Short Report: Early Diagnosis of Congenital Trypanosoma cruzi Infection, Using Shed Acute Phase Antigen, in Ushuaia, Tierra del Fuego, Argentina

Maria Cristina Mallimaci, Sergio Sosa-Estani,* Graciela Russomando, Zunilda Sanchez, Carina Sijvarger, Isabel Marcela Alvarez, Lola Barrionuevo, Carlos Lopez, and Elsa Leonor Segura

Laboratorio Central, Hospital Regional Ushuaia; Centro Nacional de Diagnóstico e Investigación de Endemopépidemias (CeNDIE) ANLIS “Dr. Carlos G. Malbrán,” Ministerio de Salud, Instituto de Efectividad Clínica y Sanitaria, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina; Instituto de Investigaciones de Ciencias de la Salud, Universidad Nacional de Asunción, Paraguay; Instituto Nacional de Parasitología “Dr. Mario Fatale Chaben,” ANLIS, Buenos Aires Argentina

Abstract. Chagas’ disease, or American trypanosomiasis, is caused by the protozoan parasite Trypanosoma cruzi. It is a major cause of morbidity and mortality in Latin America.1 It is estimated that 15,000 new cases of congenital T. cruzi transmission occur in the Americas each year. The aim of this study was to estimate the rate of congenital T. cruzi infection in infants born to infected women living in Ushuaia, Argentina, as well to assess a serologic test using Shed Acute Phase Antigen (SAPA) for a timely diagnosis of congenital infection. The rate of congenital infection among children in the study was 4.4% (3/68). Our results show that for infants younger than 30 days of age, matched blood samples from mother and infant were capable of identifying congenital transmission of infection using an enzyme-linked immunosorbent assay with SAPA. For infants older than 3 months, congenital infection could be ruled out using the same procedure.

Chagas’ disease, or American trypanosomiasis, is caused by the protozoan parasite Trypanosoma cruzi. It is a major cause of morbidity and mortality in Latin America.1 It is estimated that 15,000 new cases of congenital T. cruzi transmission occur in Latin America each year2 and 2,000 in North America.3 Currently, only direct parasite detection tests are able to confirm infection at birth. However, after the age of 9 months, with the disappearance of maternal antibodies, conventional immune-globulin G (IgG) serology may allow the diagnosis of congenital infection.4,5 The long-term follow-up on infants to confirm whether they are infected often causes decreases of the adherence, with a consequent lack of opportunity for almost 70% of newborns to infected mothers, to be diagnosed and treated timely.4,6,7

The aim of this study was to estimate the rate of congenital T. cruzi infection in infants born to women infected and living in Ushuaia, Tierra del Fuego, Argentina, as well to assess a serologic test using Shed Acute Phase Antigen (SAPA) for a timely diagnosis of congenital infection. The study populations were mothers infected with T. cruzi and their children up to 2 years old, recruited from February 2001 to December 2002. We conducted a cohort study from birth to 9 months of age. Blood samples of mothers and children were collected at birth. From age 3 months and older, only the children's blood samples were analyzed during the study. The methods for diagnosis of congenital infection were (1) the direct examination of blood parasites using the microhematocrit at the time of birth and/or (2) serologic tests: indirect hemagglutination (IHA; Polychaco, Buenos Aires, Argentina) and enzyme-linked immunosorbent assay (EIA; Conventional EIA; WIENER Laboratory, Rosario, Argentina or ORGANON, Buenos Aires, Argentina). These tests were performed according to the manufacturer’s instructions. During this study, the IgG antibody test was also performed to compare the results to the SAPA of T. cruzi (EIA-SAPA).8–12 This technique was standardized by the Departamento de Biología Molecular, Instituto de Investigaciones en Ciencias de la Salud,

*Address correspondence to Sergio Sosa-Estani, Centro Nacional de Diagnóstico e Investigación de Endemopépidemias (CeNDIE), ANLIS Dr. Carlos G. Malbrán, Ministerio de Salud, 1063 Buenos Aires, Argentina. E-mail: ssosa@msal.gov.ar

Universidad Nacional de Asunción, Paraguay (IICS-UNA).7,13 The antigen used was a recombinant SAPA of plasmid pGex (the insert used was released by Dr. Carlos Frasch [Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín, Argentina] in 1998 to the Department Biología Molecular, IICS, Paraguay) obtained by genetic engineering techniques at IICS-UNA. Serologic tests (IHA, conventional EIA, and EIA-SAPA) were performed on matched blood samples of mother and child from birth to 30 days of age and on blood samples taken from the child during follow-up. All infants had samples taken at least at 9 months of age or older. Serologic tests were considered reactive when the titer of IHA was ≥ 16, index of positivity (IP) of conventional EIA test result was ≥ 1, where IP is the optical density/cut-off, and the EIA-SAPA results were OD ≥ 0.300.7

Detection of antibodies using any serologic test is an indirect way of measuring infection. In addition, in the early months after birth, there is a great limitation because of the presence of antibodies transferred from the mother to offspring. Congenital infection was defined when a child born to a mother infected had a positive parasitologic test or IHA and conventional EIA reactive after 9 months of age. This procedure was used as the gold standard to estimate the rate of congenital T. cruzi transmission and to assess EIA-SAPA.

The data were analyzed to determine the prevalence of infection, with a 95% confidence interval or as means and SD for continuous data. Comparisons of proportions were done with χ² or Fisher tests. Means were compared by analysis of variance (ANOVA) and the Mann-Whitney test. Results of IHA were analyzed by conversion log, of 2-fold dilutions of serum samples. To analyze results of the serologic test at birth, an index was built called a “differential” by subtracting the results of the mother (Log of dilution to IHA, IP to conventional EIA, and OD to EIA-SAPA) from the results of the child (result of the child – results of the mother). Data were analyzed using Epi Info version 3.4 (CDC).

The study was approved by the Institutional Board of the Hospital Regional Ushuaia and the Ministry of Health of the Province. Oral informed consent to take samples was obtained from all women for themselves and their children.

The study population consisted of 61 infected mothers and 68 children. The mean age of the mothers was 29.1 years old,
and the mean length of residence in Tierra del Fuego was 7.1 years. Fifty percent of mothers were EIA-SAPA reactive. The rate of EIA-SAPA reactivity in women chronically infected was consistent with other studies.\(^{7,14,15}\) Among the 68 children in the study, 52.9% were girls and 60.3% were younger than 3 months of age. No children received transfusions.

The rate of congenital infection among children being studied was 4.4% (3/68). All of them were diagnosed by a parasitologic test (microhematocrit) at birth (Table 1). All infants with congenital \( T. cruzi \) infection were treated by the health providers according to official guidelines. The serologic tests were non-reactive when non-infected children reached 9 months of age or older.

Among 34 samples from mothers and their children who were 30 days of age, the median differential EIA-SAPA was \(-0.05\) (range, \(-0.5\) to 0.4) among children not infected and 1.7 (range, 1.02–2.43) among three children with congenital infection (\( P < 0.05 \)). In contrast, the median differential of IHA was \(-1.0\) (range, \(-0.5\) to 1.0) among 36 children without congenital infection and 1.0 (range, 1.0–1.0) among three children with congenital infection (\( P > 0.05 \)). For the conventional EIA for detecting IgG, the median differential was \(-0.3\) (range, \(-4.5\) to 1.8) and \(-0.3\) (range, \(-1.6\) to 0.7) among non-infected and infected children, respectively (\( P > 0.05 \)). In the three cases with congenital infection, the mothers had an OD of EIA-SAPA < 0.300 (Table 1). The differential of EIA-SAPA could distinguish the three children with congenital infection. The differential of IHA and conventional EIA of children without congenital infection overlapped with those who had congenital infection; hence, these tests could not distinguish congenital infection within 30 days of birth (Figure 1).

All children without congenital infection who tested EIA-SAPA reactive at birth or within 30 days of birth and their mothers who were EIA-SAPA reactive also had a differential index \(< 1\) at birth and were EIA-SAPA non-reactive after 3 months of age (Figure 2).

We used the subtraction to highlight the reactivity produced by the infant’s own antibody production, deducing the reactivity produced for antibodies transferred from the mother. Some SAPA-EIA–reactive mothers had infants with low reactivity (because of transferance of maternal antibodies or the lack of antibodies detected by SAPA-EIA), resulting in a value \(< 1\). All infants with values \(< 1\) were non-infected. In contrast, the differential index of conventional EIA had results \(\geq 1\) or negative values among infants with congenital infection (Figure 1).

During the study, maternal antibodies were detected in children without congenital infection at 6 months with IHA and 8 months with conventional EIA (Figure 2). All of them were non-reactive at 9 months of age or older. No non-infected children were EIA-SAPA reactive against maternal antibodies after 3 months of age. Reactivity of transferred maternal antibodies against SAPA in non-infected infants disappeared earlier than reactive transferred maternal antibodies against epimastigote antigens used in conventional-EIA. This phenomenon is caused by antibodies against recombinant SAPA detecting an antigen more defined than those of epimastigotes. Although SAPA has multiple epitopes, they are repetitive and have high affinity. Meanwhile, the reactivity of transferred antibodies against epimastigotes is more heterogeneous, and more time may be needed to show the decrease in reactivity (as least 9 months using conventional EIA).

Table 2 shows the response to the etiologic treatment of newborns diagnosed with congenital \( T. cruzi \) infection. The treatments started between 2 and 11 days after diagnosis was obtained. All infants became non-reactive according to their serologic tests during the follow-up.

Congenital \( T. cruzi \) infections occur in Ushuaia, and the migration of population can explain\(^{9}\) why the rate observed was above the national average (3.2%) and similar to the rate observed in the endemic area.\(^{17}\) The microhematocrit proved to be an excellent instrument, but it requires a long, laborious procedure and is dependent on the operator. This operator dependence explains the variations that occur in this test’s performance and why other authors have reported a detection rate ranging from 97.4% to 2.9%.\(^{7,18-21}\)

Indirect hemagglutination and conventional EIA could not distinguish children with congenital infection when the tests were made at birth.\(^{3}\) In contrast, within 30 days of birth, EIA-SAPA results from samples taken from mother and child were capable of detecting children with congenital infection. These results also indicated an ability to identify the absence of congenital infection for children \(> 3\) months of age. The use of SAPA for diagnosis of congenital infection in children in endemic areas was described previously.\(^{11,12,22,23}\) Our results show that, using corresponding samples of mother and child at birth, the test was successful in detecting congenital transmission in newborns up to 30 days, and it could rule out the possibility of congenital infection after 90 days of age.

Based on the results of these EIA-SAPA tests, it is possible to identify, within 30 days of birth, that an offspring born to an infected mother has congenital \( T. cruzi \) infection when (1) the mother is EIA-SAPA non-reactive and the child is EIA-SAPA reactive and (2) the differential of EIA-SAPA using matched samples of mother and child is \(\geq 1\). In contrast, the offspring does not have congenital \( T. cruzi \) infection within 30 days after birth when (3) the child is SAPA non-reactive, (4) both samples of the mother and child are reactive but the differential is \(< 1\); or (5) the child is EIA-SAPA non-reactive after 90 days.

### Table 1

Tests used to diagnose congenital \( T. cruzi \) infection at the time of birth or within 30 days of age, Hospital Regional Ushuaia, Ushuaia, 2001–2002.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Case 1 (age: 10 days)</th>
<th>Case 2 (age: 10 hours)</th>
<th>Case 3 (age: 2 hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct parasitemia</td>
<td>5 parasites</td>
<td>NA</td>
<td>4 parasites</td>
</tr>
<tr>
<td>(microhematocrit)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IHA reactive ≥ 1/16</td>
<td>1/512 (reactive)</td>
<td>1/256 (reactive)</td>
<td>1/128 (reactive)</td>
</tr>
<tr>
<td>EIA (IP) reactive ≥ 1</td>
<td>5.3 (reactive)</td>
<td>6.9 (reactive)</td>
<td>4.7 (reactive)</td>
</tr>
<tr>
<td>EIA-SAPA cut-off = 0.300</td>
<td>OD = 1.885 (reactive)</td>
<td>OD = 0.201 (reactive)</td>
<td>OD = 2.607 (reactive)</td>
</tr>
<tr>
<td></td>
<td>(not reactive)</td>
<td>(not reactive)</td>
<td>(not reactive)</td>
</tr>
</tbody>
</table>

NA = not applicable; IP = index of positivity (OD/cut-off); IHA = indirect hemagglutination; EIA = enzyme-linked immunosorbent assay.
A high level of absorption of EIA-SAPA at birth is not a sufficient indicator of infection when no maternal sample is available because of the inability to distinguish between the offspring’s own antibodies and those of the mother. On the other hand, it is necessary to assess whether samples of newborn with EIA-SAPA $\geq 0.300$ at birth, and a differential with a value 1 or more can diagnose congenital *T. cruzi* infection.

Neonates treated between 10 hours and 10 days of birth exhibited excellent tolerance to the treatment. All serologic tests, IHA, EIA, and EIA-SAPA, showed through serological test non-reactive that the treatment was effective. We could not test this series if EIA-SAPA showed an early negativization in comparison with conventional tests.

The main limitation of this study is the small sample size and the paucity of cases with congenital infection. Long-term follow-up of a large sample size of infants born to infected mothers is currently being analyzed (G. Russomando, personal communication). Other studies involving larger numbers of people in other regions will be necessary to validate this diagnostic method.

The prevalence of *T. cruzi* infection found in pregnant women in Ushuaia was 5.9%, similar to a region of endemic vector transmission in Argentina. This rate showed a real risk of congenital transmission, indicating that controls must be implemented, and the follow-up of children born to infected mothers should be strengthened.

Prevention of congenital transmission of *T. cruzi* is not possible at present, but it can be detected early, and treatment of infected newborns is highly effective. Congenital *T. cruzi* infection is recognized as a major cause of transmission in endemic countries where relatively successful vector...
control and blood screening programs exist.2 The disease is also observed along the major migration routes leading from endemic to non-endemic countries. After eliminating transmission of Chagas’ disease by vectors and blood transfusions, a critical final step toward eliminating the disease will involve finding new tools to diagnose and provide timely treatment to infants with congenital T. cruzi infection at the primary health care level.

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Authors’ addresses: María Cristina Mallimaci, Carina Sijvarger, Isabel Marcela Alvarez, Lola Barrionuevo, and Carlos Lopez, Laboratorio Central, Hospital Regional Ushuaia, 12 de octubre y Maipú, Ushuaia, Tierra del Fuego, Argentina; Tel: 54-2901-44-1066/1070, Fax: 54-2901-421173; E-mails: crisammallimaci@yahoo.com.ar, carina.sijvarger@gmail.com, chabelaush@hotmail.com, loladvb@hotmail.com, and rufianmelanco41@hotmail.com. Sergio Sosa-Estani, Centro Nacional de Diagnóstico e Investigación de Enemodeoparacillos (CeNDIE) ANLIS Dr. Carlos G. Malbrán, Av. Paseo Colón 568, 1063 Buenos Aires, Argentina. 

Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at second control post-treatment</td>
<td>4 years old</td>
<td>4 years old</td>
<td>17 months</td>
</tr>
<tr>
<td>Tolerance of treatment</td>
<td>Good (no adverse events)</td>
<td>Good (no adverse events)</td>
<td>Good (no adverse events)</td>
</tr>
<tr>
<td>EIA (IP) at first control post-treatment</td>
<td>Not reactive</td>
<td>Not reactive</td>
<td>Not reactive</td>
</tr>
<tr>
<td>EIA-SAPA at first control post-treatment</td>
<td>DO = 0.188 reactive</td>
<td>DO = 0.188 (not reactive)</td>
<td>DO = 0.132 (not reactive)</td>
</tr>
<tr>
<td>IHA at first control post-treatment</td>
<td>Not reactive</td>
<td>Not reactive</td>
<td>Not reactive</td>
</tr>
<tr>
<td>IHA-SAPA at second control post-treatment</td>
<td>DO = 0.164 (not reactive)</td>
<td>DO = 0.132 (not reactive)</td>
<td>DO = 0.106 (not reactive)</td>
</tr>
</tbody>
</table>

REFERENCES


