

Diagnosis of congenital Chagas disease: Relevant aspects.

Dayana Sofía Torres-Martínez ^a, John Jaime Quimbaya-Ramírez ^b,
Ángela Liliana Monroy-Díaz ^c

- a. Bacteriologist and Clinical Laboratorian, Research professor at Universidad de Boyacá, Tunja, Colombia. ORCID: <https://orcid.org/0000-0002-4013-7656>
- b. Veterinarian, Master's in research of Infectious Diseases, Professor at Universidad de Santander, Bucaramanga, Colombia. ORCID: <http://orcid.org/0000-0002-0883-3395>
- c. Bacteriologist and Clinical Laboratorian, Master's in research of Infectious Diseases. Professor, Universidad de Boyacá, Tunja, Colombia. ORCID: <https://orcid.org/0000-0002-3420-9123>

DOI: [10.22517/25395203.25093](https://doi.org/10.22517/25395203.25093)

Abstract

Introduction: This article describes relevant aspects surrounding congenital Chagas disease, such as epidemiology, symptomatology, review of clinical cases, and diagnostic techniques.

Methods: A review of the literature was carried out through bibliographic databases such as PubMed, Science direct, Scopus, Plos One, SciELO, having as inclusion criteria articles or publications between January 2013 and January 2022 in Spanish and English.

Results: It was determined that the prevalence of congenital Chagas disease is still a public health problem in endemic and non-endemic areas, and maternal serology is essential for timely follow-up of cases.

Conclusions: Current diagnostic follow-ups differ in endemic countries and screening is being applied in non-endemic areas where women from areas of active transmission of Chagas disease migrate.

Key words: Chagas disease, molecular biology, diagnosis, PCR, *Trypanosoma cruzi*.

Resumen

Introducción: El presente artículo describe aspectos relevantes entorno de la Enfermedad de Chagas congénita, tales como epidemiología, sintomatología, revisión de casos clínicos y las técnicas diagnósticas.

Métodos: Se realizó una revisión de la literatura por medio de bases de datos bibliográficas como PubMed, Science direct, Scopus, Plos One, SciELO, teniendo como criterio de inclusión las publicaciones artículos o comprendidos entre enero de 2013 y enero del año 2022 en idioma español e inglés.

Resultados: Se determinó que la prevalencia de la Enfermedad de Chagas congénita aún es un problema de salud pública en áreas endémicas y no endémicas, siendo la serología materna indispensable para dar seguimiento oportuno a los casos.

Conclusiones: Los seguimientos diagnósticos actuales difieren en los países endémicos y se están aplicando tamizajes en zonas no endémicas donde migran mujeres procedentes de áreas de transmisión activa de la Enfermedad Chagásica.

Palabras clave: Enfermedad de Chagas, biología molecular, diagnóstico, PCR, *Trypanosoma cruzi*.

Introduction

Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*), discovered by the Brazilian physician Carlos Justiniano Ribeiro Chagas in 1909 (1). This parasite is mainly transmitted vectorially to mammalian hosts by a group of hemiptera and hematophagous insects belonging to the family *Reduviidae*, subfamily *Triatominae* (2) commonly called “Chinches”, “Pitos” or “Barbeiros”, depending on the geographical area (3). There are alternative forms of infection such as blood transfusion, organ donation, oral transmission caused by ingestion of food contaminated with the parasite, accidental transmission in health workers and researchers, and vertical transmission (4).

According to the World Health Organization (WHO), it is estimated that 70 million people are at risk of infection by the *T. cruzi* parasite worldwide, placing Latin America as the main affected area (5), mother-to-child transmission is estimated between 1 to 12% in endemic areas (4), in Colombia transmission has ranged between 1 to 4% (4), in Bolivia in 4%, a similar percentage in the population of Argentina and 4.3% from Portugal (6). In addition, cases of this infection have been reported in non-endemic areas such as the United States, Spain, Switzerland, and Sweden due to the migration of infected women from South American areas to these countries (7).

This infection in women of fertile age predisposes to a congenital presentation (2); where 60 to 90% of neonates are asymptomatic with a pro-

gression to a severe chronic disease, which implies an underreporting and a limiting factor for diagnosis. When a symptomatic presentation develops, it usually appears in the first 30 days of life without a pathognomonic picture (3), having varied clinical manifestations that include low birth weight, prematurity, respiratory distress, hepatosplenomegaly, jaundice (2,8), generalized edema and even hydrops fetalis and death (3,8), which is why congenital Chagas disease is considered a public health problem (6).

Regarding the diagnosis of the disease, it is necessary to have the patient's clinical data, epidemiological data such as the incidence and prevalence of Chagas disease in the geographical area where the patient lives, and confirmation through laboratory tests, whose technique will depend on the clinical phase (9). The tests commonly used in the acute phase include the demonstration of the parasite present in the blood by microscopy and the Polymerase Chain Reaction (PCR) (10), while in the chronic phase serological diagnostic techniques are usually used to determine IgG antibodies (5,10).

It is known that molecular techniques can offer an earlier diagnosis of congenital infection than current methods (6), especially real-time PCR (qPCR) due to its characteristics of sensitivity (3), quantitative result (parasite load) and information of epidemiological interest in the determination of parasite lineages (11) in endemic and non-endemic areas (12), besides the inclusion of this technique in the diagnostic algorithm in endemic areas could reduce the follow-up period of the newborn and would mean a timely treatment (7).

In view of the above, the objective of this review is to present the epidemiology and diagnostic techniques for the detection of congenital Chagas disease.

«The tests commonly used in the acute phase include the demonstration of the parasite present in the blood by microscopy and the Polymerase Chain Reaction (PCR).



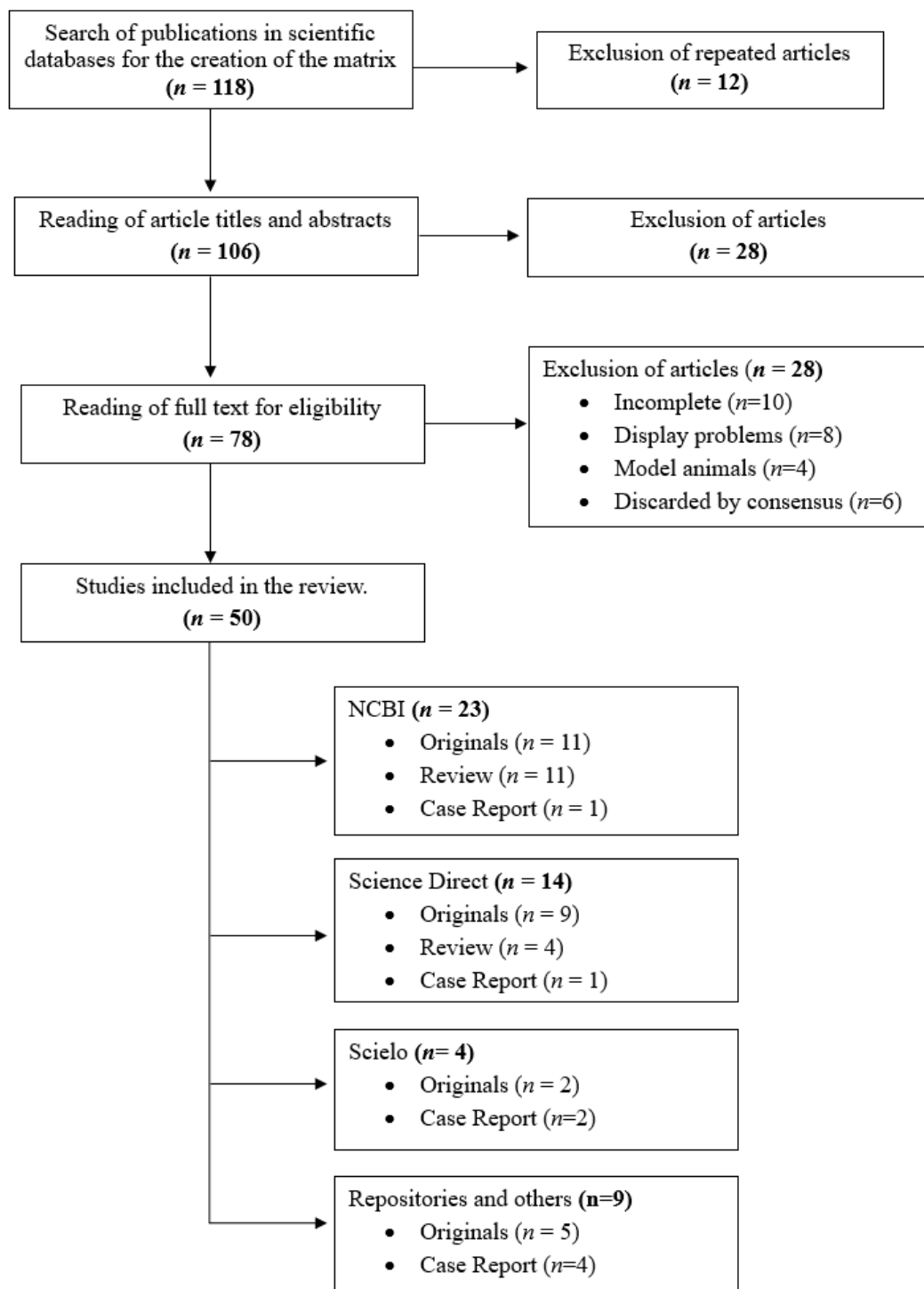
Methodology

The search for scientific production related to etiology, epidemiology, symptomatology, conventional and molecular diagnosis, as well as the development of new techniques for congenital Chagas disease was carried out. The review used the bibliographic databases Science direct, PubMed, Plos One, SciELO, among others, taking a period of time from 2013 to January 2022 and selected publications in Spanish and English language.

The descriptors DeCs (Health Sciences Descriptors) and Mesh (Medical Subject Headings) were used, applying the words “Chagas Disease”, “Epidemiology”, “Congenital”, “Molecular Diagnostic Techniques” associated with various combinations of Boolean indicators “And” and “Or”. All types of studies were included (review articles, original articles, case reports, consensus, among others); research studies measuring the degree of *T. cruzi* infection in vivo in mice or other animal models, articles that presented problems in their visualization and had an incomplete development of the topic, as well as articles that were repeated in the search matrix and those that by consensus did not contribute to the objective of the review of the topic were not included. According to Figure 1, a total of 78 articles were obtained for the complete reading of which 50 manuscripts were selected for the development of the document.

« *The search for scientific production related to etiology, epidemiology, symptomatology, conventional and molecular diagnosis, as well as the development of new techniques for congenital Chagas disease was carried out.* »

Figure 1. Types of articles included in the literature review.



Results

Epidemiology

According to the World Health Organization (WHO), Chagas disease is considered a “neglected tropical disease” that mainly affects low-income populations living in tropical and subtropical conditions (11). Congenital transmission of Chagas disease has become an increasing public health problem in areas where vectorial transmission has been interrupted and in non-endemic areas (9), added to the lack of knowledge of the disease, late diagnosis and poor patient follow-up can lead infected neonates to develop chronic phases (13) of the pathology with cardiac or gastrointestinal manifestations (12).

It is estimated that in the United States, 40 thousand women of fertile age are infected by *T. cruzi* annually (11), reporting 60 to 315 cases of congenital Chagas disease (14), which has been associated with tourism to endemic regions (13) and the migration of women infected with the parasite (15). Likewise, countries such as Canada, Australia, New Zealand, Spain and Japan (4) have reported the migration of about 14 million people from endemic areas, considering American Trypanosomiasis as an emerging disease (16), which has led to the development of screening programs in pregnant women and newborns (4) together with the application of new diagnostic tests such as loop-mediated isothermal amplification (*T. cruzi*- LAMP) (17).

In endemic regions, approximately two million women have a positive serology for the *T. cruzi* parasite and are potential transmitters of congenital Chagas disease (15) with an average risk of infecting their newborns of 5% (4). A variable rate of mother-to-child transmission has been reported in countries such as Paraguay (10%), Bolivia (3.4-8.6%), Chile (2.3%), Brazil (1.4%) (18), Argentina (6.6-11%) and Mexico (6.3%) (18,19), This variability depends on the subregion where exposure to the vector occurs, the lack of access to the health system due to social stigma (20), sanitary conditions (4,16,20) and living in constructions made of adobe, straw, mud or cane (21).

On the other hand, studies carried out in Colombia have estimated that 166,221 women between 15 and 44 years of age are infected with the parasite (16), with 1,046 new congenital cases occurring annually (21). In areas such as Boyacá, a prevalence of 2.4% of pregnant women seropositive for *T. cruzi* was established (22); making it essential to understand the transmission dynamics of the parasite in order to develop appropriate strategies

such as screening and medical controls (23,24), guarantee healthy environments (21), ensure medical coverage and care due to the limited access to resources and interventions in certain regions of the country (25) along with the treatment of Chagas disease in Colombia (22).

Although the estimated burden of Chagas disease reported in the country is low (0.956%) (14), Paez mentions that 10% of the Colombian population living in endemic areas are at risk (26) of vector transmission (27). On the other hand, Parra et al, determined that in Colombia, the most exposed populations are the indigenous tribes of the Sierra Nevada de Santa Marta (25), since they have poor living conditions and poor contact with the vector; In the Manzanal, Kasakumake, Umandita and Gumake settlements, 34% of positive serologies were obtained without significant differences in the infection of men and women (28). This was associated with the social (5), ecological, environmental, and cultural conditions of their environment, as well as their proximity to a great diversity of domestic (21) and wild animals and the lack of availability of adequate diagnosis and therapy (25), (29).

On the other hand, Carlier et al. (30) mention that in addition to intervening pregnant women, it is necessary to evaluate the serological status of 4 groups such as girls and adolescents, women of fertile age who are not pregnant, family members and all children born to women infected with the parasite and newborns as well as children at risk of being infected. Thanks to the application of the national subprogram for the promotion, prevention and control of Chagas disease, the Colombian Ministry of Health was able to screen a total of 27,334 people from 106 municipalities in Arauca, Boyacá, Casanare, Norte de Santander, Santander and Vichada; seropositivity was established in 21.15% of children under 5 years of age, 66.9% were between 5 and 14 years of age and the remaining 11.9% were over 15 years of age (21).

*«According to the World Health Organization (WHO),
Chagas disease is considered a “neglected tropical disease”
that mainly affects low-income populations living
in tropical and subtropical conditions.»*



Congenital Chagas disease

The first described cases of congenital Chagas disease date back to 1911, when two newborns of mothers with the disease presented convulsions and died between 6 to 8 days of life, and the presence of the *T. cruzi* parasite was found in their autopsies (9). Congenital transmission commonly occurs during the second or third trimester of pregnancy (8); infection should be suspected in births to a mother with positive serology for ***T. cruzi***, and transmission can occur in both the acute and chronic phases of maternal transmission and can occur in multiple pregnancies (9,11).

On the other hand, the infection develops when the infectious stage (metacyclic trypomastigote) enters the blood circulation (30) and mobilizes until it reaches the placenta and invades the Hofbauer cells (31), once there it multiplies and crosses the trophoblast (25); this invasion combined with a high parasite load can cause placentitis and villitis in the areas where the cellular layer has been destroyed (9). It should be noted that early transmission of the parasite increases the risk of spontaneous abortion (32).

However, some of the documented risk factors that could influence the pathogenesis of the disease include parasitemia, host genetics, immune response (7,33), gestational age and maternal age (18), knowledge along with exposure to the insect vector (2), family history of Chagas disease (9), having had multiple pregnancies or twin births (34), the genotype or strain of the infection (23), taking into account that TcI is the most prevalent discrete typing unit (DTU) (12), while TcII and TcVI (14,18) have greater infective capacity and pathogenicity (14, 36). Likewise, it has been documented that the social and demographic conditions of the population (31) together with the presence of co-infections, especially by human immunodeficiency virus (HIV), could aggravate the disease (7,9,11,33).

As for the main alterations in the newborn, these are variable, developing a wide spectrum of manifestations ranging from apparently healthy neonates of adequate weight for gestational age to severe conditions that can lead to death (32). In the symptomatic picture, the manifestations of the disease may include low birth weight, prematurity, low Apgar score (29), anemia (33), jaundice (25), thrombocytopenia, respiratory distress syndrome (15,18), hepatosplenomegaly and fever (32); symptoms that can be confused with infection by Cytomegalovirus, Herpes simplex (34), Toxoplasmosis, among other infectious agents (25).

On the other hand, newborns with asymptomatic congenital infection

are usually discharged without evaluation, but after years of silent infection about 20-30% develop severe and life-threatening manifestations (8) such as myocarditis, meningoencephalitis, gastrointestinal mega syndromes (35), anasarca, pneumonitis (33) and 5% present central nervous system and myocardial involvement (9, 34).

According to the publications consulted, five case reports were found corresponding to congenital Chagas disease (see Table 1), the first case corresponds to a neonate product of the seventh pregnancy of an indigenous woman from a rural area of western Colombia, who at day 50 of life presented hemodynamic failure and undetermined sepsis, positive parasitemia was identified for *T. cruzi* with a diagnosis of chagas meningoencephalitis when parasites were observed in cerebrospinal fluid (CSF) (36), treatment and favorable evolution were established, the epidemiological nexus was related to positive serology for *T. cruzi* in both parents.

The second case corresponded to a symptomatic male neonate with hepatosplenomegaly. A basic infectious profile of IgG and IgM antibodies against: Cytomegalovirus, HIV, Herpes simplex virus type I and II, Toxoplasma, Epstein Bar virus, Parvovirus, together with the determination of rapid plasma reagin (RPR) and total treponemal antibodies with negative results. The diagnosis was facilitated due to the inquiry of the geographical origin and the presence of positive results in the serological determination against *Trypanosoma cruzi* of the mother (24).

«As for the main alterations in the newborn, these are variable, developing a wide spectrum of manifestations ranging from apparently healthy neonates of adequate weight for gestational age to severe conditions that can lead to death.»



Table 1. Case reports corresponding to congenital Chagas disease

Age	Clinical manifestations and findings	Clinical history or relevant history	Diagnostic method - confirmation Chagas disease	Treatment	Reference
Neonate	Respiratory distress syndrome, with satisfactory evolution. At one month of life, he presented sepsis, positive urine culture for Klebsiella pneumoniae. At 50 days he presented hemodynamic instability, respiratory distress, mucocutaneous pallor and fever.	Colombian indigenous mother, 32 years old, multiparous.	<ul style="list-style-type: none"> Parasitemia positive by microhematocrit for Trypanosoma cruzi. Genotyping of T. cruzi showed TcI lineage. Histopathological study: ascending intraamniotic infection, with acute chorioamnionitis and subchorionitis; in the placental disc there was intervillitis with amastigotes in the intermediate trophoblast and in the basal plate. 	Benznidazole, for 60 days	(36)
Male neonate	Fever, jaundice, hypoglycemia. Physical examination: irritability, petechiae, distended abdomen with hepatomegaly and splenomegaly of 7 cm. Negative IgM serology against TORCH group agents.	The mother came from an endemic area: Ecuador.	<p>Serology: Indirect immunofluorescence (IFA) positive 1/64 and chemiluminescence (QLIA) positive.</p> <p>PCR positive</p>	Benznidazole (10 mg/kg/day) for 1 month	(24)
13-year-old boy	Chronic constipation. Colon dysentery	Parents and grandparents of Japanese descent lived in Chagas disease endemic areas in Bolivia until 1992. Patient had low birth weight.	<ul style="list-style-type: none"> Positive Elisa serology Positive real-time nested PCR Isolation on Novy, McNeal and Nicolle culture medium 	Oral benznidazole (5 mg/kg/day for 60 days).	(37)

« Subsequently, three cases were found, in which late symptomatology was present, achieving the diagnosis of Chagas disease at an advanced age: 13 years, 24 and 26 years ... »

<p>Young man 24 years old</p>	<p>No acute phase clinical manifestations. He is admitted to the Health Department for a positive serology when making a blood donation in 2014</p>	<p>The mother came from the city of Concepción, Bolivia. The patient one year ago presented with left-sided chest pain. Electrocardiogram and laboratory tests such as hemogram, BUN, creatinine, electrolytes and liver function tests were performed, where normal results were obtained</p>	<p><i>T. cruzi</i> enzyme immunoassay (AB EIA): Positive. AB IB immunotransfer assay (TESA): Positive</p>	<p>Nifurtimox orally at 10 mg/kg, daily, divided into three doses, for 90 days.</p>	<p>(38)</p>
<p>Young man 26 years old</p>	<p>No clinical manifestations of acute phase. He was admitted to the Health Department due to a positive serology after a blood donation in 2008.</p>	<p>The mother came from the city of Concepción, Bolivia. The patient denies having received previous blood transfusions and has not traveled to Bolivia or any other endemic destination in the Americas</p>	<p>Serology was confirmed by the Center for Disease Control and Prevention (CDC)</p>	<p>Nifurtimox, 10 mg/kg orally daily, divided into three doses for 90 days.</p>	<p>(38)</p>

Subsequently, three cases were found, in which late symptomatology was present, achieving the diagnosis of Chagas disease at an advanced age: 13 years (37), 24 and 26 years (38); some of the associated causes included presenting an apparently “healthy” state in the first years of life, the lack of adequate prenatal control and not carrying out an adequate follow-up of the newborn. It should be noted that, in all three cases, the mothers came from an endemic area and years later settled in non-endemic areas where Chagas disease was ignored as a differential diagnosis (33). In two siblings the diagnosis was accidental (38) when presenting a positive serology in a blood donation day; when inquiring about the donors’ condition, all alternative forms of infection were ruled out, including the vector’s bite, considering the cases as asymptomatic congenital Chagas with progression to the chronic phase of the disease, evidencing the first features of cardiac involvement.

Certainly, the etiological treatment of the disease in acute and congenital phase consists of the administration of two drugs Benznidazole or Nifurtimox, whose period of ingestion is determinant for its efficacy, considering

that in patients younger than 15 years who have had a shorter time of exposure to the parasite present a better response to antitrypanosomic (1), allowing a cure of up to 100% (18) in addition to having a good tolerance and safety profile (33), while in adults adverse effects such as anorexia, gastrointestinal alterations, neurological disorders, fever and skin rash have been reported, causing the interruption or abandonment of treatment in 75% of cases (39).

In all reports, treatment was administered to patients and parents (if required) presenting a significant clinical improvement, and no adverse effects were reported in children or adults (24,36-38). As previously mentioned, the cure rate decreases with age (40), being necessary the implementation of strategies that facilitate the opportune diagnosis avoiding the affectation of diverse systems by the progression of the disease to a chronic phase (41,42); additionally in 3 of the 5 cases (24,36,37) the PCR technique was used in the follow-up and diagnosis of the disease.

Diagnosis of congenital Chagas disease

Diagnosis is based on direct and indirect methods (see Table 2). Direct methods refer to the search for the parasite *T. cruzi* parasite, either by using fresh or stained samples, or by concentrating them as the micro-method or Strout's method to be observed by microscopy (14,43), while the indirect methods are classified as parasitological, serological and molecular (1,44), which require the growth of the microorganism in a specific culture media in the case of blood culture (33), the determination of antibodies in the patient's serum (serology) as a response to infection (39) or the amplification of a specific fragment (45) of the parasite in the patient's sample by first passing through a DNA extraction step (41), or the amplification of a specific fragment (42) of the parasite in the patient's sample by first passing through a DNA extraction step (43).

Table 2. Laboratory diagnostic techniques for congenital Chagas disease.

In all reports, treatment was administered to patients and parents (if required) presenting a significant clinical improvement, and no adverse effects were reported in children or adults. »

Table 2. Laboratory diagnostic techniques for congenital Chagas disease.

Technique	Test	Advantages	Limitations	References
Parasite concentration	Microhematocrit or Micro-method	<ol style="list-style-type: none"> 1. Can be performed in first level laboratories. 2. Low sample volume. 3. Can be performed on venous or umbilical cord blood. 4. If the test is negative at birth, it can be repeated at one month of age. 5. It allows to observe the viability of the parasite. 	<ol style="list-style-type: none"> 1. Health personnel must be trained to identify the parasite. 2. In low parasitemia or chronic phase, false negatives can be generated. 3. Parasite detection limit around 50 parasites/ml. 4. Low sensitivity 15- 35%. 5. Depends on the quality of the sample. 	3, 7, 17, 25, 26, 29
Microscopic observation	Fresh examination	<ol style="list-style-type: none"> 1. Can be performed in first level laboratories. 2. Low sample volume: 5 µL. 3. Morphological characteristics are checked. 	<ol style="list-style-type: none"> 1. Health personnel must be trained for parasite identification. 2. In low parasitemia or chronic phase, false negatives can be generated. 3. Low sensitivity reported. 	26, 33, 39
	Gross drop			
	Peripheral blood smear			
Other parasitological methods: <i>T. cruzi</i> growth	Blood culture	<ol style="list-style-type: none"> 1. Performed "in house". 2. Use of commercial media: NNN (Novy-McNeal-Nicolle) and LIT medium supplemented with fetal bovine serum. 3. Yield increases with the volume of blood used. 	<ol style="list-style-type: none"> 1. Prolonged time is required to determine growth: 30-60 days. 2. Not routinely used. 3. Parasites must be adapted to the culture medium for growth. 4. Lower sensitivity among indirect methods. 5. Approximate volume of 30 ml of blood is required. 	17, 23, 26, 30, 33
	Xenodiagnosis	<ol style="list-style-type: none"> 1. Metacyclic trypomastigotes are examined in the insect Triatomineo. 	<ol style="list-style-type: none"> 1. Not routinely used. 2. Triatomines must be free of <i>T. cruzi</i>. 3. The patient must be bitten by the insects. 4. Approximately 3 months are required to obtain results. 5. The insect vector must be fed in order to subsequently examine the intestine. 6. Costly test 	26, 27, 43

Serology	Enzyme-linked immunosorbent assay (ELISA)	<ol style="list-style-type: none"> 1. Sensitivity reported from 97 to 100%. 2. Specificity 96.3 to 100%. 3. Allows short patient follow-up. 4. Detection of IgG antibodies by obtaining color (conjugate + substrate). 5. Method of choice for the diagnosis of most clinical situations 	<ol style="list-style-type: none"> 1. Determination of antibodies from 9-10 months of age, to avoid false positives. 2. Two tests based on different antigens and/or techniques are required to confirm the diagnosis. 3. Trained personnel and special equipment are required for reading. 4. May present cross-reactions due to other pathologies. 5. High losses are generated during follow-up (80%). 6. Depending on the Kit, cross-reactions with Leishmania species may occur 	3, 10, 25, 26, 27, 29, 33,43
	Indirect Immunofluorescence (IIF)	<ol style="list-style-type: none"> 1. Sensitivity 93.3 to 100%. 2. Specificity 99 to 100%. 3. Allows a short follow-up of the patient. 4. Detection of IgG antibodies revealed by fluorescein. 5. Method of choice for the diagnosis of most clinical situations 		3, 13, 25, 26, 27,43
	Indirect hemagglutination (IHA)	<ol style="list-style-type: none"> 1. visible reading to the naked eye: Agglutination. 2. Determination of IgG antibodies. 		5, 25
Polymerase Chain Reaction (PCR)	Conventional	<ol style="list-style-type: none"> 1. Detection of 1 parasite/mL 2. Allows monitoring of acute infection. 3. Sensitivity reported between 90 to 95%. 4. Best test for acute phase and congenital infection 	<ol style="list-style-type: none"> 1. Specialized infrastructure. 2. The standardization process varies according to the laboratory conditions. 3. laboratory conditions. 4. Sensitivity and specificity changes according to the processing, conservation, DNA extraction, reagents and primers used. 5. False positives <1% in infected infants due to transplacental transfer or contamination with umbilical cord blood. 6. A positive result does not indicate parasite viability. 7. A negative result does not rule out infection. 	3, 6, 7, 11, 14, 25, 27, 42, 47, 48
	Real time	<ol style="list-style-type: none"> 1. Quantitative result. 2. Determination of parasite lineages. 3. Single round reaction in a closed tube. 4. Simultaneous detection of target DNA and internal controls. 5. Best test for acute phase and congenital infection 		25, 27, 29, 48, 49

Isothermal amplification	Looplamp	<ol style="list-style-type: none"> 1. No thermal cycler is required. 2. Results can be visualized in 1 hour. 3. Reaction in a single tube. 4. Tests have shown a sensitivity of 93% and specificity of 100%. 5. Less expensive than a qPCR kit. 	<ol style="list-style-type: none"> 1. Adequate infrastructure is required. 2. Experimental phase. 3. Lack of standardization of the technique. 4. Qualitative results. 5. A previous DNA extraction procedure is required. 	6, 7, 30, 42,43, 45
Nanoparticles	Chunap	<ol style="list-style-type: none"> 1. Potential to be a sensitive and simple test. 2. Urine is used, avoiding a non-invasive sample collection. 	<ol style="list-style-type: none"> 1. It is in the experimental phase. 2. Lack of standardization of the technique. 3. Nanoparticles are costly to develop and are not available in all laboratories 	39

On the other hand, the detection of congenital Chagas disease in endemic regions is based on a complex algorithm (8,33), which begins with the maternal serological detection that confirms the infection by *T. cruzi* (29), it is important to highlight that in the absence of a single serological test sufficiently sensitive and specific, it is necessary to use 2 or more with different antigenic principle (13). Subsequently, the newborn is evaluated by microhematocrit using umbilical cord or peripheral blood samples (14), serological studies (46) are recommended after 9 months of age (17), with sufficient time for the elimination of maternal antibodies (3, 47).

However, Benatar et al, consider that incorporating nucleic acid amplification methods into the algorithm would reduce constant sampling, allow early detection of a greater proportion of cases that are not detected by parasitological methods and reduce the loss of patient follow-up (17), in addition to the fact that this technique has various DNA amplification targets such as a 195 bp satellite (17,18,20,24), the E13 repetitive element, the D7 domain of the 24s subunit coding gene of ribosomal DNA, the sequence of the flagellar protein F29, the coding gene for histone H2A (27) and the conserved sequences of the mini-circles of the kinetoplast (kDNA) (46,48).

Likewise, Cura et al, mention that the choice of the type of PCR (conventional, multiplex, real time, among others), the biological origin of the sample and the biological target for amplification, has demonstrated a predictive role in congenital diagnosis, finding a higher clinical sensitivity when kDNA was amplified but lower specificity due to the presence of false positives with *Trypanosoma rangeli* (49), requiring the standardization of the techni-

que together with the research of new isothermal molecular technologies (46) that allow a timely and accurate delivery of results, shortening the diagnostic period (6,50).

In South America there is still no unified flow chart on the diagnosis of *T. cruzi* infection and the diagnostic techniques to be used.

Conclusions

Timely diagnosis of gestational Chagas disease and in women of childbearing age is vital for the control of the disease, since this would allow follow-up and control of both neonates and infected mothers.

Similarly, it is essential to advance in the generation of new diagnostic techniques that can be used as support in the algorithm of Chagas disease in endemic and non-endemic areas, since assays that have shown good sensitivity and specificity have been developed, but their major limitation is the infrastructure, the training of laboratory personnel and the cost for their development.

In addition, Colombia should guarantee the follow-up of women of fertile or gestational age throughout the national territory who are at risk of presenting Chagas disease to prevent progression to a chronic phase and minimize the incidence of cases, as well as to strengthen prevention strategies and the establishment of surveillance of family members in seropositive pregnant women and with confirmed neonates, given that congenital transmission can occur in all children and possibly older children have not been diagnosed.

Conflict of interest: none.

E-mail correspondence: almonroy@uniboyaca.edu.co

Bibliographic References

1. Subdirección de Vigilancia y Control en Salud Pública. Guía protocolo para la vigilancia en salud pública de Chagas. Ministerio de salud. Disponible en: <https://www.minsalud.gov.co/Documents/Salud%20P%C3%BAblica/Ola%20invernal/Protocolo%20Chagas.pdf>
2. Cevallos A.M, Hernández R. Chagas Disease: Pregnancy and Congenital Transmission. *Biomed Res Int.* 2014; 401864. DOI: 10.1155/2014/401864
3. Cucunubá Z.M, Valencia C.A, Puerta C.J, Sosa S., Torrico F., Cortés J.A, et al. Primer censo colombiano sobre Chagas congénito y orientación clínica a mujeres en edad fértil con diagnóstico de Chagas. *Infectio.* 2014;50-65. DOI: 10.1016/j.infect.2013.12.001
4. Freitas K.C, Andrade F.A, Bavia L., Damasceno F.S, Holsbach M., Messias I.J, et al. Chagas Disease: From Discovery to a Worldwide Health Problem. *Front Public Health.* 2019; 7: 166. DOI: 10.3389/fpubh.2019.00166
5. Carlier Y, Estani SS, Luquetti AO, Buekens P. Congenital Chagas disease: an update. *Mem Inst Oswaldo Cruz.* 2015; 110(3): 363-368. DOI: 10.1590/0074-02760140405
6. Padilla JA, Gallego M, Schijman AG, Gascon J. Molecular diagnostics for Chagas disease: up to date and novel methodologies. *Expert Review of Molecular Diagnostics.* 2017. DOI: 10.1080/14737159.2017.1338566
7. Messenger LA, Bern C. Congenital Chagas disease: current diagnostics, limitations and future perspectives. *Curr Opin Infect Dis.* 2018; 31 (5): 415-421. DOI: 10.1097/QCO.0000000000000478
8. Edwards MS, Montgomery SP. Chagas Disease: Implementation of Screening to Benefit Mother and Infant. *Clinics in Perinatology.* 2021; 48(2): 331-342. DOI: <https://doi.org/10.1016/j.clp.2021.03.013>
9. Edwards MS, Stimpert KK, Bialek SR, Montgomery SP. Evaluation and Management of Congenital Chagas Disease in the United States. *J Pediatric Infect Dis Soc.* 2019; 8(5): 461-469. DOI: 10.1093/jpids/piz018.
10. Marcus R., Henao A.F, Nolan M., Livingston E., Klotz S.A, Gilman R.H, et al. Recognition and screening for Chagas disease in the USA. *Ther Adv Infect Dis.* 2021; 8: 20499361211046086. DOI: 10.1177/20499361211046086
11. Livingston E.G, Duggal R., Dotters S. Screening for Chagas Disease during Pregnancy in the United States—A Literature Review. *Trop Med Infect Dis.* 2021; 6(4): 202. DOI: 10.3390/tropicalmed6040202
12. Chakravarti I., Schaeubinger M.M, Ruiz A., Briones C., Fernández E.A, Villanueva C.C, et al. Chagas Disease in Pregnant Women from Endemic Regions Attending the Hospital General de Mexico, Mexico City. *Trop Med Infect Dis.* 2022; 7(1): 8. DOI: 10.3390/tropicalmed7010008
13. Restrepo Zambrano M, Rousset F, Carrasco OF, Echeverría Murillo D, Costales JA, Brenière SF. Congenital Chagas Disease in the Ecuadorian Amazon: Maternal Screening at Delivery and Evaluation of Risk Factors Associated with Vector Exposure. *Am J Trop Med Hyg.* 2019; 101(6):1350-1358. DOI: 10.4269/ajtmh.19-0340
14. Bern C., Messenger L.A, Whitman A., Maguire J.H. Chagas Disease in the United States: a Public Health Approach. *Clin Microbiol Rev.* 2020; 33(1): e00023-19. DOI: 10.1128/CMR.00023-19

15. Santana K.H, Rodrigues L.G, De Castro D.B, Pereira M. Epidemiology of Chagas disease in pregnant women and congenital transmission of *Trypanosoma cruzi* in the Americas: systematic review and meta-analysis. *Trop Med Int Health*. 2020 ;25(7):752-763. DOI: 10.1111/tmi.13398.
16. Suescún S.H, Medina M.I, Murcia N.A, Forero S.M. Infección por *Trypanosoma Cruzi* en donantes de un banco de sangre del departamento Boyacá, Colombia. *Rev. Méd. Risaralda* 2021; 27 (1): 27-33. DOI: 10.22517/25395203.24601
17. Benatar AF, Danesi E, Besuschio SA, Bortolotti S, Cafferata ML, Ramíez JC, et al. Prospective multicenter evaluation of real time PCR Kit prototype for early diagnosis of congenital Chagas disease. *EBiomMedicine*. 2021; 69: 103450. DOI: 10.1016/j.ebiom.2021.103450
18. Bua J, Volta BJ, Perrone AE, Scollo K, Velázquez EB, Ruiz AM, et al. How to Improve the Early Diagnosis of *Trypanosoma cruzi* Infection: Relationship between Validated Conventional Diagnosis and Quantitative DNA Amplification in Congenitally Infected Children. *PLoS Negl Trop Dis*. 2013; 7(10): e2476. DOI: <https://doi.org/10.1371/journal.pntd.0002476>
19. Buekens P, Cafferata ML, Alger J, Althabe F, Belizán JM, Bustamante N, et al. Congenital Transmission of *Trypanosoma cruzi* in Argentina, Honduras, and Mexico: An Observational Prospective Study. *Am J Trop Med HYG*. 2018; 98(2): 478-485. DOI: 10.4269/ajtmh.17-0516
20. Iglesias L., Barja M.R, Boquete T., Benito A., Jordan B., Hernández T.B. Mapping health behaviour related to Chagas diagnosis in a non-endemic country: Application of Andersen's Behavioural Model. *PLOS ONE* 17(1): e0262772. DOI: <https://doi.org/10.1371/journal.pone.0262772>
21. Subprograma nacional de promoción, prevención y control de la Enfermedad de Chagas. Plan de interrupción de la transmisión vectorial intradomiciliaria de *Trypanosoma cruzi* por *Rhodnius prolixus* en 34 municipios priorizados de Colombia, 2019. 2020:1- 403. Disponible en: <https://www.minsalud.gov.co/sites/rid/Lists/BibliotecaDigital/RIDE/VS/PP/plan-interrupcion-transmisionn-cruzi-prolixus-colombia2019.pdf>
22. Monroy-Díaz A., Rodríguez-Niño S., Suescún-Carrero S.H, Ramírez-López L. Seroprevalencia de infección por *Trypanosoma cruzi* y factores asociados en Miraflores, Boyacá, Colombia. *Revista Investig Salud Univ Boyacá*. 2018;5:(1):31-47. DOI: <https://doi.org/10.24267/23897325.284>
23. Volta B, Russomando G, Bustos PL, Scollo K, Rissio AM, Sánchez Z, et al. Diagnosis of congenital *Trypanosoma cruzi* infection: A serologic test using Shed Acute Phase Antigen (SAPA) in mother-child binomial samples. *Acta trópica*. 2015;31-37. DOI: <https://doi.org/10.1016/j.actatropica.2015.03.026>
24. Martínez Ortiz, A., et al. "Enfermedad de Chagas neonatal de transmisión vertical en países no endémicos: El uso de la PCR en el diagnóstico: ventajas sobre técnicas convencionales." *Anales del Sistema Sanitario de Navarra*. Vol. 36. No. 1. Gobierno de Navarra. Departamento de Salud, 2013.
25. Parra G, Oliveros H, Hotez P, Motoa G, Paredes CF, Henao Martínez AF. In Search of Congenital Chagas Disease in the Sierra Nevada de Santa Marta, Colombia. *Am J Trop Med Hyg*.2019; 101 (3): 482-483. DOI: 10.4269/ajtmh.19-0110
26. Páez MS. Factores de riesgo y prevención primaria de la enfermedad de Chagas congénito. Universidad de Murcia 2017. Available in: <https://digitum.um.es/digitum/bitstream/10201/55216/1/Marina%20Sim%C3%B3n%20P%C3%A1ez%20Tesis%20Doctoral.pdf>

27. Balouz V., Agüero F., Buscaglia C.A. Chapter One - Chagas Disease Diagnostic Applications: Present Knowledge and Future Steps. *Advances in Parasitology*. 2017; 97: 1-45. DOI: <https://doi.org/10.1016/bs.apar.2016.10.001>
28. Mejía-Jaramillo A.M, Agudelo-Urbe L.A, Dib J.C, Ortíz S., Solari A., Triana-Chávez O. Genotyping of *Trypanosoma cruzi* in a hyper-endemic area of Colombia reveals an overlap among domestic and sylvatic cycles of Chagas disease. *Parasit Vectors*. 2014; 21 (7):108. DOI: 10.1186/1756-3305-7-108.
29. Messenger LA, Gilman RH, Verstegeui M, Cardenas GC, Sanchez G, Valencia E, Sancez L, et al. Toward Improving Early Diagnosis of Congenital Chagas Disease in an Endemic Setting. *Clin Infect Dis*. 2017; 65(2): 268-275. DOI: 10.1093/cid/cix277
30. Carlier Y, Altcheh J, Angheben A, Freilij H, Luquetti AO, Schijman AG, et al. Congenital Chagas disease: Updated recommendations for prevention, diagnosis, treatment, and follow-up of newborns and siblings, girls, women of childbearing age, and pregnant women. *Plos Negl Trop Dis*. 2019; 0007694. DOI: 10.1371/journal.pntd.0007694.
31. Abras A, Ballart C, Llovet Teresa, Roig Carme, Gutiérrez C, Tebar S, et al. Introducing automation to the molecular diagnosis of *Trypanosoma cruzi* infection: A comparative study of sample treatments, DNA extraction methods and real-time PCR assays. *Plos One*. 2018; 0195738. DOI: 10.1371/journal.pone.0195738
32. Manrique-Abril F., Ospina J.M, Herrera G., Florez A.C, Pavia P.X, Montilla M., et al. Diagnóstico de enfermedad de Chagas en mujeres embarazadas y recién nacidos de Moniquirá y Miraflores, Boyacá, Colombia. *Infectio*. 2013; 17 (1): 28-34. DOI: [https://doi.org/10.1016/S0123-9392\(13\)70045-6](https://doi.org/10.1016/S0123-9392(13)70045-6)
33. López R, Norman FF, Bern C. 103- American Trypanosomiasis. *Hunter's Tropical Medicine and Emerging Infectious Diseases*. 2020: 762-775. DOI: <https://doi.org/10.1016/B978-0-323-55512-8.00103-4>
34. Avila- Arzanegui O., Liendo- Arenaza P., Martínez-Indart L., Martínez- Astorkiza T., Pocheville- Guruceta M.I, Egurbide- Arberas M.V. Prevalencia de la infección por *Trypanosoma cruzi* y transmisión vertical en mujeres gestantes latinoamericanas en un área de salud de Vizcaya. *Enferm Infecc Microbiol Clín*. 2013; 31 (4): 210-2016. DOI: <https://doi.org/10.1016/j.eimc.2012.01.029>
35. Rendell V.R, Gilman R.H, Valencia E., Cardenas G.G, Verastegui M., Sanchez L., et al. *Trypanosoma cruzi*-Infected Pregnant Women without Vector Exposure Have Higher Parasitemia Levels: Implications for Congenital Transmission Risk. *PLoS ONE*. 2015; 10(3): e0119527. DOI: <https://doi.org/10.1371/journal.pone.0119527>
36. Sandoval Martínez D.K, Jaimes Sanabria M.Z, Jiménez Vargas F.L. Trasmisión vertical de la enfermedad de Chagas: reporte de caso. *Ginecol.obstet*. 2020; 88(4). DOI: <https://doi.org/10.24245/gom.v88i4.3607>
37. Imai K., Maeda T., Sayama Y., Mikita K., Fujikura Y., Misawa K., et al. Transmisión de madre a hijo de la enfermedad de Chagas congénita, Japón. *Emergente Infect Dis*. 2014; 20(1): 146–148. DOI: 10.3201/eid2001.131071
38. Murillo J., Bofill L.M, Bolivar H., Torres C., Urbina J.A, Benhayon D., et al. Congenital Chagas' disease transmission in the United States: Diagnosis in adulthood. *ID Cases*. 2016; 5: 72-75. DOI: <https://doi.org/10.1016/j.idcr.2016.07.011>
39. Castro-Sesquen Y.E, Gilman R.H, Galdos Cardenas G, Ferrufino L., Sánchez G.,

- Valencia-Ayala E. Use of a Novel Chagas Urine Nanoparticle Test (Chunap) for Diagnosis of Congenital Chagas Disease. *Plos Negl Dis.* 2014; 8(10): e3211. DOI: 10.1371/journal.pntd.0003211
40. Klein M.D, Tinajeros F, Menduiña M.C, Málaga E., Condori B.J, Verástegui M. Risk factors of maternal Chagas Disease and vertical transmission in a Bolivian Hospital. *Clin Infect Dis.* 2021; 2450-2456. DOI: 10.1093/cid/ciaa1885
 41. Viettri M., Lares M., Medina M., Herrera L., Ferrer E. Evaluation of commercial kits for the immunological and molecular diagnosis of Chagas disease in endemic areas of Venezuela. *Enfermedades infecciosas y microbiología clínica.* 2021. DOI: <https://doi.org/10.1016/j.eimce.2021.11.002>
 42. Fumadó V., Juncosa T., Posada E., Fisa R., Gállego M., Gascón J. Chagas pediátrico en zona no endémica. *Enfermedades Infecciosas y Microbiología Clínica.* 2014; 32(5): 293-296. DOI: <https://doi.org/10.1016/j.eimc.2013.04.024>
 43. Besuschio S.A, Picado A., Muñoz Calderón A., Weherendt D.P, Fernández M., Benatar A., et al. Trypanosoma cruzi loop-mediated isothermal amplification (Trypanosoma cruzi Loopamp) kit for detection of congenital, acute and Chagas disease reactivation. *Plos Negl Trop Dis.* 2020; 14(8): e0008402. DOI: 10.1371/journal.pntd.0008402
 44. Campos-Valdez G., Canseco-Ávila L.M, González-Noriega F., Alfaro-Zebadua O., Nava-Medecigo I.Y, Jiménez-Cardoso E. Transmisión materno-fetal de Trypanosoma cruzi, un problema de salud poco estudiado en México: caso Chiapas. *Salud Pública Mex.* 2016; 58(3). DOI: <https://doi.org/10.21149/spm.v58i3.7898>
 45. Bisio M.C, Rivero R., González N., Ballering G., D'Amico I., Kessler C., et al. Diagnostic Accuracy of Two Molecular Tools for Diagnosis of Congenital Chagas Disease. *Molecular Diagnosis & Therapy.* 2021; 25:791-801. DOI: <https://doi.org/10.1007/s40291-021-00553-3>
 46. Denegri M., Oyarce A., Larraguibel P., Ramírez I., Rivas E., Arellano G., Báez A., et al. Cribado y transmisión congénita de la enfermedad de Chagas en población usuaria del Hospital Dr. Félix Bulnes Cerda y Atención Primaria de Salud del Servicio de Salud Metropolitano Occidente de Santiago, Chile. *Rev. Chil. Infectol.* 2020; 37(2). DOI: <http://dx.doi.org/10.4067/s0716-10182020000200129>
 47. González L.F, Gastañaga-Holguera T., Jiménez-Montero B., Pérez Z.D, Ramos M.I, Amador P.M, et al. Seroprevalencia y transmisión vertical de enfermedad de Chagas en una cohorte de gestantes latinoamericanas en un hospital terciario de Madrid. *Anales de Pediatría.* 2018; 88(3): 122-126. DOI: <https://doi.org/10.1016/j.anpedi.2017.03.003>
 48. Simón M., Gil-Gallardo L.J, Iborra M.A, Carrillero B., López M.C, Barja M.R, et al. An observational longitudinal study to evaluate tools and strategies available for the diagnosis of Congenital Chagas Disease in a non-endemic country. *Acta tróptica.* 2019;199: 105127. DOI: <https://doi.org/10.1016/j.actatropica.2019.105127>
 49. Cura C.I, Ramírez J.C, Rodríguez M., López-Albizu C., Irazu L., Scollo K., et al. Comparative Study and Analytical Verification of PCR Methods for the Diagnosis of Congenital Chagas Disease. *The Journal of Molecular Diagnostics.* 2017; 19(5): 673-681. DOI: <https://doi.org/10.1016/j.jmoldx.2017.05.010>
 50. Velásquez E.B, Rivero R., De Rissio A.M, Malagrino N., Esteva M.I, Riarte A.R, et al. Predictive role of polymerase chain reaction in the early diagnosis of congenital Trypanosoma cruzi infection. *Acta Tropica.* 2014; 137:195-200. DOI: <https://doi.org/10.1016/j.actatropica.2014.05.016>