early blood samples from 302 babies born to *T. cruzi*-infected mothers, we assayed the efficacy of 3 early diagnostic methods. In particular, we addressed the following questions. First, what is the detection limit, in parasites/ml of blood, for PCR and HC? Second, how effective are the performances of these methods in routine laboratory testing of hospital samples? Third, to what extent do HC and PCR improve upon the conventional early diagnostic method of MH, thus increasing the number of patients that can receive early treatment? Fourth, what is the predictive value of PCR and early parasitological methods to announce persistent, serologically confirmed congenital infection? Finally, are PCR results affected by the extraction method or inhibitory factors present in the sample? The work seeking answers to these questions is presented here. It was undertaken with experimental samples prepared in the laboratory and with clinical samples regularly collected from hospital maternity wards.

MATERIALS AND METHODS

Subjects and study area

This study was done in Salta, a province of Argentina, with approximately 1 million inhabitants, and highly endemic for Chagas disease.

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TABLE I. Relative sensitivities of methods for the diagnosis of congenital *T. cruzi* infection.

Method	Number of samples tested	Positive with tested method	Positive with reference methods†	% Sensitivity*
MH	272	8	29	27.6
HC	287	18	29	62.1
PCR (standard DNA extraction)	235	15	24	62.5
PCR (standard + improved DNA extraction)	232	18	21	85.7
Any early methods (MH+HC+PCR)‡	302	27	29	93.1

* Relative sensitivity (%) was calculated as follows: positive with tested method × 100/positive with reference methods.
† Reference methods are delayed serological tests plus the remaining early method, excluding PCR.
‡ For assessment of sensitivity of any early methods (last line), serology is the only reference standard.

Samples were collected from July 1997 to December 2001. Vector control has covered most of this territory in recent years, but the latest estimates indicate that 12.3% of the mothers are infected (Contreras et al., 1999).

Study design and criteria for admission

The sensitivity of detection for *T. cruzi* by PCR and HC was analyzed using laboratory-made samples with serial, calibrated dilutions of *T. cruzi* trypomastigotes in blood. The study of clinical samples was organized with collaboration between health centers and laboratory. Mothers were identified as bearing *T. cruzi* infection by an initial serological reaction (indirect hemagglutination [IHA] and/or ELISA) made in the hospital. This result was confirmed by a second study (IHA and ELISA) made in our laboratory with a separate sample. Deliveries of *T. cruzi*-infected mothers were reported by the obstetrics personnel, providing for the opportune collection of specimens.

Blood samples from newborns were obtained in most cases from umbilical chords and occasionally from venous puncture performed during the first 15 days of life. As a rule, all babies were studied serologically at 6.7 ± 0.9 (SD) and 11.3 ± 2.1 mo of age (delayed serology). Collection of these samples was arranged by check-up appointments to the hospital or, in most cases, by active search in house-to-house visits. Every individual included in this study fulfilled the following admission criteria, i.e., serological confirmation by diagnosis of *T. cruzi* infection in the mother and chord-blood or venous puncture sample obtained during the first 15 days of life. Congenital infections were subjected to treatment. In cases where the evidence for infection was exclusively based on PCR results, treatment was delayed until obtaining serological confirmation.

Sample collection

Informed consent was obtained from mothers of babies participating in this study, under a protocol approved by the Ministry of Public Health of the Province of Salta. Umbilical chords were collected following an established protocol. Blood engorgement of the chord was achieved by placing a clamp at the fetal end. After spontaneous delivery of the placenta, a second clamp was placed at the placental end. After sectioning the clamped segment, the chord was rinsed with tap water, placed in a sealed and labeled plastic bag, and sent to the laboratory. To minimize possible contamination with *T. cruzi* DNA from amniotic fluid, the chord was submerged for 1 min into a 3% hypochlorite solution, rinsed in water, and punctured for obtaining blood in a UV decontamination chamber. The blood used for MH and HC was anticoagulated with heparin. For PCR, 1:100, 0.342 M EDTA (Anticoagulante W, Laboratorios Wiener, Rosario de Santa Fé, Argentina) was used. Samples were prepared by diluting 2–5 ml blood, 1:1, in a 6 M guanidine, 0.2 M EDTA lysis buffer, and mixed thoroughly. After 24 hr, the sample was heated for 10 min in boiling water and mixed again.

TABLE II. Agreement between MH and HC to detect *T. cruzi* in chord blood.

Microhematocrit	Hemoculture +	Hemoculture −	Total
+	7	1	8
−	9	245	254
Total	16	246	262

Serological reactions

The enzyme-linked immunosorbent assay (ELISA) test was performed using a commercial kit (Wiener Chagatest-ELISA). Diluted plasma or sera were incubated in plastic wells coated with *T. cruzi* membrane and cytoplasmic antigens. Antibodies were revealed with peroxidase-anti-IgG conjugates oxidizing tetramethyl benzidine into colored compounds and measured with an automatic spectrophotometer. Optical densities ≥0.22 were considered positive. The indirect hemagglutination (IHA) test was performed with a commercial kit (Wiener Chagatest-HAI) based on the agglutination of *T. cruzi* antigen-sensitized goat erythrocytes by 2-fold dilutions of sera. Titers of ≥1:16 were considered positive.

Microhematocrit (MH)

This method concentrates the parasites in the leukocyte cell layer for microscopic observation (Freilij et al., 1983). Heparinized capillary tubes were filled with 70 μl of blood each, centrifuged, and cut with a diamond marker to place the buffy coat layer between a slide and a coverslip. Six replicate tubes were studied for each sample. The coverslip area was completely scanned under the microscope.

Hemoculture (HC)

One-milliliter blood samples were divided into 5 equal aliquots and each was inoculated in a glass hemolysis tube containing 2 ml of liver-infusion tryptose medium (LIT) plus 10% fetal bovine serum, 1% hemin, and antibiotics. After 15, 30, 45, and 60 days of incubation with the tubes in slanted position at 29 C, parasite development was observed under an inverted microscope. All of the liquid layer was examined in at least 10 fields.

Extraction of DNA

One hundred microliters of the blood-guanidine buffer mixture were used for each miniprep. DNA was extracted in duplicated tubes with phenol–chloroform–isoamilic alcohol and precipitated with sodium–acetate–ethanol. The procedure just mentioned above is referred to as standard DNA extraction. All maternity samples were tested once with this method and the initial results are presented in Tables I, III, and IV. Moreover, some selected samples, where a false-negative PCR result was suspected (see Results), were further subjected to repeated DNA extraction using spin columns (QIAamp DNA Blood Minikit, Valencia, California) and examined for PCR-inhibitory factors. This follow-up procedure, restricted to some samples, is referred to as improved DNA extraction (Table I).

PCR

The procedure used was adapted for chord blood from established protocols (Britto et al., 1995; Wincker et al., 1997). PCR work was

TABLE III. Agreement between HC and PCR to detect *T. cruzi* in chord blood.

Hemoculture	PCR +	PCR −	Total
+	6	8	14
−	8	199	207
Total	14	207	221

done in UV light-irradiated isolation chambers, using discardable plasticware, under strict decontamination procedures. Because cross-contamination or PCR artifacts are a constant risk, a maximum of 3 samples was processed, together with a negative and a positive control, per extraction session. PCR mixtures and DNA extraction were performed in separate chambers. All reagents were prepared in aliquots, using exclusive pipettes and filter tips. Amplicons were electrophoresed in a separate building. Segments of 330-bp kinetoplast DNA were amplified using primers #121 (5′-AAA TAA TGT ACG GG(T/G) GAG ATG CAT GA-3′) and #122 (5′-GGT TCG ATT GGG GTT GGT GTA ATA TA-3′). The choice of these primers was made on the basis of the high copy number ($n > 10^4$) of their target sequence and their high sensitivity to detect infection in field studies (Sturm et al., 1989; Breniere, 1994; Britto et al., 1995; Wincker et al., 1997; Diez et al., 1998; Schijman et al., 2003). Amplification was carried out in a thermal cycler (M. J. Research, Watertown, Massachusetts) with a hot-start step to denature DNA. Thermal cycles used were cycle 1 (×2) 1′ at 98 C and 2′ at 64 C; cycle 2 (×33): 1′ at 94 C and 2′ at 64 C; cycle 3 (×1): 1′ at 72 C and 2′ at 25 C. Amplicons were electrophoretically run in 2% agarose gels, revealed by ethidium bromide staining, and recorded by scanning with a Kodak EDAS system.

Standard *T. cruzi* suspensions

Calibrated suspensions of *T. cruzi* in blood were used for testing the sensitivity of PCR. For this purpose, blood from mice acutely infected with the Tulahuen *T. cruzi* strain was serially diluted in human blood to obtain the desired concentration of parasites. The reaction conditions were optimized as to obtain a sensitivity of 2.5 parasites/ml.

Sample and reaction controls

The PCR result of every blood sample was obtained from a gel that included blank, reaction controls, and simultaneously processed negative/positive sample controls. These included blood with 1–5 parasites/ml, to control the sensitivity of the PCR reaction in every gel (Fig. 1).

Test for PCR-inhibitory blood samples

One microliter of a PCR-positive DNA sample was mixed with 100 μl of the test sample (guanidine-EDTA-preserved blood). The mixture was subjected to DNA extraction and PCR procedures. Evidence for inhibition resulted from parallel reactions showing positive results with *T. cruzi* DNA alone or mixed with noninhibitory samples and negative results in mixtures with inhibitory samples.

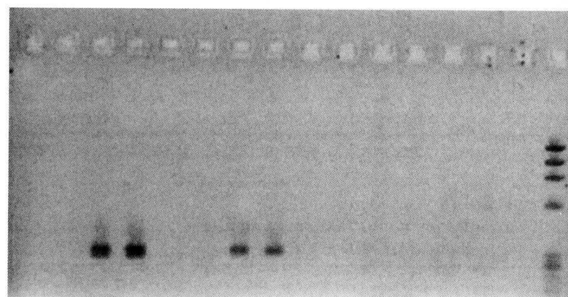

FIGURE 1. PCR products amplified from human-chord blood using primers 121 and 122, separated on 2% agarose gel containing ethidium bromide. Lane 1: blank tube. Lane 2: negative sample. Lanes 3 and 4: Positive control sample of *T. cruzi* diluted in blood at a concentration of 2.5 parasites/ml. Lanes 5–14: Chord-blood samples from infected mothers, run in duplicate reactions. The second sample (lanes 7 and 8) is positive.

Data recording and statistical analysis

Using a database in Excel software, information was entered on every newborn under study, including birthdate, name of the mother, previous serology of the mother, sample type (chord or venous blood), serology at birth, results of MH, HC, and PCR for the same specimen, serological data after 6–12 mo, and special observations. Sensitivity endpoints of PCR for parasites were calculated with the Reed and Muench (1938) method.

RESULTS

Sensitivity of PCR, tested on calibrated concentrations of *T. cruzi* in blood

This parameter was first analyzed by applying the standard PCR method to laboratory-made, serial dilutions of trypomastigotes in blood. An average from 10 experiments indicated a DL_{50} (50% detection limit) of 1.05 ± 0.2 parasites/ml blood. In a second set of experiments, *T. cruzi* was serially diluted in blood and the relative sensitivities of PCR and HC were compared by testing both methods in parallel on the same material. In every experiment (Fig. 2), the sensitivity of PCR was higher. The DL_{50}, in parasites/ml, averaged 18.19 for HC and 0.68 for PCR. Thus, this PCR assay was sensitive enough as to detect 1 parasite present in a sample.

Prevalence of congenital infection in hospital samples by combined criteria

In total, 302 newborns from *T. cruzi*-infected mothers were studied; 90.4% of them were analyzed with MH, 95.4% with

TABLE IV. Predictive value of early methods (PCR, HC, and MH) to announce late serological evidence of congenital *T. cruzi* infection.

Early method	Number of samples tested with early method and delayed serology	Positive by early method; confirmed by serology (a)/positive by early method; tested by serology	Positive predictive value (%)	Negative by early method; confirmed negative by serology/negative by early method; tested by serology	Negative predictive value (%)
PCR	169	8/12	66.7	142/157	90.4
Hemoculture	202	16/16	100	173/186	93.0
Microhematocrit	201	8/8	100	173/193	89.6
Any early method	212	18/22	81.8	179/190	94.2

* Successfully treated cases, which become seronegative, are excluded.

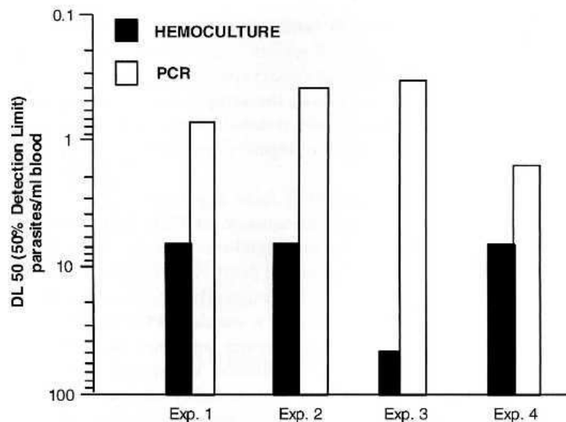

FIGURE 2. Sensitivity of PCR, as compared with hemoculture. The height of the bars indicates the sensitivity of the method, e.g., the dilution point where parasites were no longer detected in blood. DL_{50} (50% detection limit) was calculated with the Reed–Muench method in 4 independent experiments (experiments 1–4) with calibrated dilutions of *T. cruzi* in blood.

HC, 78.1% with PCR, 70.5% with delayed serology, and 51.0% with all methods together. Although some methods were occasionally omitted due to limited sample volume (early tests) or follow-up problems (delayed serology), all samples were included in the analysis because they provided valid comparative data for the remaining tests. MH, HC, and PCR were always applied to the same sample, but several serum specimens were obtained at different times from the same patient. In 29/302 babies (9.6%), a positive diagnosis of congenital infection was obtained with 1 or more methods (Table I). The sensitivity (%) of each method was calculated using the formula $a \times 100/b$, where a = the number of positive diagnoses with the tested method and b = the number of positive diagnoses with any of the remaining 3 methods (Table I). It was observed that HC (18/29; 62.1%) improved detection over MH (8/29, 27.6%). The standard (first attempt) PCR method detected as many cases of infection (15/24, 62.5%) as HC. However, after repeated DNA extraction in discordant, PCR(−) samples and exclusion of inhibitory samples, the level of detection by PCR was 85.7% (18/21), which represents an improvement over HC. All early methods (MH, HC, and PCR), taken together, were able to identify 27 of the 29 subjects revealed as positive after serological confirmation (93.1% sensitivity).

Analysis of false-negative PCR results

In 8/235 blood samples from maternity wards, parasitological methods (HC or MH) were positive and PCR was negative. Six of these samples could be subjected to further DNA extractions, to repeated PCR, and to search for PCR-inhibitory factors. After subsequent DNA extractions, 3 of the samples became PCR(+), indicating that the first extraction had been insufficient. Of the remaining, persistently PCR(−) samples, all 3 had PCR-inhibitory factors. Thus, an initial survey of the first results obtained with the standard PCR led to a rather low performance of this method regarding sensitivity (Tables I, III, IV). However, an improved procedure (second DNA extraction and exclusion of inhibitory samples) raised the sensitivity of PCR beyond the level of HC (Table I). This is consistent with the possibility that an improved PCR procedure, applied to all maternity samples, might have been more sensitive than HC, as indicated by the experiments with calibrated dilutions of *T. cruzi* in blood.

Analysis of PCR-positive, seronegative subjects

In 4/169 subjects tested with PCR and serology, only the first method showed a positive result, in the absence of treatment, raising doubt as to whether a false positive might have resulted from contamination or other reasons. Because contamination was absent from reaction and sample controls run in parallel (see Methods), this finding is not necessarily a false positive and will be discussed in connection with similar observations from other groups (see Discussion).

Agreement between MH, HC, and PCR

Table II summarizes the concordance between MH and HC. Overall agreement was 96.2% and agreement of positive diagnoses was 41.2%. The discrepancy was mostly due to the higher sensitivity of HC over MH, given the blood volumes used for the practical application of each method. Table III shows the agreement between HC and PCR. Again, overall concordance was high, but a low proportion of the positive diagnoses were in agreement between both methods (see Discussion).

Predictive value of early diagnostic methods

To investigate the value of early methods (MH, HC, and PCR) for predicting serologically confirmed, congenital infection, the results obtained with each of them were referred to the serological status of the baby at 6–13 mo of age (Table IV). The positive predictive value was 100% for both MH and HC, in contrast with the low performance of PCR in this respect (66.7%). All 3 early methods, taken together, correctly predicted 94.2% (179/190) of the serologically negative cases.

DISCUSSION

Congenital *T. cruzi* infection is most often discovered by clinical signs or serological tests, many months or years after birth. A good opportunity is thus lost for initiating early treatment, halting the pathogenic process, and minimizing the drug's side effects. In babies born to infected mothers, serological control at 12 mo of age is a secure and rather inexpensive strategy to detect congenital transmission. However, compliance with the appointment for delayed blood tests is very low, and the disease progresses, untreated, in most subjects. For these reasons, the diagnostic possibilities should be exhausted before mothers and their newborn are dismissed from the maternity ward. This study shows that a careful search for *T. cruzi* in chord blood will reveal most cases of congenital transmission. If positive, this diagnosis precludes further serological screening and allows early treatment. In the province of Salta and many other areas, a major difficulty for medical control of newborns and infants is the failure of mothers to respond to health-center recalls for other than urgent medical matters. This is the major impediment for serological control of congenital Chagas

disease. The diagnostic strategies proposed here are based on samples that can be obtained before mother and child leave the maternity ward and that can be totally processed within 2 wk to 2 mo after birth. This reduces the necessity for recalls of children born to infected mothers to only congenitally infected children and represents a more than 10-fold reduction in the burden of the recall system.

The detection of *T. cruzi* in blood by PCR was pioneered in the 1980s and soon was further adapted to clinical diagnosis (Moser et al., 1989; Sturm et al., 1989; Breniere, 1994; Britto et al., 1995; Kirchhof et al., 1996; Wincker et al., 1997; Diez et al., 1998). Preliminary studies with umbilical chords, including trials from our own laboratory, indicate that PCR might be a promising method to detect congenital transmission (Diez et al., 1998; Mora et al., 1998; Virreyra et al., 2003). Thus, we explored the incorporation of this molecular method and of HC, on a regular basis, to established operational guidelines (Blanco et al., 2000) for the detection of congenital *T. cruzi* infection.

The routine, standard PCR procedure used here allowed the detection, in laboratory-calibrated blood samples, of *T. cruzi* concentrations 27 times lower (average) than the limit for hemoculture. However, paradoxically, in routine processing of chord-blood samples shipped from maternity wards, this advantage did not result in a remarkable diagnostic improvement. Evidently, the results of laboratory assays on calibrated blood suspensions were not replicated in the real scenario of hospital specimens. Here, the levels of sensitivity of PCR and HC were similar. But PCR results can be obtained in 48 hr, whereas HC takes 15–60 days of incubation. Although PCR was clearly more sensitive than MH, this improvement was obtained at the expense of lower specificity and reduced ability to predict the outcome of delayed serological tests.

In a previous study of 50 infants born to *T. cruzi*-infected mothers and aged 0–6 mo (Schijman et al., 2003), the sensitivity of MH was higher than in our study (82.4 vs. 27.6). This difference is probably associated with the fact that their specimens were taken from peripheral blood, whereas most of our specimens were from chord blood. An increase in parasitemia some time after delivery might explain these results.

The finding that PCR did not substantially improve the rates of detection for *T. cruzi* in chord blood, as compared with the less sophisticated HC, was independently confirmed by a recent study using the Tcz1/Tcz2 primers and 1-ml specimens (Virreyra et al., 2003). Three hundred and eleven chord-blood samples were analyzed with PCR, of which 18 had been classified as positive and 293 as negative, based on MH and HC. PCR replicated these results without detecting additional cases, except in 1 sample that had been tested by MH and not by HC.

Although the overall agreement of PCR with HC and with serological data exceeded 92%, many positive results were noncoincident among different methods, particularly PCR. This may be due, in part, to limitations in sample volume and, in part, to a few demonstrated instances of false-negative PCR results. Discordance between PCR and HC or MH can simply be explained on the basis of the expected distribution of highly diluted organisms in relatively low-volume samples; the overall results obtained here indicate that *T. cruzi* concentrations as low as 0.1–1 parasite/ml of blood are not uncommon in infected chord blood. In this range, the expected probability that 1.0- and 2.8-ml blood samples (as used here for HC or, on average, for PCR, respectively) will both capture circulating parasites, achieving full concordance of results, can be as low as 0.3. This is in full agreement with our observations; given the low concentrations of *T. cruzi* in blood, the sample volumes used, and the number of samples tested, it would have been more surprising not to detect any false negative-results than having detected a few.

A reanalysis of selected PCR false negatives indicated that the first result had been a consequence of PCR inhibition, insufficient DNA extraction, or limitation in sensitivity. Of 6 false-negative samples, 3/6 became positive after new DNA extractions, and the 3 remaining persistently negative ones had inhibitory factors able to abrogate a standard PCR reaction. In our experience, PCR inhibitory factors were not found in extensive experiments on *T. cruzi* dilution in human blood, but could be detected in 3 selected and probably exceptional samples. In contrast with serological methods, a common feature of PCR, MH, and HC is the remarkable increase in sensitivity obtained by simply repeating the tests.

In this work, congenital infection was diagnosed in 29/302 (9.6%) newborns to *T. cruzi*-infected mothers. Diagnosis was successfully established by early methods in 27 (93.1%) of them. However, 2 cases (6.9%) were overlooked by early methods and only discovered serologically at a later date. The cases where early methods miss the diagnosis seem consistent with infections acquired either shortly before delivery or after birth. In this case, infection by oral route, such as may occur by suction of the mothers blood through nipple lesions, cannot be excluded (Jorg, 1992).

In 4/169 samples tested with PCR and serology, we obtained a positive PCR result in subjects who were later confirmed as seronegative in absence of treatment. Contamination was absent from sample and reaction control tubes run in parallel with PCR test reactions. Similar findings, involving 31 subjects from 6 publications of 4 different research groups, have been reported (Avila et al., 1993; Wincker et al., 1994; Wincker et al., 1997; Gomes et al., 1999; Castro et al., 2002; Salomone et al., 2003). The apparent discrepancy between PCR and serological results has been mostly explained by the inability of particular infected patients to react in conventional serological tests. Another alternative, which cannot be ruled out in our case, is that of a temporary infection, self-cured during the first month of age.

In conclusion, this work indicates that early diagnostic methods (MH, HC, and PCR) can establish, in most cases, the evidence for congenital *T. cruzi* infection before mother and child leave the maternity ward. MH can discover roughly one third of the infections and HC may easily double this proportion. PCR is clearly a method to be carried out in reference centers, where analysis of large-volume blood samples (≥5 ml) and availability of control samples with calibrated *T. cruzi* concentrations may secure a sensitive detection of *T. cruzi* in chord blood. The regular application of these early methods can minimize, probably to less than 10%, the number of positive diagnoses that depend on check-up appointments for delayed serological tests.

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