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Trypomastigote Excretory Secretory Antigen Blot Is Associated With *Trypanosoma cruzi* Load and Detects Congenital *T. cruzi* Infection in Neonates, Using Anti–Shed Acute Phase Antigen Immunoglobulin M

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Background. Congenital *Trypanosoma cruzi* infection accounts for an estimated 22% of new cases of Chagas disease in Latin America. However, neonatal diagnosis is challenging, as 9-month follow-up for immunoglobulin G testing is poor, quantitative polymerase chain reaction (qPCR) analysis is not routinely performed, and the micromethod misses ≥40% of congenital infections.

Methods. Biorepository samples from new mothers and their infants from Piura, Peru, (an area of nonendemicity), and Santa Cruz, Bolivia (an area of endemicity) were accessed. Infant specimens were assessed using the micromethod, qPCR analysis, and a trypomastigote excretory secretory antigen (TESA) blot for detection of immunoglobulin M (IgM)–specific shed acute phase antigen (SAPA) bands, using qPCR as the gold standard.

Results. When compared to qPCR, IgM TESA blot was both sensitive and specific for congenital Chagas disease diagnosis. Cumulative sensitivity (whether only 4 bands or all 6 bands were present) was 80% (95% confidence interval [CI], 59%–92%). Specificity was 94% (95% CI, 92%–96%) in the area of endemicity and 100% in the area of nonendemicity. SAPA bands occurred sequentially and in pairs, and parasite loads correlated highly with the number of SAPA bands present. The micromethod detected infection in fewer than half of infected infants.

Conclusions. The IgM TESA blot for detection of SAPA bands is rapid, relatively inexpensive, and more sensitive than the micromethod and may be a useful point-of-care test for detection of congenital *T. cruzi* infection.

Keywords. Chagas disease; *Trypanosoma cruzi*; congenital; TESA blot; IgM SAPA; diagnosis.

Chagas disease is caused by the parasite *Trypanosoma cruzi*, which infects an estimated 5 million–8 million people globally, predominantly in the Americas, with a particularly high prevalence in Bolivia [1–3]. When untreated, infection passes into a lifelong chronic phase. An estimated 20%–30% of untreated individuals subsequently develop irreversible cardiomyopathy or gastrointestinal disease; the other 70%–80% never develop symptomatic disease [3].

T. cruzi is principally transmitted through the infected feces of triatomine bug vectors [1]. During the chronic phase, vertical transmission of Chagas is a concern and has been described in 1%–10% of infected pregnancies [4–6]. With vector-borne transmission decreasing, congenital infections now account for an estimated 22% of new cases of disease [7]. Women who were congenitally infected may themselves transmit congenitally [8, 9].

Congenital infection has been associated with premature delivery, low Apgar scores, and low birth weight. Although more-severe cases are decreasing, respiratory distress syndrome, hepatosplenomegaly, and meningoencephalitis are still observed [3, 6]. Screening pregnant women and infants in Chagas disease–endemic areas is important to curb transmission and ensure treatment; during infancy, trypanocidal chemotherapy has high efficacy and is well tolerated [10, 11].

In regions of endemicity, diagnosis involves maternal serological screening, followed by infant testing at birth and again at 6–12 months of age, although follow-up for retesting is rare [12–14]. Serological screening is used for maternal diagnosis, because the parasite load is low during the chronic phase of infection [15–18]. Diagnostic methods for infants include

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the examination of concentrated blood samples by the micromethod (MM), Western blots for immunoglobulin M (IgM)–specific trypomastigote excretory secretory antigen (ie, TESA blots), and quantitative polymerase chain reactions (qPCR) for analysis of the parasite load [14, 18, 19].

The MM assay is the only test widely available for diagnosis in the first month of life [14]. qPCR is not a practical diagnostic option in resource-limited settings, and IgG serologic analysis may not be used at birth, because maternal antibodies may cross the placental barrier [19–21]. Infants can be screened for immunoglobulin G (IgG) after 9 months of age, but as few as 20% in control programs return for follow-up screening [12, 13].

TESA IgM blots use TESAs to identify acute infection through the presence of shed acute phase antigens (SAPAs). In the presence of anti-SAPA antibodies, they are seen in a gel as polypeptides of 130–200 kDa that form a ladder-like pattern of up to 6 bands during acute or congenital infection [22, 23]. The present work aims to assess the sensitivity and specificity of the SAPA bands on IgM TESA blots as compared to MM and qPCR analyses and to analyze the significance of <6 bands. Additionally, it will evaluate this reactivity in light of the parasite load observed in newborn subjects. It further aims to assess the effect of treatment on specimen positivity.

MATERIALS AND METHODS

Specimen Sources and Maternal-Infant Cohorts

Specimens were accessed using the Universidad Peruana Cayetano Heredia Biorepository in Lima, Peru, and came from 2 study populations: (1) participants in the Percy Boland Hospital Study in Santa Cruz, Bolivia, where Chagas disease is endemic and (2) patients at Cayetano Heredia and Santa Rosa hospitals in Piura, Peru, an area where the disease is not endemic. Both studies recruited consenting pregnant participants and their newborns at delivery and gathered maternal and infant blood samples. Specimens were also obtained at Percy Boland Hospital from children who returned for the second evaluation approximately 1 month after birth.

Diagnosis of Maternal *T. cruzi* Infection

For mothers in the Percy Boland cohort, sera were screened onsite for Chagas disease, using the Chagas Detect Plus rapid test (InBios International, Seattle, WA), as well as the PolyChaco indirect hemagglutination assay at a single dilution of 1:16 (Lemos Laboratories, Santiago del Estero, Argentina). Maternal infection status was later confirmed using a commercial enzyme-linked immunosorbent assay (ELISA), the recombinant v3.0 ELISA (Wiener Laboratories [Rosario, Argentina]; reported sensitivity, 99.3%; reported specificity, 100%), performed according to the manufacturer's instructions. Maternal infection with *T. cruzi* was confirmed if results of ≥2 tests were positive. In 397 seropositive mothers, an IgG TESA blot was also performed, as previously described in the literature [22, 23], to detect SAPA bands, as well as to look for a single band between 150 and 160 kDa (indicating chronic infection).

Diagnosis of Congenital *T. cruzi* Infection

In infants in our Percy Boland cohort, congenital *T. cruzi* infection was determined postnatally in infants on the basis of qPCR or micromethod positivity. Micromethod was performed immediately after birth on fresh blood samples, while qPCR was performed within a month on a blood clot that was frozen at −20°C and shipped to Peru for analysis, as described previously [24]. Results were then sent back to Bolivia, and infants with positive results were referred to a neonatologist for follow-up and treatment with benznidazole. Whole-blood specimens were stored in RNA/DNA Shield (Zymo Research) and placed in our biorepository.

Western blot (for detection of IgM SAPA bands) was then performed on sera from seropositive and seronegative infants from Santa Cruz (a Chagas disease–endemic area). Archived sera from mothers and infants from Piura (Chagas disease–nonendemic area) were used as a control.

For the micromethod, blood specimens were aliquoted into 4–6 heparinized microhematocrit tubes, processed by centrifugation (9.503 *g* for 7 minutes), and analyzed by microscopy.

To extract DNA from clots, samples were processed using a high-speed benchtop homogenizer (FastPrep 24), followed by the High Pure PCR Template Preparation kit (Roche Diagnostics). By use of a Matrix E tube (MP Biomedicals, Santa Ana, CA), 300 µL of clot, 300 µL of guanidine/ethylenediaminetetraacetic acid, 40 µL of proteinase K (20 mg/mL), and 5 µL of internal amplification control (40 pg/µL) were processed by the FastPrep homogenizer at 5.5 m/second for 30 seconds. Then, following centrifugation at 9.503 *g* for 1 minute, 450µl of supernatant was removed, and 150 µL of Binding buffer from the High Pure PCR template preparation kit (Roche Diagnostics) was added. The mixture was then incubated at 70°C for 10 minutes, and extraction was performed using the High Pure PCR Template Preparation kit (Roche Diagnostics). A standard curve for clot samples was built as follows: 9 mL of blood, extracted without additives, was spiked with 1 ml of 10^6 parasites/ml suspended in PBS immediately after blood draw. The spiked sample was then thoroughly mixed by inverting the tube at least 20 times and allowed to sit for at least 30 minutes (no longer than 60 minutes) to allow clot formation. The clot was recovered after centrifugation at 1100 × *g* for 20 minutes. DNA from the clot was extracted as detailed for the clinical clot samples.

As described by Duffy et al [25], duplex qPCR was performed to target the satellite sequence of the nuclear genome of *T. cruzi* and the sequence of the internal amplification control. Quantitative PCR was then performed using the FastStart Essential DNA Probes Master (Roche Diagnostics, Mannheim, Germany), as previously described by Ramírez *et al* [26]. 5µL of

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re-suspended DNA was used in a final volume of 20µL, alongside 0.75µM of the Cruzi1 and Cruzi2 primers, 0.1µM of the IACF and IACRev primers and 0.5µM of the Cruzi3 and IACtq probes. Cycling conditions included a first step of 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 58°C for 1 minute.

IgG and IgM TESA Blot

Sera specimens were stored at -20°C prior to testing. Trypomastigote Excretory-Secretory Antigen (TESA) was processed using SDS-PAGE and immunoblotting as described by Umezawa et al [22] with the following modification: TESA antigen was diluted (vol/vol) in SDS-sample buffer (0.5M Tris-HCl [pH 8], 2.5 M dithiothreitol, 10% SDS, and 0.01% bromophenol blue), then boiled for 5 minutes. Samples were loaded in a 7% polyacrylamide mini-gel (Mini-Protean II; Bio-Rad). Then, separated antigens were transferred onto 0.45-mm-pore-size nitrocellulose membranes (Bio-Rad). Blotted antigens were tested with specimens, and TESA antigen-antibody complex were detected using peroxidase-conjugated goat anti-human immunoglobulin M (IgM) (KPL, Gaithersburg, MD, USA) for infants, or anti-human IgG, (KPL, Gaithersburg, MD, USA) for mothers. Hydrogen peroxide and 3,3'-diaminobenzidine (DAB; Sigma) were used as substrate and chromogen.

Acute infection in newborns was indicated by the presence SAPA bands, or ladder-like bands between 120–200 kDa on the IgM TESA blot [20]. Chronic maternal infection was indicated by a TESA band, a single 150–160 kDa band on the IgG TESA blot [22]. The total number of bands between 130 and 200 kDa was recorded. Any blot with ambiguous or weak banding was repeated. Band interpretation is depicted in Figure 1.

Statistical Analysis

Analyses were performed using Stata13 (Stata Corp, College Station,TX). Two-way and multiway tabulations of frequencies and means were performed. Further analysis was made graphically and with the Fisher exact, McNemar, and χ^2 tests. The *t* test, Wilcoxon signed rank test, Mann-Whitney test, and logistic regression were also used.

We defined new variables for all possible combinations of the six IgM SAPA band weights and conducted bivariate and multivariate analyses to identify patterns of band occurrence. To assess the concordance of qPCR and MM we used the McNemar exact test. Additionally we evaluated the sensitivity, specificity and the area under the curve (AUC) for MM in predicting qPCR outcome. We also investigated the sensitivity, specificity and the AUC of IgM SAPA bands in newborns using qPCR as the reference test and then, separately, using MM. The Fisher exact and χ^2 tests were used for testing associations. All

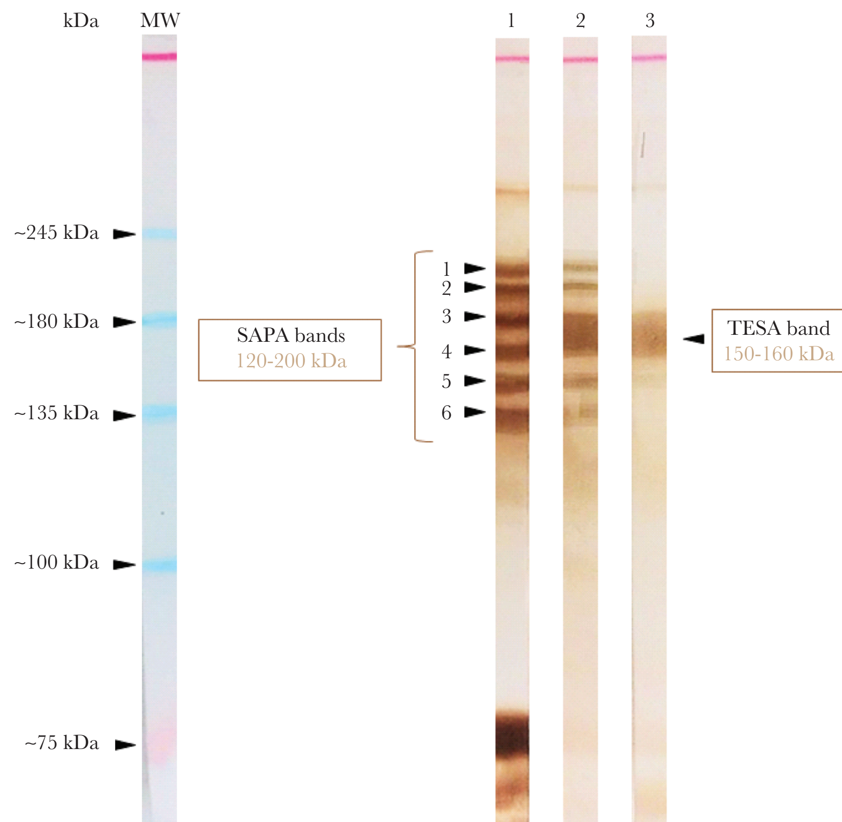

Figure 1. Band interpretation. MW, molecular weight; SAPA, shed acute phase antigen; TESA, trypomastigote excretory secretory antigen.

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confidence intervals (CIs) are at the 95% confidence level. The 95% CIs for incidence, sensitivity and specificity values were computed using the exact binomial method. The association between the number of IgM SAPA bands observed in newborns and their qPCR outcomes were also assessed using logistic regression.

To better understand the relationship between qPCR and MM, the parasite load (natural log of the parasite load plus 1) in qPCR-positive newborns whose micromethod results were negative was compared against those with both tests positive. The association of parasite load with different numbers of IgM SAPA bands was assessed by the Mann-Whitney test and linear regression.

To evaluate the impact of the early start and duration of therapy, we used Wilcoxon signed rank test to compare the number of bands and the parasite load at birth and the month 1 evaluation. We also used the Mann-Whitney test to compare the difference in parasite load at the month 1 evaluation between those who received earlier treatment and others.

We also assessed the sensitivity and specificity of mothers' qPCR and IgG bands in predicting newborns' qPCR results by the Fisher exact test.

Ethics Statement

The institutional review boards of the Johns Hopkins Bloomberg School of Public Health; The Universidad Peruana Cayetano Heredia (PNS); The Universidad Catolica Boliviana San Pablo; Hospital de la Mujer Percy Boland; Asociacion Benefica PRISMA, Hospital Cayetano Heredia and Hospital Santa Rosa approved the study.

RESULTS

Our participants consisted of 601 seropositive mothers and their 617 newborns who were recruited by the Percy Boland study in Santa Cruz, Bolivia, where *T. cruzi* is endemic (Figure 2). Specimens were collected from all mothers and their infants at birth. Follow-up specimens were collected from 299 infants whose mothers returned for the second evaluation approximately one month after birth. 318 infants did not return for this follow-up evaluation. A control sample of seronegative mothers and their newborns from Percy Boland and from two Piura hospitals were enrolled as negative controls.

Comparison of qPCR and MM as Diagnostic Methods of Chagas Disease in Newborns

We compared qPCR and MM tests for identifying *T. cruzi* positive newborns of seropositive mothers at birth (Table 1). Of the 617 newborns, 443 newborns were tested using both methods. 5 were not tested by qPCR and 174 were not tested by MM. Among 26 infants positive by at least one test, qPCR detected 25 (96%) infants while MM detected only 12 – MM sensitivity in predicting qPCR outcome: 44%(24–65%), specificity: 99%(99–100%), $P < .0001$, AUC = 72%. Both qPCR and MM were positive in specimens from 11 infants. One MM-positive specimen was negative by qPCR and may have resulted from an error in reading the MM test, since IgM SAPA bands were also negative for the specimen.

Sensitivity and Specificity of IgM SAPA Bands vs qPCR as the Reference Test

A primary objective was to evaluate the sensitivity and specificity of IgM SAPA bands with qPCR as the gold standard. Data on IgG positivity at 6–9 months of age were not available to confirm infection status, so we selected qPCR as our primary gold standard because it was more objective than MM and noted as being more sensitive in the literature [27, 28].

Of the 592 analyzable records 567 infants were qPCR-negative and 25 were qPCR-positive - an incidence of congenital transmission of 4.1% among seropositive mothers. In this study, only one pair of twins was qPCR positive. Given that 15 mothers gave birth to more than one newborn, the incidence of congenital transmission among such mothers was 6.7% (0.2–31.9%).

Sensitivity of IgM SAPA bands was assessed by SAPA band presence in qPCR-positive infants (Table 2). None of the 25 qPCR-positive infants were observed to have band weights one and two exclusively without any other bands present, while 20% (5 infants) had four bands (band weights one, two, three and four) and 60% (15 infants) had all six bands. The cumulative sensitivity of either four or six bands was 80% (59–93%). The positive likelihood ratio was 13.8 (9.4–20.2), while the negative likelihood ratio was 0.21 (0.1 to 0.5).

Specificity was assessed in the 567 qPCR-negative infants born to seropositive mothers in Santa Cruz. Of these newborns, 6% (33 infants) had IgM SAPA bands. 3% (15 infants) had 2 bands, and 3% (18 infants) had >2 bands, resulting in IgM SAPA band specificity of 94% (92–96%). Specificity was 100% in both control groups. Compared with MM, TESA blot had a higher AUC (88% vs 72%; $P = .001$, by the χ^2 test)

Presence and Sequence of IgM SAPA Bands

We studied the presence and sequence of the 6 IgM SAPA band weights in qPCR-positive and qPCR-negative newborns. With 3 exceptions, band weights 1 and 2, 3 and 4, and 5 and 6 were always paired. Furthermore, these pairs were present in sequential order: band weights 1 and 2 were always present if 3 and 4 were present, and 1, 2, 3, and 4 were present if 5 and 6 present. For the three exceptions to band pairing, only three bands were observed: 1, 2, and 3, without band 4, and these specimens were qPCR-negative. There were 2 exceptions to sequential band pair appearance. One qPCR-positive/band-positive specimen had band weights 5 and 6 without band weights 3 and 4. Additionally, one

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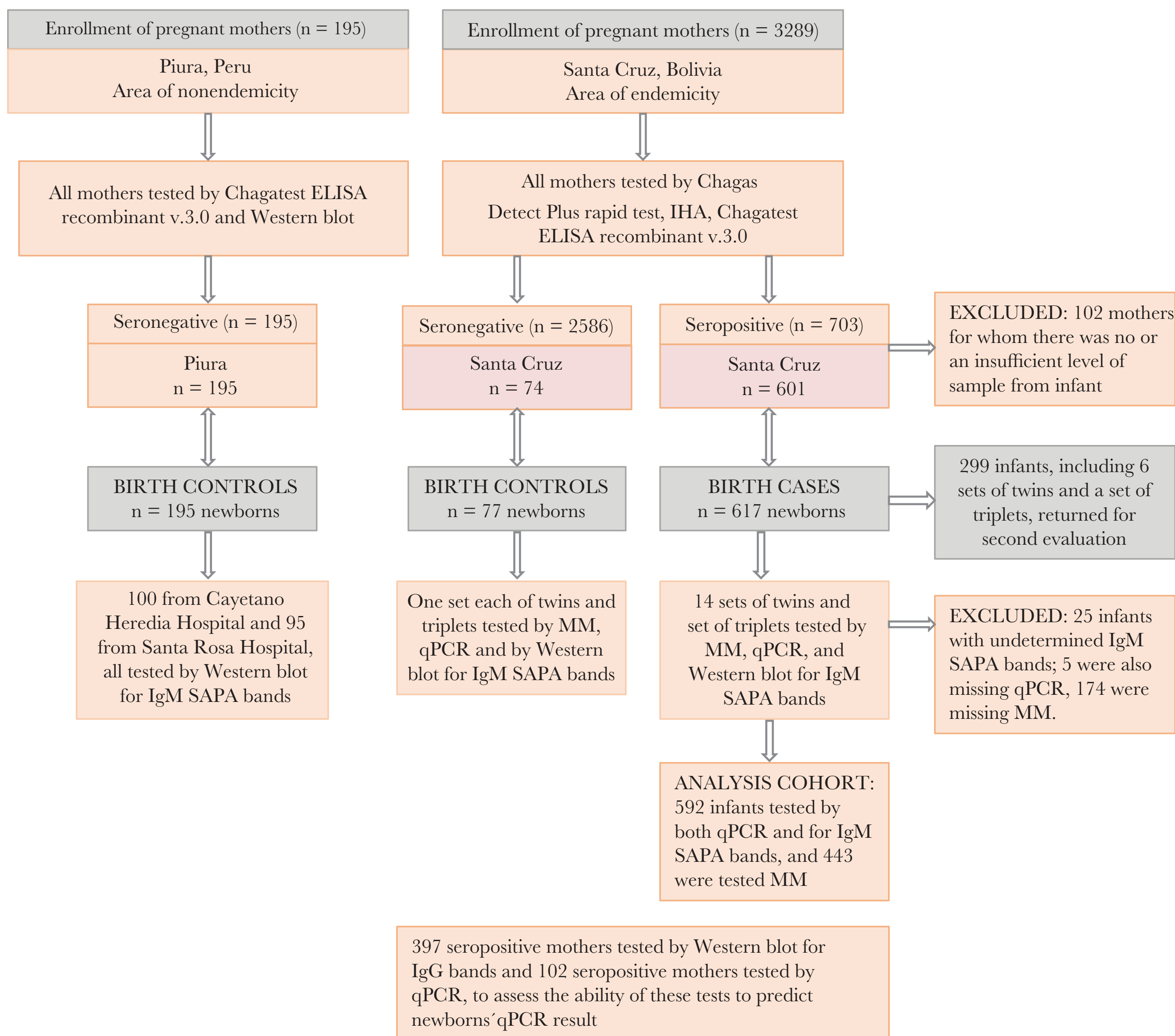

Figure 2. Flow of participants through the study. ELISA, enzyme-linked immunosorbent assay; IgM, immunoglobulin M; IHA, indirect hemagglutination assay; MM, micromethod`; qPCR, quantitative polymerase chain reaction assay; SAPA, shed acute phase antigen; TESA, trypomastigote excretory secretory antigen.

qPCR-negative/band-positive specimen had band weights 1, 2, 3, and 6. All infants with only bands one and two were qPCR and MM negative.

Parasite Load Association With MM and SAPA Bands

Parasite load was higher in qPCR-positive infants who were also MM positive, compared with those who were MM negative ($P = .0138$, by the Mann-Whitney test; Figure 3).

Parasite load was also associated with the number of IgM SAPA bands present at birth, as demonstrated in the lower panel of Figure 3 (n = 25, OR = 1.82, $P = .028$; Mann-Whitney test between 4 and 6 bands vs no bands $P = .0066$; $r^2 = 0.29$ among qPCR-positive infants; probability of F = .0056). A stronger relationship was observed when including both qPCR-positive and qPCR-negative infants (n = 592, adjusted $r^2 = 0.43$; probability of F < .00001; graph not shown).

Treatment and Parasite Load

Twenty-four out of 25 infants who were qPCR-positive at birth received treatment with benznidazole. Although timing varied, 9 of these newborns had parasite load determinations at the second evaluation after being treated. The analysis of treatment effect was limited to these infants and indicated a negative correlation between parasite load post-treatment and the number of days of treatment received prior to the determination. All infants whose treatment started within the first 15 days of life (Figure 4) and received at least 18 days of therapy (Figure 4)

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Table 1. Concordance Between Micromethod (MM) and Quantitative Polymerase Chain Reaction (qPCR) Results at Birth

	qPCR Result, No. (%)	
MM Result	Negative	Positive
Negative	417 (99.8[a])	14 (56)
Positive	1 (0.2)	11 (44[b])
Total	418	25

$P < .0001$, by the McNemar and Fisher exact tests.

[a]Specificity.

[b]Sensitivity.

were qPCR-negative at the second evaluation, regardless of their parasite load at birth (natural log of parasite load at birth in infants with negative qPCR at second evaluation = 9.95, among those with positive qPCR at second evaluation = 9.48, $P = .3272$). Treatment for shorter periods did not change the qPCR ($P = .0073$, by the Mann-Whitney test, and $P = .0431$, by the Wilcoxon signed rank test).

Although the number of IgM SAPA bands at birth was highly correlated with log parasite load, we did not see evidence of any correspondent change in the number of bands following treatment, even when adjusted for treatment duration (data not shown).

Maternal qPCR Result as a Predictor of Newborn Infection

Mothers' qPCR results were predictive of their newborns' qPCR status with a sensitivity of 75% (46–91%) and specificity of 74% (64–82%), observed among 102 mothers who had qPCR results and their infants (Table 3). In addition, all 25 qPCR-positive infants were born to mothers who had a chronic TESA IgG band (24%) or both TESA and SAPA IgG bands (76%).

DISCUSSION

This study demonstrated that, when compared to qPCR, the IgM blot was both sensitive and specific for congenital Chagas diagnosis if four or more IgM SAPA bands were present. SAPA bands occurred in pairs, sequentially, and parasite load correlated highly with the number of SAPA bands present. Both MM and qPCR were strongly associated with the number of SAPA bands at birth. Yet, compared to qPCR, MM detected less than half of congenitally infected infants. In contrast, both IgM SAPA blot and qPCR were always positive when MM was positive, excepting one infant who was MM-positive but qPCR and IgM SAPA band negative. Children treated for at least 18 days reversed their qPCR, but there was no reversal of a positive IgM SAPA blot. Mothers who had a positive qPCR were three-fold more likely to vertically transmit Chagas, a finding similar to previous studies [14, 29, 30].

The cumulative sensitivity of either four or six SAPA bands occurring at birth was 80%. To be considered positive by IgM TESA previous studies required that all six SAPA bands be present [20, 22]. In our study when only six bands were used sensitivity was 60%, a 20% loss of sensitivity. Specificity of the IgM SAPA bands was 94%. Two other studies, one in Argentina and the other in Chile, examined SAPA for the diagnosis of congenital Chagas infection using a recombinant protein and either ELISA or IFA to detect IgM antibody in babies [31, 32]. In the Argentine study the observed sensitivity was 100% but only three positive babies were studied while in the Chilean study a sensitivity of 42% was observed among 12 positive babies sampled at birth [31, 32]. The lower sensitivity in the Chilean study may be due to their using a smaller segment of the SAPA molecule.

Further investigation is indicated to understand the apparent pairing of the SAPA bands and correlation with parasite load. Whilst the SAPA molecule has been previously characterized as having two domains, enzymatic and antigenic [23], further description is warranted.

Early identification and treatment of *T. cruzi* congenital infection is required to prevent chronic complications and curb transmission [20, 21]. Maternal IgM antibodies do not cross the placental barrier; thus, IgM in infant blood samples indicates infant immune response to *T. cruzi* [20]. Six percent of infants

Table 2. Distribution of Immunoglobulin M–Specific Trypomastigote Excretory Secretory Antigen Blot Band Patterns, by Micromethod (MM) and Polymerase Chain Reaction (PCR) Results, in Specimens Obtained at Birth From Newborns of Seropositive Mothers

		Bands, No. (%), by qPCR and MM[a] Result			
		qPCR		MM	
Bands, No.	Bands Present	Negative	Positive	Negative	Positive
0	None	534 (94)	5 (20)	388 (90)	1 (8)
2	1 + 2	15 (3)	0 (0)	15 (3)	0 (0)
3	1 + 2 + 3	3 (1)	0 (0)	3 (1)	0 (0)
4	1 + 2 + 3 + 4	5 (1)	5 (20)	7 (2)	3 (25)
6	1 + 2 + 3 + 4 + 5 + 6	10 (2)	15 (60)	17 (4)	8 (67)
Total	…	567	25	430	12
P[b]			<.0001		<.0001

[a]In 12 infants with positive results of the micromethod assay, 92% (95% confidence interval, 56%–99%) had either 4 or 6 bands.

[b]By the Fisher exact test.

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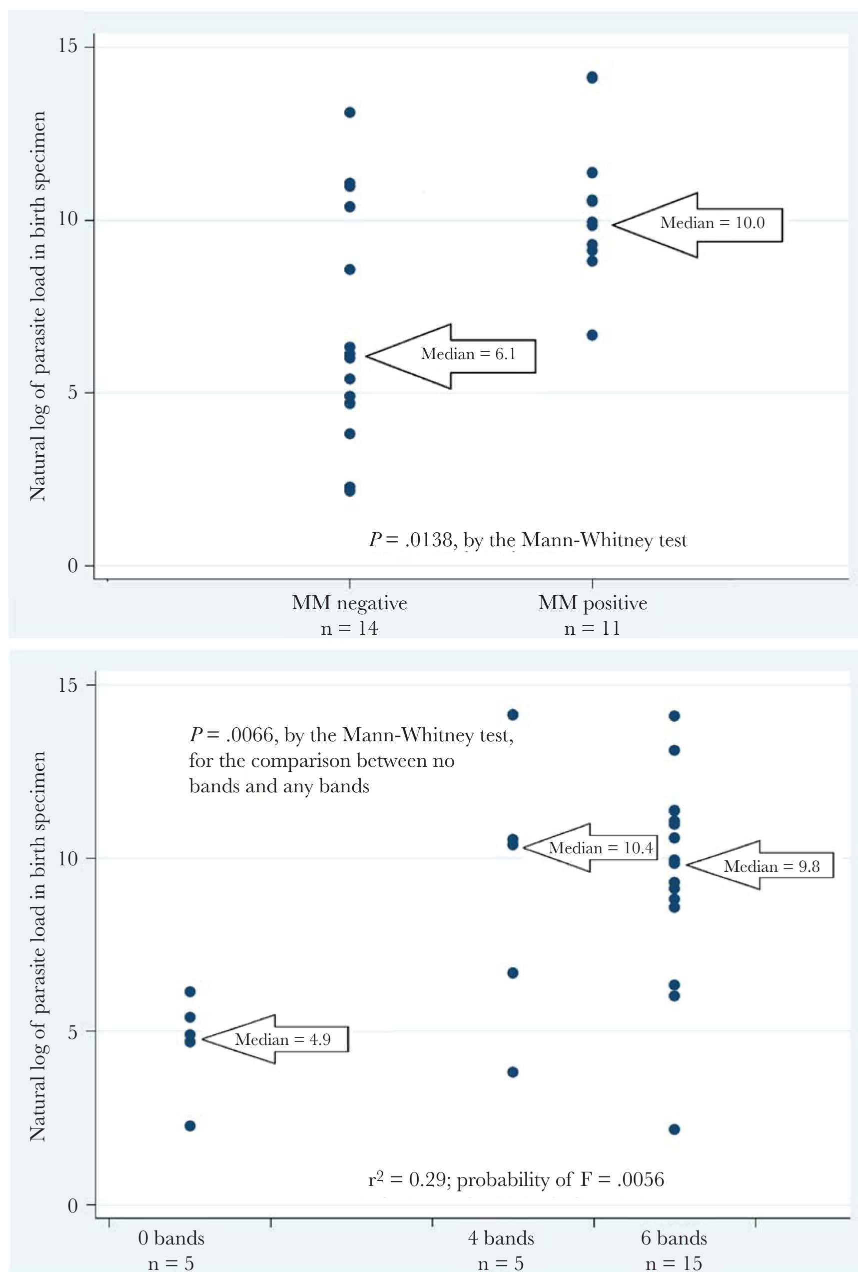

Figure 3. Natural log of parasite loads among quantitative polymerase chain reaction–positive newborns, by diagnostic test (top panel) and total number of immunoglobulin M (lower panel).

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were IgM SAPA positive but qPCR negative. All were from seropositive mothers. These infants thus could be infected, but not detected by qPCR, or they may have had antigen exposure without active infection. No child born to a seronegative mother had any IgM SAPA band, thus supporting the high specificity of the test.

Once IgG has waned six- to nine-months after birth, infant specimens may be tested for IgG to confirm congenital infection, since infection via triatomine bug feces in the first 3–4 years of life is uncommon [2]. However, testing at 6–9 months of age rarely occurs, primarily due to loss to follow-up [12, 13]. The gold standard used in this study was qPCR, which has the highest described specificity and sensitivity for infants under 6 months. Presently qPCR is not routinely performed in Bolivia due to expense and lack of trained personnel. Due to its lower cost, MM is still the most commonly described test for congenital Chagas [14, 19]. However, MM is subjective and has poor sensitivity, failing to detect over 40% of infections, particularly those where parasite load is low [14, 20, 28].

The lack of 6–9-month follow-up is a limitation of this study. In addition, cross reactivity for other endemic infectious agents, such as *Leishmania* species [33], was not assessed, and venous samples, rather

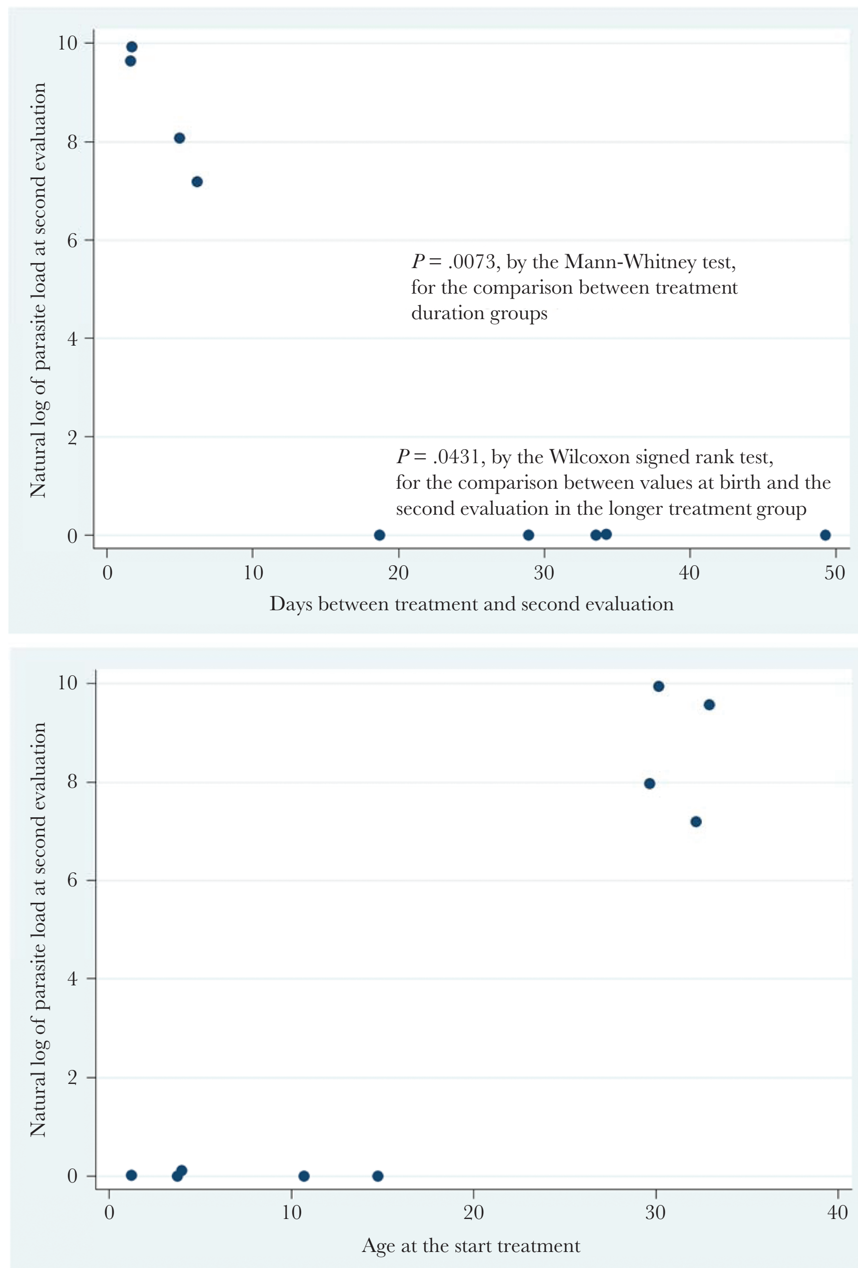

Figure 4. Parasite load by number of days after commencement of treatment (tests indicated in the top panel apply equally to the bottom panel).

than cord blood samples were taken from infants in Bolivia following delivery, reducing amounts of clot to be sampled. It should also be noted that the performance of serological tests for Chagas disease could vary by geographic region [34], thus the results of this study are specific to Bolivia and probably extend to other countries where *T. cruzi* DTU types II, V, and VI are predominant. The projectability of these results to areas with type I will need further investigation.

The presence of the bands in pairs suggests that these may be dimers. Our group is in the process of both defining the composition of these bands, using a proteomic approach, and producing recombinant antigens for future use.

In summary, screening for congenital *T. cruzi* infection is difficult. For lack of a better screening test for infants, and given substantial loss to follow up, greater than 50% of *T. cruzi*–infected infants may go undiagnosed in current screening programmes [12, 13, 20]. SAPA antigen clones were demonstrated to be useful for diagnosing congenital infection in small numbers of infected children but sequences were not published. Thus SAPA recombinant antigens may be a useful approach in the future [27, 28]. The IgM blot for SAPA bands demonstrates that in the future, the SAPA antigen may provide a rapid, relatively inexpensive Point of Care test for congenital infection a test that is more reliable and sensitive than MM.

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Table 3. Maternal Quantitative Polymerase Chain Reaction (qPCR) Results as Predictors of Newborn qPCR Status

	Newborn qPCR Result	
Maternal qPCR Result	Negative	Positive
Negative	63(74)	4(25)
Positive	23 (26)	12 (75)
Total	86	16

$P < .0001$, by the Fisher exact test.

MEMBERS OF THE STUDY GROUP

Members of the Chagas Working Group in Bolivia and Peru are as follows: Monica Miranda-Schaeubinger, Indira Chakravarti, Ellen L. Ferriss, Clariza Chavez, Jean Karla Velarde, Federico Urquizu, Mirko Gorena, Natalie Bowman, and Edith Hinojosa.

Notes

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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