‘Towards elimination of congenital transmission of Chagas disease in Bolivia, Brazil, Colombia and Paraguay’

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Contents

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Name Sponsor/Funder	Fundação Oswaldo Cruz (Fiocruz)
Address Sponsor/Funder	Avenida Brasil, 4365 Manguinhos - Rio de Janeiro - RJ 21040-360 Brazil
Principal Investigator	Dr. Andréa Silvestre de Sousa
Principal Investigator email	andrea.silvestre@fiocruz.br
Principal Investigator Telephone	+55 21 99918-2147
Lead institution	Fundação Oswaldo Cruz (Fiocruz)
Lead institution address	Avenida Brasil, 4365 Manguinhos – Rio de Janeiro – RJ 21040-360 Brazil
Unit	Instituto Nacional de Infectologia Evandro Chagas
Laboratory/Department	Chagas disease Clinical Research Laboratory
Other investigators	Debbie Vermeij, Evelin Esther Fortun, Justo Chungara, Fernanda Sardinha, Eliana Amorim de Souza, Gabriel Parra, Magdalena Wiessner, Vidalia Lesmo, Alberto Novaes Ramos Jr., Carmem Emanuelle Leitão, Hellen Xavier, José Alexandre Menezes, Jaqueline Caracas Barbosa, Andrea Marchiol, Colin Forsyth, Rafael Herazo, Valentina Carranza
Research sites	**Bolivia**: Padilla, Yotala, Tarabuco, La Asunta, Mairana, El Torno, Vallegrande, San Lorenzo, Villamontes, Uriondo **Brazil**: Igarapé Miri, Riachão das Neves, Janaúba, Rosário do Sul, Paraúna **Colombia**: Moniquirá, Santana, Chitaraque, Soatá, Boavita, Tipacoque, San Mateo, Miraflores, Zetaquira, Guachaca, Don Diego, El Yopal **Paraguay**: Caacupe, Paraguari, Villa Elisa, Mariscal Estigarribia, Concepción
Partner institution	Instituto Nacional de Laboratorios de Salud (INLASA)
Partner institution address	Avenida René Zavaleta La Paz Bolivia
Partner institution telephone	+591 22226670
Partner institution email	inlasalapazbolivia@gmail.com
Partner institution	Instituto Nacional de Salud (INS)
Partner institution address	Calle 26, No 51-20, Zona 6 111321 Bogotá Colombia
Partner institution telephone	+57 12207700
Partner institution email	

Partner institution	Servicio Nacional de Erradicación del Paludismo (SENEPA)
Partner institution address	Manuel Dominguez casi Brasil Asunción Paraguay
Partner institution telephone	+595 21204729
Partner institution email	
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Abbreviations

AE	Adverse events
ANVISA	Brazil National Health Surveillance Agency
BZN	Benznidazole
CAB	Community Advisory Board
CD	Chagas disease
CRF	Case Report Form
CS	Civil Society
CSO	Civil society organization
CUIDA Chagas	Comunidades Unidas para Innovación, Desarrollo y Atención para la enfermedad de Chagas; Comunidades Unidas para Inovação, Desenvolvimento e Atenção para a doença de Chagas
DALY	Disability adjusted life year
DNA	Deoxyribonucleic acid
DNDi	Drugs for Neglected Diseases initiative
eCRF	Electronic Case Report Form
ECG	Electrocardiogram
ELN	National Liberation Army of Colombia
EMTCT	Elimination of mother-to-child transmission
ERC	Ethics Review Committee
FARC	Revolutionary Armed Forces of Colombia
FDA	Food and Drug Administration
FGD	Focus group discussions
FIND	Foundation for Innovative New Diagnostics
Fiocruz	Fundação Oswaldo Cruz
Fiotec	Fundação para o Desenvolvimento Científico e Tecnológico em Saúde
GDI	Gender Development Index
GII	Gender Inequality Index
HBV	Hepatitis B virus
HDI	Human Development Index
HDR	Human Development Report
HIV	Human Immunodeficiency Virus
HMIS	Health Management Information System
IEC	Information, education and communication
IHDI	Inequality-Adjusted Human Development Index
INI	Instituto Nacional de Infectologia Evandro Chagas
INLASA	Instituto Nacional de Laboratorios de Salud "Néstor Morales Villazón"
INS	Instituto Nacional de Salud de Colombia
IPS	Institute of Social Welfare in Paraguay
LAFEPE	Laboratório Farmacêutico de Pernambuco
MCH	Maternal and child health
MoH	Ministry of Health
MPI	Multidimensional Poverty Index
M&E	Monitoring and Evaluation
NFX	Nifurtimox
NHR	Netherlands Hanseniasis Relief
NTD	Neglected Tropical Disease
PAHO	Pan American Health Organization

PCR	Polymerase chain reaction
PHC	Primary health care
PI	Principal investigator
PPP	Purchasing power parity
PoC	Point of care
QALY	Quality adjusted life year
RDT	Rapid diagnostic tests
RMNCH	Reproductive, maternal, newborn and child health
SAE	Serious adverse events
SBCC	Social behaviour change communication
SENEPA	Servicio Nacional de Erradicación del Paludismo
SOP	Standard operating procedures
STI	Sexually transmitted infection e.g. syphilis
T. cruzi	*Trypanosoma cruzi*
UNDP	United Nations Development Programme
UHS	Unified Health System
VAS	Visual Analogue Scale
WCBA	Women of childbearing age
WHO	World Health Organization

Abstract

Chagas disease (CD) is a neglected tropical disease (NTD) that mainly affects poor and vulnerable populations in endemic countries in Latin America. Untreated, a significant number of patients will develop severe and sometimes life-threatening clinical complications. Due to the relative success of measures to control vector and transfusion transmission, congenital transmission has become proportionally more relevant in these areas, in addition to being the main source of new cases in non-endemic countries. According to estimates from the World Health Organization (WHO), 6 to 7 million people are infected worldwide, of which 1.12 million are women of childbearing age, and an estimated 8,000-15,000 infected babies are born each year in Latin America. Several international strategies and action plans like the elimination of mother-to-child transmission (EMTCT) initiative from the Pan American Health Organization (PAHO) and the road map for neglected tropical diseases from the WHO have been launched to achieve the elimination of congenital transmission of CD, but the barriers impeding access to health-related services for this NTD are profound, especially when it comes to congenital transmission. Endemic countries often do not have adequate reproductive, maternal, newborn and child health (RMNCH) services in place, nor do they systematically carry out congenital transmission surveillance, which results in underestimated data on CD prevalence in pregnant women and newborns.

This mixed-method implementation research, using qualitative and quantitative approaches, aims to contribute to the elimination of congenital transmission of CD by increasing the access of women of childbearing age (WCBA), their infants and children, and their household contacts to CD diagnosis, treatment and comprehensive care. The study will be implemented in a total of 32 municipalities in Bolivia (10), Brazil (5), Colombia (12) and Paraguay (5), selected according to public health priorities, ensuring geographically and epidemiologically diverse contexts, with primary health care (PHC) as the central focus of interventions, integrating with existing initiatives such as health and vaccination campaigns and the Elimination of Mother to Child Transmission plus program (EMTCT-plus).

Approximately 234,000 WCBA, their infants and children and their household contacts will be actively and systematically tested throughout the duration of the study (48 months). This study will conduct test, treat and comprehensive care interventions that will be integrated into primary and maternal and child health care, and will include, amongst others, counseling services, the use of rapid diagnostic tests (RDTs) for screening to expedite the diagnostic process of chronic CD, the use of molecular biology to facilitate an early diagnosis of CD in newborns and provision of treatment courses. In addition test, treat and care service provision, the study will include cross-cutting interventions like health campaigns, community mobilization and community and civil society engagement. During the first stage of this research, a formative study will aim to carry out a situational analysis of each of the territories, to better understand the local context, identify existing barriers and design appropriate interventions for each alternative implementation model. A cost-effectiveness study will compare these interventions with the status quo in each country, considering the effects on health (Disability Adjusted Life Years (DALYs) and Quality Adjusted Life Years (QALYs)). Monitoring and evaluation (M&E) frameworks will periodically assess indicators of success, such as: acceptability, adoption and coverage from community and health professionals, feasibility, appropriateness, sustainability and implementation cost. Through this comprehensive and integrated approach, alternative implementation models will be provided and tested in different Latin American contexts, and will be made available for replication and scale-up.

Keywords

Chagas disease; Infectious Disease Transmission, Vertical; Public Health Surveillance; Primary Health Care; Health Services Accessibility; Comprehensive Health Care; Latin America.

Rationale & Background

Chagas disease

Chagas disease (CD), a neglected tropical disease (NTD) also known as American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). *T. cruzi* parasites are mainly transmitted through contact with feces/urine of infected blood-sucking triatomine ('kissing') bugs. Other forms of transmission include consumption of contaminated food, transmission from an infected mother to her newborn during pregnancy or childbirth, blood or blood product transfusion from infected donors, organ transplants using organs from infected donors, and laboratory accidents. (1) CD has an acute phase, which can last up to a few weeks or months and is usually mild or asymptomatic, and a chronic phase. An estimated 30-40% of infected and untreated people will develop severe and sometimes life-threatening medical problems over the course of their lives, including cardiac alterations, digestive manifestations, and neurological or mixed alterations, which may require specific treatment. If untreated, infection is lifelong. (2)

CD is mainly found in endemic areas of 21 continental Latin American countries, with approximately 65 million people at risk of contracting the disease. An estimated 6 to 7 million people worldwide are infected with the *T. cruzi* parasite, of which the large majority resides in Latin America. Every year, over 10,000 CD-related deaths are reported. (1,3,4) Symptomatic CD imposes a substantial financial burden on healthcare systems and societies. (5) With an estimated USD 690 million in healthcare costs and USD 8 billion in annual economic losses, the CD economic burden equals or exceeds that caused by other prominent infectious diseases, such as zika (USD 3.7 billion). (6,7) However, despite the high morbidity and mortality of CD and the significant associated economic burden, only 7% of people with CD have been diagnosed and only about 1% receive etiological treatment. (8) Timely identification and treatment of CD has important benefits, including prevention of future congenital transmission in treated mothers, serological cure in infants and children, and reduction of progression to advanced forms of the disease in adults. (9–13) However, once the disease has progressed to an advanced phase with severe cardiac or digestive disease, etiological treatment does not appear to have clinical benefits. (14) This supports the need for improved diagnostics and early access to safe and effective treatment. (15)

Congenital transmission

An estimated 1.12 million women of childbearing age are infected with the *T. cruzi* parasite (4), and the congenital transmission rate approaches 5%, with higher rates in high-risk endemic areas. (16) Congenital infection could perpetuate CD indefinitely, even in countries without vector transmission. (17) The incidence of congenital *T. cruzi* infection is an estimated 8,000 to 15,000 cases per year in Latin America. (18) In the wake of progress in vector transmission control, congenital transmission has become proportionately more relevant, accounting for about one-third of new infections in 2010. As maternal and child health (MCH) services do not routinely screen mothers or newborns for CD in most endemic areas, prevalence in pregnant women and newborns may be underestimated. Efficacy of treatment in congenitally infected infants exceeds 90%, preventing morbimortality in babies and future chronic stages of the disease. (19) *T. cruzi* infection is curable if treatment is initiated soon after infection, which is why the screening of newborns and children of infected mothers that have not yet received antiparasitic treatment has become an essential strategy. The benefits of universal screening for *T. cruzi* as part of routine prenatal testing in endemic countries far outweigh the program costs. In a recent study, maternal screening, infant testing, and treatment of CD are cost saving for all rates of congenital transmission greater than 0.001% and all levels of maternal prevalence above 0.06%. Rapid diagnostic tests make universal screening cost-saving with maternal prevalence as low as 0.008%. (20)

However, the most cost-effective strategy is the etiological treatment of women of childbearing age before pregnancy, which would allow for a reduction of congenital cases.

Barriers to adequate health care

Despite diverse geographical, political, socio-economic and cultural contexts, several common barriers impede effective and efficient diagnosis, treatment, and aftercare of persons affected by CD. These barriers are: a lack of verified data on the burden of CD; a lack of integrated actions on surveillance, control and care at primary health care level; geographic distance of patients from facilities; an often cumbersome, time-consuming and costly diagnostic process; a lack of integration in reproductive, maternal, newborn and child health (RMNCH) policies and practices; a disproportionate impact of the disease on vulnerable populations; limited knowledge on CD in both the general population as well as health professionals; limited media attention; limited health education initiatives; limited availability of tools and materials at (peripheral) health centers; fear; stigma and discrimination against persons affected by CD; low social mobilization; and a limited political voice of persons at risk of CD. (21,22)

Access to diagnosis is the main barrier to CD treatment. This is key in preventing congenital transmission as (i) diagnosing and treating girls and women in endemic areas before pregnancy significantly reduces the risk of congenital transmission, (ii) diagnosing *T. cruzi* infection in pregnant women allows for early screening for infection in the newborn, and (iii) diagnosing *T. cruzi* infection in children born to infected mothers allows for the implementation of a highly efficacious and safe treatment. However, diagnosing the different forms of CD is complex. As an infected mother's antibodies to *T. cruzi* can persist in her infant for up to 9–12 months, serologic testing has major drawbacks for detecting congenital infection in neonates. Current methods miss a substantial number of congenital CD cases. Although rapid diagnostic tests (RDTs) have been developed for CD, they provide only screening information, and have not been widely implemented in public health systems, nor are they recommended in national or regional guidelines in Latin America. These complexities result in limited access to and demand for treatment as chronic CD requires at least 2-lab based serological tests and congenital CD diagnosis requires implementing a diagnostic algorithm combining microscopy at birth and serology over a 9-12 months period. Polymerase chain reaction (PCR) in the first trimester of life increases the sensitivity of the diagnosis of congenital CD. However, PCR is not a widely available tool and is usually not accessible in primary health care. Evidence suggests that new point of care (PoC) tools could simplify algorithms for diagnosis of CD in primary health care through the use of two RDTs, but this requires additional validation. (23–25)

The treatment landscape for CD is plagued by a multitude of barriers preventing widespread access. There are only two drugs available for the treatment of CD, benznidazole (BZN) and nifurtimox (NFX). Both drugs have been proven to be effective in treating patients for acute disease, reactivation in immunosuppressed hosts, congenital disease, and most cases undergoing chronic phase of infection. However, both drugs require long periods of administration (60 days for BZN and 60-90 days for NFX) which causes frequent unwanted drug-related adverse reactions and treatment discontinuity. (26) These side effects add to affected persons' and physicians' reluctance to initiate treatment in the first place. Extensive laboratory monitoring is required, which can add to patients' out-of-pocket costs, and side effects can limit patients' ability to work or care for children. In addition, neither drug can be administered to pregnant women or patients in the late stage of the disease with severe cardiac or digestive disease. In many countries, BZN is the preferred first line of treatment because of its lower incidence of adverse events (AE) and shorter treatment duration. (27) Recent evidence suggests that a shorter 15-day treatment course of BZN would have the same effectiveness as the regular treatment course, but with less adverse effects. (28) However, the results of this study should be verified through an additional clinical trial. BZN is produced by two companies: Elea-Phoenix, an Argentine

pharmaceutical company (29), and Laboratório Farmacêutico de Pernambuco (LAFEPE) (30), a Brazilian public enterprise. The Elea-Phoenix 100mg and 12,5mg formulations are registered at the Food and Drug Administration (FDA) and in several Latin American countries (excluding Brazil), while the adult and pediatric versions of the LAFEPE product are approved for use in Brazil by its National Health Surveillance Agency (ANVISA). Based on the last Pan American Health Organization (PAHO) Strategic Fund tender, the cost of the Elea-Phoenix product is USD 90 ex-works for a complete adult 60-day treatment. The cost for the LAFEPE product is USD 65 ex-works for the same treatment duration in the adult version. NFX is produced by Bayer, and donated annually to the PAHO strategic fund, therefore being the preferred treatment in a number of countries despite its higher frequency of side effects. (31) Despite being registered in most countries in the Americas, BZN and NFX are not routinely available in sufficient quantities at primary healthcare facilities for several reasons, ranging from suboptimal ordering patterns, repressed demand, limited supply/production, and in-country supply chain issues.

Global and Regional Chagas disease priorities and protocol alignment

On the 24th of May 2019, at the 72nd session of the World Health Assembly, the World Chagas Disease Day was instituted. World Chagas Day is now one of 11 World Health Organization (WHO) global public health campaigns. In addition, WHO, in their newly presented NTD Roadmap, has identified three critical actions for the elimination of CD (32):

Critical action 1: Advocate with high-level national ministries to recognize CD as a public health problem, and establish effective prevention, control, care and surveillance in all affected territories.
Critical action 2: Improve medical care for CD, from training health care workers in-service to integrating training at all levels of health services.
Critical action 3: Ensure that countries in which domiciliary vector transmission is still registered in certain territories comply with prevention, control and surveillance.

PAHO, in their framework for elimination of mother-to-child transmission (EMTCT) of Human Immunodeficiency Virus (HIV), Syphilis (STI), Hepatitis B (HBV) and CD (33), has identified the following lines of action:

Line of action 1: Integrate HIV/STI/HBV/Chagas interventions within sexual and reproductive health, antenatal care, maternal and child health, and family and community health policies, programs, and services.
Line of action 2: Intensify strategic information on HIV, syphilis, HBV, and CD in maternal and child health services.
Line of action 3: Improve the laboratory network and quality and supply chain management.

Although access to prenatal care and childbirth related services for pregnant women is high in the context of the Americas, problems persist in relation to screening for mother-to-child transmission diseases, which requires the integration of surveillance actions with those of care. Recommended interventions to control congenital CD are available in all endemic countries, but data on health service coverage are limited. Based on the limited information from a few countries that reported to PAHO, screening for CD in pregnant women varies widely from just over 5% to almost 60% among the few countries that have reported it. (33) Strategic actions for the elimination of congenital CD should be focused on prenatal, childbirth, puerperium and monitoring of the mother-child binomial. Just like the prevention of congenital syphilis, HIV or hepatitis B, the early diagnosis of the pregnant woman during prenatal care, timely treatment (when indicated), definition of the safest way of delivery, laboratory and clinical investigation of the newborn with treatment and surveillance of partners and other

children are necessary routines to be carried out by reproductive, maternal, neonatal and child health systems in an integrated manner with primary health care. For HIV infection and syphilis, despite this high screening coverage, no progress has been made in recent years, which has generated persistence of mother-to-child transmission of HIV and a consistent and paradoxical increase in syphilis. This fact reinforces the importance of seeking even more consistent and integrated strategies for disease control for this mode of transmission. (33)

According to the EMTCT plus initiative, the elimination of CD as a public health problem is defined as ≥90% of infected children treated, cured and supported by screening, ≥90% of pregnant women in prenatal care and treatment after delivery, and ≥90% testing on exposed neonates (with *T. cruzi* seropositive mothers), milestones that have not yet been achieved in any Latin American country. As part of the implementation of the EMTCT plus initiative, Latin American countries are encouraged to review and update their information systems to monitor the programmatic indicators for CD control. This scenario opens a window and provides an additional and excellent opportunity for the development of this study, with a view to contribute to decision-making based on the best available evidence, within real scenarios.

For CD, the following interventions are included in this study:

- Adolescence and pre-pregnancy (sexual and reproductive health): diagnosis and treatment of girls infected with *T. cruzi* and women of childbearing age (WCBA) in endemic areas and adequate care, referral and monitoring of pregnant women and infected sexual partners.
- Pregnancy: increased early access to antenatal care; appropriate provision of actions during prenatal care, including promoting partner involvement; routine serological screening for CD and; monitoring of pregnant women with CD, defining referral when necessary (for example, risk pregnancy with chronic Chagas cardiomyopathy).
- Childbirth: parasitological and serological screening for *T. cruzi* in newborns of infected mothers.
- Postnatal - Mothers: treatment of *T. cruzi* seropositive mothers after pregnancy.
- Postnatal - Newborns: serology for *T. cruzi* of newborns with infected mothers (at 8 months); treatment of *T. cruzi* infected children before one year of age and clinical / serological follow-up until a negative result; immediate treatment of all newborns with positive parasitological results for *T. cruzi*.
- Cross-cutting interventions: health campaigns involving information, education and communication; support for social mobilization and community engagement; consider the serological test of siblings of children infected with *T. cruzi*.

Implementation research

The purpose of implementation research is to support and promote the successful application of interventions that have been demonstrated to be effective. (34) Implementation research is increasingly being recognized as one of the most important interfaces between the availability of tools, strategies and interventions and their use within health systems and control programs. (35) As mentioned above, current implementation models for CD in Latin America suffer from a number of significant inadequacies. CD in general is rarely a priority, and congenital CD even less so. Services exist but are not routinely integrated into maternal and child health care, diagnostics and therapeutics are available but are not efficiently or effectively used, and there is a general lack of knowledge on the disease, its consequences, and how it should be managed. Through carefully designed implementation research, this study seeks to present a comprehensive and integrated approach that will provide alternative implementation models, that will have been tested in different Latin American contexts.

These models will then be made available for replication and scale-up. The outcomes of this research will address WHOs critical actions 1 and 2, and PAHOs lines of action 1, 2 and 3, thereby tackling the following barriers: a lack of efficient diagnostic algorithms, a lack of knowledge and understanding of CD both by people at risk as well as health professionals and managers, a lack of integrated surveillance, control and care; a lack of efficient and effective policies; insufficient supply of necessary therapeutic and diagnostic tools; low social mobilization; and a limited political voice of persons at risk of CD.

Purpose, Objectives, Research Questions and Expected Outcome

Purpose of this research

The purpose of this research is to generate evidence on the feasibility and effectiveness of test, treat and care interventions in order to contribute to the elimination of congenital transmission of CD. This evidence will be generated through a carefully designed mixed methods implementation research, that will be executed in four CD endemic countries, Bolivia, Brazil, Colombia and Paraguay.

Objectives and research questions

Primary objective

To demonstrate the extent to which the implementation of test, treat and comprehensive care interventions can contribute to tackle implementation barriers and improve access and demand for Chagas disease diagnosis, treatment and care for women of childbearing age, their infants, children and household contacts.

Secondary objectives

1. To conduct a situational analysis of each of the territories included in the implementation research in order to better understand the local context considering physical, socioeconomic and cultural environments, health systems, stakeholders and institutional culture. These key aspects will contribute to and affect the planning, implementation, monitoring and outcomes of the interventions.
2. To demonstrate the feasibility of using RDTs for the screening of chronic CD.
3. To demonstrate the feasibility of using PCR for the diagnosing of newborns.
4. To contribute to the integration of test, treat and care interventions into the EMTCT Plus programs in the four countries.
5. To assess the cost-effectiveness of the implemented interventions.

Primary research question

'To what extent can an integrated model of implementation strategies increase the access of women of childbearing age, their infants, children and household contacts to Chagas disease diagnosis, treatment and comprehensive care through primary health care in endemic areas in Bolivia, Brazil, Colombia and Paraguay, in order to control the congenital transmission of Chagas disease?'

Expected outcomes

The primary expected outcome of this study will be increased access to and demand for effective diagnostics, treatment, and care for CD. This study will generate implementation models that can be replicated in different countries and contexts.

The secondary expected outcome will be engaged communities and civil society at local, national and regional levels to increase demand for services and advocate for mainstreaming recommended Chagas disease approaches into local policies, strategies and plans.

The study

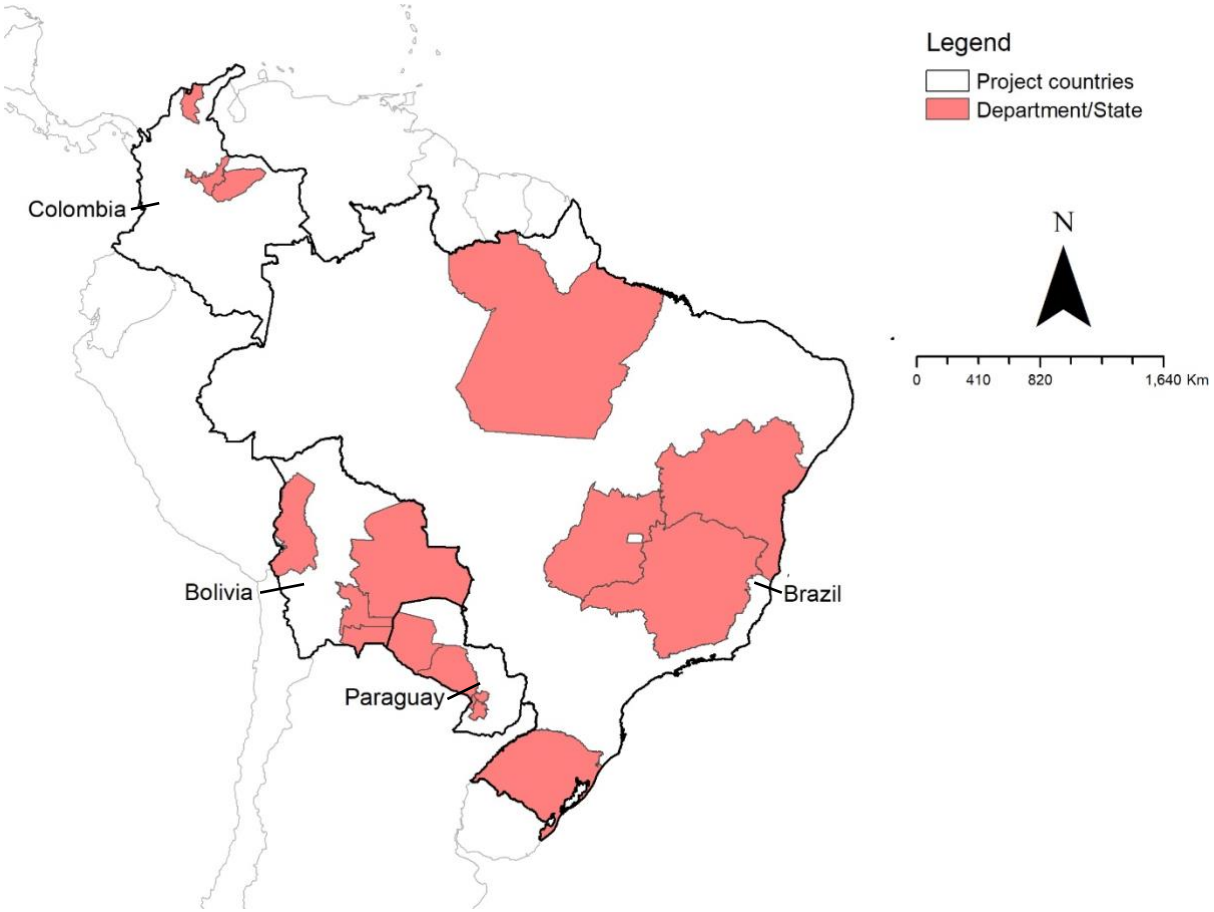
Through carefully designed mixed-method implementation research that will be executed in each of the countries, this study will generate evidence on the feasibility and effectiveness of test, treat and care approaches in order to contribute to the elimination of congenital transmission of CD.

A patient's ability to obtain diagnosis and treatment is subject to more than just the availability of tools and treatment options. It also relates to the patients' understanding of and beliefs around a disease, as well as the time, travel and out-of-pocket costs involved in seeking treatment, and the ease of adhering to treatment guidelines. Low provider awareness, low availability of CD services in primary healthcare, and lack of public information currently suppress demand for CD testing and treatment, thereby discouraging patients. By engaging communities and civil society (CS), and by employing targeted information, education and communication (IEC) interventions, this study will seek to boost patient demand. At the same time, capacity building of health professionals and integration of interventions into primary health care (PHC) will ensure an increase of proactive screening via the health system while ensuring patient requests for CD testing and treatment are effectively met.
This study is part of a larger project called CUIDA Chagas, which will be implemented in Bolivia, Brazil, Colombia and Paraguay and comprises of three separate but interlinked studies, namely (i) this implementation research, (ii) a study that will validate a diagnostic algorithm based on rapid diagnostic tests, and (iii) a double-blind, phase III clinical trial to evaluate a shorter benznidazole treatment regimen. The project is financed by Unitaid and the Ministry of Health (MoH) of Brazil, and has been endorsed by the MoHs of the four participating countries. In addition, the project actively seeks collaboration with PAHO and WHO, to ensure alignments with the international agenda's on CD. CUIDA Chagas will be implemented through a consortium of key players in public health, including governmental and non-governmental actors such as the *Fundação para o Desenvolvimento Científico e Tecnológico em Saúde* (Fiotec) and the *Fundação Oswaldo Cruz* (Fiocruz) from Brazil, the *Instituto Nacional de Laboratorios de Salud* (INLASA) from Bolivia, the *Instituto Nacional de Salud* (INS) from Colombia, the *Servicio Nacional de Erradicación del Paludismo* (SENEPA) from Paraguay, and the Foundation for Innovative New Diagnostics (FIND). Researchers from the governmental consortium members will be actively involved in this study, which will contribute to the sustainability of the actions and the potential inclusion of study results in national guidelines.
By generating solid evidence, demonstrating best practices, and generating tools and resources to support implementation, recommendations may ultimately be included in WHO, PAHO and national MoH guidelines, thereby contributing to sustainability of the study's results. This, combined with a strong emphasis on regional linking and learning promoting cross-country collaboration throughout all phases of the study, will facilitate the replication and scale up of this study's interventions.

Study Locations

The study will be implemented in four countries: Bolivia, Brazil, Colombia and Paraguay (Figure 6).

Figure 6 - Study countries including department/states



Study countries have been selected based on a mix of criteria. While the prevalence and burden of CD are important factors, the potential for sustainable and catalytic change in the region was evaluated as whole and the below criteria helped identify the top countries:

- **Prevalence and burden of CD**: countries with high prevalence and/or absolute numbers of people with CD were selected in order to ensure the project benefits a large number of affected people and supports countries where CD is a major public health challenge. In total, the four countries have an estimated 2.4 million people with *T. cruzi* infection, of which an estimated 498,255 are women between the ages of 15-44. The following tables (1-3) demonstrate the ranking of the project countries when it comes to estimated prevalence, estimated total number of people with *T. cruzi* infection and the estimated number of women aged 15-44 with *T. cruzi* infection (36).

Table 1. Estimated *T. cruzi* prevalence per country

	Country	Estimated prevalence of *T.cruzi* infection per 100 habitants
1	Bolivia	6.10
2	Argentina	3.64
3	Paraguay	2.13
4	Ecuador	1.38
5	El Salvador	1.30
6	Guatemala	1.23
7	Colombia	0.96
8	Honduras	0.92
9	French Guyana, Guyana and Suriname	0.84
10	Mexico	0.78
11	Venezuela	0.71
12	Chile	0.70
13	Nicaragua	0.52
14	Panama	0.52
15	Peru	0.44
16	Belize	0.33
17	Uruguay	0.24
18	Costa Rica	0.17
19	Brazil	0.03

Table 2. Estimated number of people infected by *T. cruzi*

	Country	Estimated number of people infected by *T.cruzi*
1	Argentina	1,505,235
2	Brazil	1,156,821
3	Mexico	876,458
4	Bolivia	607,186
5	Colombia	437,960
6	Ecuador	199,872
7	Venezuela	193,339
8	Paraguay	184,669
9	Guatemala	166,667
10	Peru	127,282
11	Chile	119,660
12	El Salvador	90,222
13	Honduras	73,333
14	Nicaragua	29,300
15	Panama	18,337
16	French Guyana, Guyana and Suriname	12,600
17	Uruguay	7,852
18	Costa Rica	7,667
19	Belize	1,040

Table 3. Estimated no of women aged 15-44 years with *T. cruzi* infection

	Country	Estimated no of women aged 15-44 years with *T. cruzi*
1	Argentina	211,102
2	Bolivia	199,351
3	Mexico	185,600
4	Brazil	119,298
5	Colombia	116,221
6	Paraguay	63,385
7	Ecuador	62,898
8	Venezuela	40,223
9	Guatemala	32,759
10	Peru	28,132
11	El Salvador	18,211
12	Honduras	16,149
13	Chile	11,771
14	Panama	6,332
15	Nicaragua	5,822
16	French Guyana, Guyana and Suriname	3,818
17	Uruguay	1,858
18	Costa Rica	1,728
19	Belize	272

- **Political commitment and readiness:** for project success, the political commitment of government is indispensable, and in turn this commitment is reinforced as governments engage in direct, productive actions to help affected people. During the CUIDA Chagas project conception phase, representatives of ministries of CD endemic countries and some of the most prominent CD related technical-scientific institutions were invited to join forces with Fiocruz. Out of the group that was invited, the ministries of Bolivia, Colombia and Paraguay accepted participation. Each of these countries' governments has demonstrated a commitment and readiness to control CD through national programs, vector control initiatives, research support, and other activities. In addition, each government has put forward a renowned national institute to participate in the CUIDA Chagas project, emphasizing the importance and their commitment.
- **Availability of strong implementing partners**: each country has historically exhibited a commitment to controlling CD, with recent achievements, and hosts diverse stakeholders in government, academia, and civil society actively working to improve control of CD.
- **Relevance of countries to this study:** Bolivia is the country in Latin America that is most affected by Chagas disease, in terms of prevalence. This had led to a large interest of research consortia and a number of important studies being conducted in Bolivian territories (like the TESEO, EQUITY and CHICO studies). (37–39) Although estimated prevalence in Brazil is low, due to the size of the population, the absolute number of estimated cases is high, both for the general population as well as women aged 15-44. In addition, Brazil's government has demonstrated significant interest in supporting interventions that have the potential of contributing to the elimination of congenital transmission. Colombia also has a relatively low prevalence, but relatively high absolute estimated numbers of CD infected persons. In addition, Colombia has a geographic and populational diversity that is important to include in the different studies. Paraguay is the country with the third highest prevalence in the region and a considerable proportion of women aged 15-44 that are estimated to be infected. In addition, the country has played a pioneering role in identifying the relevance of congenital transmission of Chagas disease.
- **Health systems:** each of the four countries has organized their health systems in different ways, with the primary health care (PHC) coverage (defined as % of the population that has access to PHC facilities) ranging from only 7% to 100% in the selected territories. Generally speaking, Colombia has the highest PHC coverage, whereas PHC coverage in Paraguay is very low. This diversity is important to be able to assess the feasibility of the implementation research interventions in each territory and ensure maximum replicability to other countries.
- **Socio-economic factors**: according to the World Bank classification, Bolivia is a low- and middle-income country (LMIC), while Brazil, Colombia and Paraguay are upper- and middle- income countries (UMICs) who nevertheless suffer from high income inequalities. In these countries, as in the entire region, CD disproportionately affects marginalized populations, including indigenous communities, poor people in both rural and urban areas, and rural-urban or transnational migrants.
- **Parasitic variance**: the countries included in the project represent the genetic variety of parasitic strains that are responsible for the largest number of clinical manifestations of CD (see Table 4).
- **A strong link with epidemiological and entomological surveillance**: the countries have established epidemiological surveillance systems, with particular experience in CD.

Table 4 demonstrates the epidemiological characteristics of CD in the four countries.

Table 4 - Epidemiological characteristics of Chagas disease in the countries

Country	Geographical areas at risk	Estimated no. of people infected by *T. cruzi* (2010)	Estimated no of women aged 15-44 with *T. cruzi* infection (2010)	Estimated annual no of cases of *T. cruzi* infection due to congenital transmission (2010)	Estimated prevalence of *T. cruzi* infection per 100 habitants (2010)	Population 2010	Predominant genetic and parasitic variances CD	Existing CD control program (Y/N)	Vector control (Y/N)	Existence of a diagnostic algorithm for Chagas disease	Existence of adequate laboratories for this project (Y/N)
Bolivia	Cochabamba, Sucre, Tarija and Santa Cruz	607,186	199,351	616	6.104	9,947,000	TcI & TcV	Y	Y	Y - see annexes for details	Y
Brazil	Bahia, Goiás, Minas Gerais, Pernambuco, Pará	1,156,821	119,298	571	0.030	190,755,799	TcI & TcII	Y	Y	Y - see annexes for details	Y
Colombia	Arauca, Sierra Nevada de Santa Marta, Casanare, Santander, Boyacá	437,960	116,221	1,046	0.956	45,805,000	TcI	Y	Y	Y - see annexes for details	Y
Paraguay	Chaco and Eastern regions	184,669	63,385	525	2.130	8,668,000	TcII & TcV	Y	Y	Y - see annexes for details	Y

Bolivia

The Plurinational State of Bolivia is a land-locked country located in central-western South America, bordering Brazil, Argentina, Peru, Paraguay and Chile. The country has a total land mass of 1,098,581 km^2 and an estimated population of 11,758,869. (40) There are 36 constitutionally recognized nations with their respective languages, and 40.7% of Bolivians say they belong to an indigenous, native, campesino or Afro-Bolivian nation. (41)

Bolivia ranks at or near the bottom among Latin American countries in several areas of health and development, including poverty, education, fertility, malnutrition, mortality, and life expectancy. On the positive side, more children are being vaccinated and more pregnant women are getting prenatal care and have skilled health practitioners attend their births. (40)

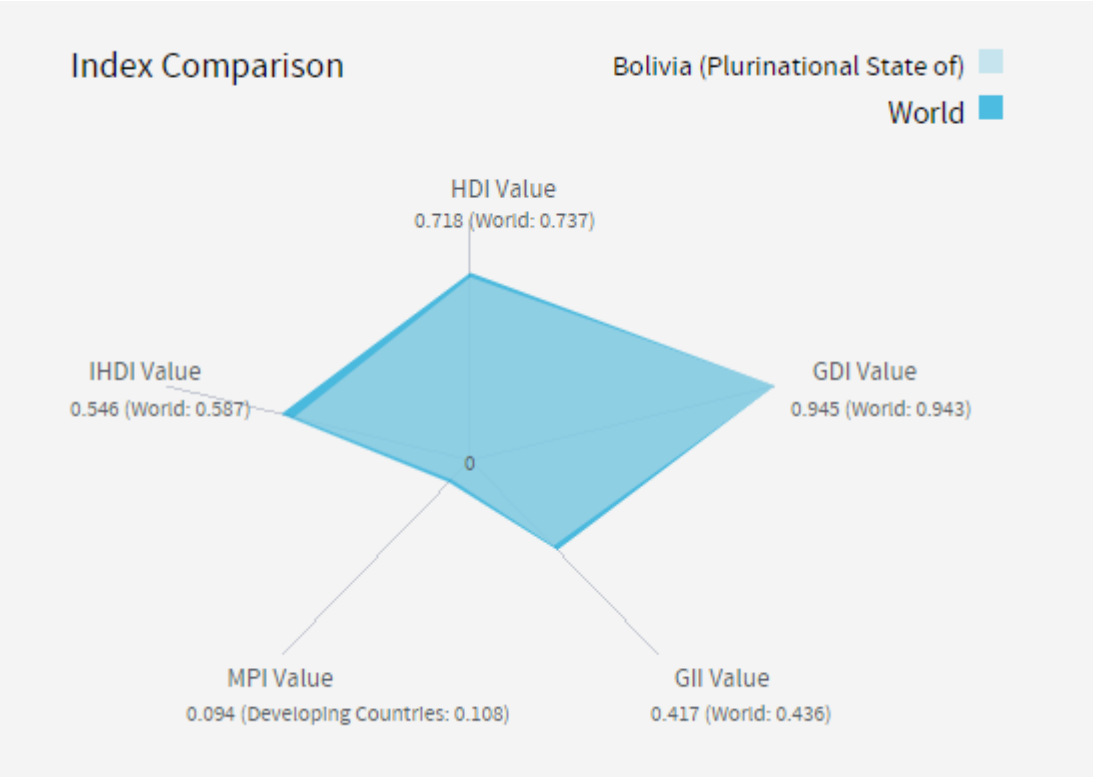
Bolivia's income inequality is the highest in Latin America and one of the highest in the world. Public education is of poor quality, and educational opportunities are among the most unevenly distributed in Latin America, with girls and indigenous and rural children less likely to be literate or to complete primary school. The lack of access to education and family planning services helps to sustain Bolivia's high fertility rate—approximately three children per woman. (40)

The United Nations Development Programme's (UNDP) Human Development Report (HDR) Office releases five composite indices each year (42,43):

I. the Human Development Index (HDI), a summary measure for assessing long-term progress in three basic dimensions of human development: a long and healthy life, access to knowledge and a decent standard of living.
II. the Inequality-Adjusted Human Development Index (IHDI), which takes inequality into account in all three dimensions of the HDI by 'discounting' each dimension's average value according to its level of inequality.
III. the Gender Development Index (GDI), which measures gender inequalities in achievement in three basic dimensions of human development: health (measured by female and male life expectancy at birth), education (measured by female and male expected years of schooling for children and mean years for adults aged 25 years and older) and command over economic resources (measured by female and male estimated GNI per capita).
IV. the Gender Inequality Index (GII), which reflects gender-based inequalities in three dimensions – reproductive health, empowerment, and economic activity. Reproductive health is measured by maternal mortality and adolescent birth rates; empowerment is measured by the share of parliamentary seats held by women and attainment in secondary and higher education by each gender; and economic activity is measured by the labour market participation rate for women and men.
V. the Multidimensional Poverty Index (MPI), which identifies multiple overlapping deprivations suffered by individuals in 3 dimensions: health, education and standard of living. The health and education dimensions are based on two indicators each, while standard of living is based on six indicators.

According to the 2020 report, Bolivia ranks as number 107 (out of 189 countries) on the HDI. Figure 7 demonstrates the index comparison between Bolivia and the world average.

Figure 7 - Human Development Indicators Bolivia



The Bolivian health system

The Bolivian health system consists of multiple public and private sector entities that perform different functions such as leadership, financing, insurance, procurement, and health care delivery. The public sector includes a public subsector and the short-term social security subsector. (41) The public subsector is managed at different national levels: the MoH is in charge of policy management at the national level, and the Departmental Health Services (SEDES) manage the sector at the departmental level. Municipal executive authority is recognized as the highest authority responsible for local health management. The municipal health networks have primary and secondary care establishments. For primary care, there are also *Mi Salud* clinics, co-managed by the Ministry of Health and the corresponding autonomous municipal government. The departmental health networks include several municipal health networks and more specialized hospitals. (41)

The short-term social security subsector is comprised of managing entities (health funds) in charge of granting packages of in-kind services (maternity and sick benefits), and monetary benefits (maternity and sick leave, workers compensation, etc.) to subscribed formal workers. (41) In 2019, the existing law no 475 on comprehensive health services was replaced by law 1152 to include a larger part of Bolivian society, in order to move towards a Unified Health System (Sistema Único de Salud – SUS). (44,45) Through this modification, beneficiaries now include all Bolivian women and men who are not protected by the short-term social security subsector, foreigners who are not protected by the short-term social security subsector, within the framework of international instruments, under the principle of reciprocity and under the same conditions as the Bolivians, and foreigners whose country of origin does not have agreements with Bolivia regarding reciprocity in health but who belong to the following population groups: pregnant women, from the beginning of gestation until six months after birth, women regarding sexual and reproductive health care; children up to 5 years of age; women and men from 60 years of age; and people with disabilities who are qualified according to current regulations. (46)

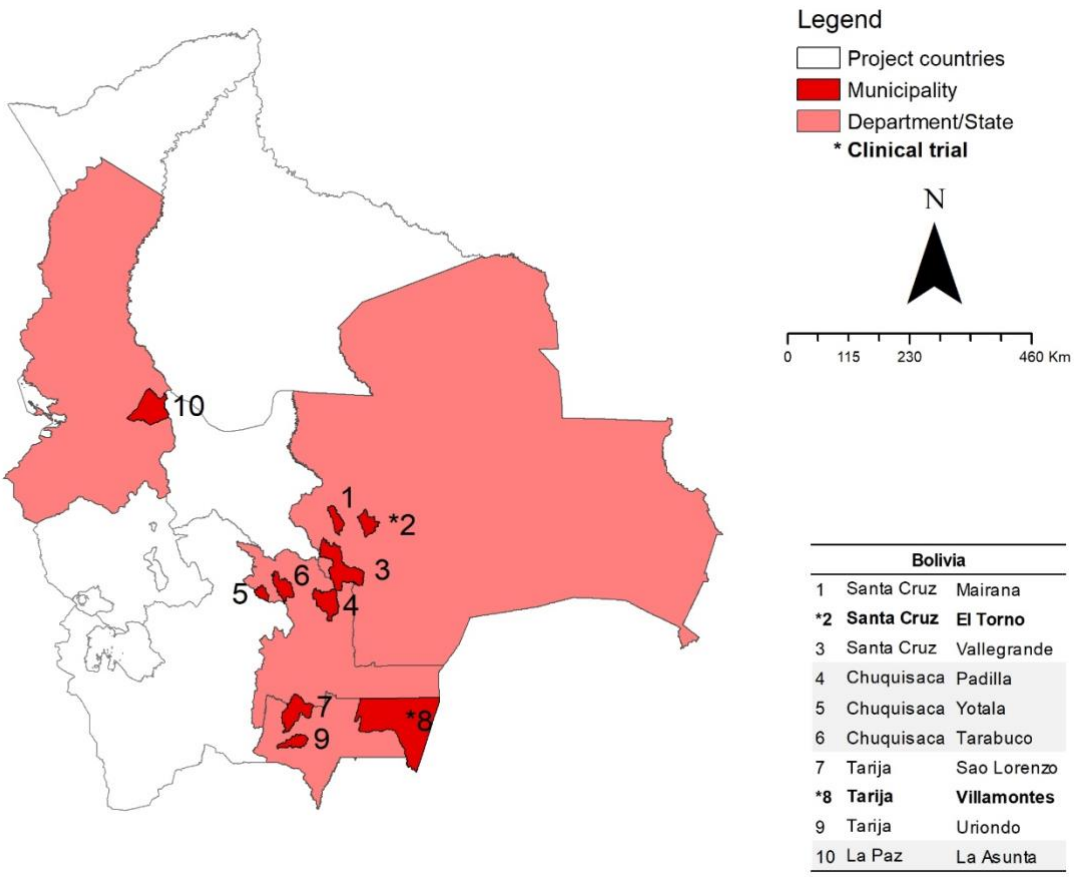
Research locations in Bolivia

This research will take place in ten municipalities in four departments in Bolivia, targeting a total of 88,300 people for CD screening. The municipalities have been selected by the Bolivian MoH according to public health priorities, ensuring geographically and epidemiologically diverse contexts, with PHC as the central focus of interventions, integrating with existing initiatives most relevant to the country context, such as health and vaccination campaigns and EMTCT-plus. Table 5 and Figure 8 provide more detailed information on the Bolivian study sites.

Table 5 – Research sites Bolivia

Country	Department / State	Municipality	Population	No. of WCBA	Estimated no. of CD cases	Estimated no. of persons to be screened	Estimated no. of persons to be treated	Estimated no. of PHCs	No. of maternity wards (as part of PHC or stand alone)	No. of laboratories	Current CD testing and treatment approach
Bolivia	Chuquisaca	Padilla	11,067	2,654	2,805	3,600	260	6	1	1	Current approach as described in national guidelines
		Yotala	10,017	2,165	2,539	2,900	210	1	0	1	
		Tarabuco	10,383	2,497	2,632	3,400	240	12	1	1	
	La Paz	La Asunta	42,644	10,413	10,809	14,000	1,025	17	9	1	
	Santa Cruz	Mairana	13,197	2,639	3,345	3,600	260	4	4	1	
		El Torno	63,298	16,196	16,044	22,000	1,595	21	1	1	
		Vallegrande	18,478	5,003	4,684	6,800	490	10	2	1	
	Tarija	San Lorenzo	25,796	6,718	6,539	9,200	660	19	1	2	
		Villamontes	51,916	12,661	13,159	17,200	1,245	25	1	1	
		Uriondo	15,599	4,137	3,954	5,600	405	10	1	1	
Totals			**262,395**	**65,083**	**66,509**	**88,300**	**6,390**	**125**	**21**	**11**	

Figure 8 – Research sites Bolivia



Bolivia		
1	Santa Cruz	Mairana
***2**	**Santa Cruz**	**El Torno**
3	Santa Cruz	Vallegrande
4	Chuquisaca	Padilla
5	Chuquisaca	Yotala
6	Chuquisaca	Tarabuco
7	Tarija	Sao Lorenzo
***8**	**Tarija**	**Villamontes**
9	Tarija	Uriondo
10	La Paz	La Asunta

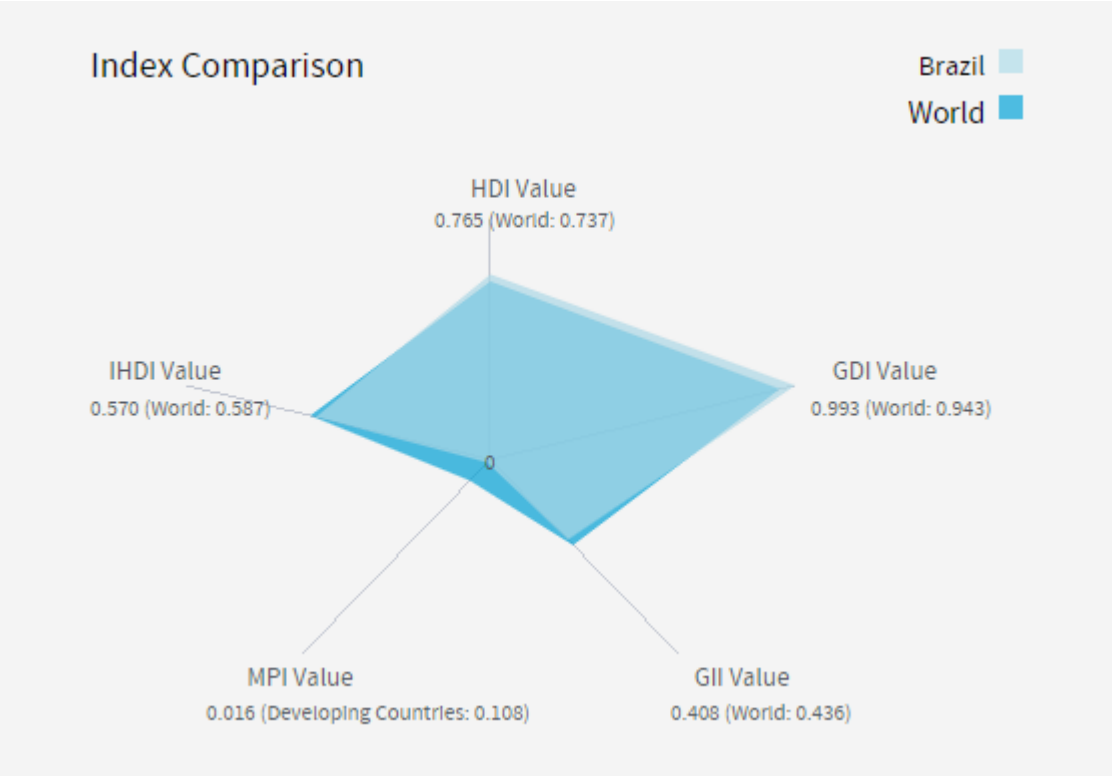
Brazil

The Federative Republic of Brazil is the fifth largest country in the world, with a total land mass of 8,515,770 km^2 and an estimated population of 213,445,417. (47) It borders every South American country, except Chile and Ecuador.

Brazil's rapid fertility decline since the 1960s is the main factor behind the country's slowing population growth rate, aging population, and fast-paced demographic transition. Well-funded public pensions significantly decreased poverty among the elderly, and Bolsa Familia and other social programs have lifted tens of millions out of (extreme) poverty. More than half of Brazil's population is considered middle class, however, poverty and income inequality levels remain high; the Northeast, North, and Center-West, women, and black, mixed race, and indigenous populations are disproportionately affected. Disparities in opportunities foster social exclusion and contribute to Brazil's high crime rate, particularly violent crime in cities and *favelas*. (47)

According to the UNDP HDR, Brazil ranks as number 84 (out of 189 countries) on the HDI. Figure 9 demonstrates the index comparison between Brazil and the world average. (48)

Figure 9 - Human Development Indicators Brazil



The Brazilian health system

Brazil's Unified Health System (UHS or SUS) was created almost 30 years ago in the Federal Constitution of 1988. Based on recognition of the right to health as a civil right, the principles that define the policies of the UHS consist of universal and comprehensive access to health services (which are organized regionally and hierarchically); promotion of equity; decentralized management; and social participation. Management of the system is shared by the three levels of government: the Ministry of Health at the federal level, and the state health secretariats and municipal health secretariats at the lower levels. The system is funded by taxes and contributions at the federal, state, and municipal levels. The network of services provided by the UHS includes its own public facilities and outsourced private services, with nonprofit sources being preferred in the latter case. Social

participation is promoted through health conferences and councils that operate continuously at the three levels of government, and through other mechanisms such as the Office of the Public Prosecutor, which fields citizen complaints. (49)

The legal framework of the UHS allows for private enterprise to provide health care, either directly (direct payment) or through health plans and insurance. The complementary health care subsystem has nearly 50 million beneficiaries—almost 25% of the Brazilian population. It is regulated and overseen by the National Complementary Care Agency (ANS), primarily with regard to contractual matters, guaranteed coverage of the portfolio of services, quality of care, price adjustments, and financial sustainability. (49)

As an economic measure, a constitutional amendment went into effect in 2018 demanding that all federal government spending on health and education would be frozen for up to 20 years, with the exception of inflation correction. (50) This means that regardless of issues such as population growth and an aging population, government spending will remain the same. The result of this decision will be a decline in health services in poor municipalities, as the government contribution will remain the same, and municipalities will not have the available funds to take up the additional share.

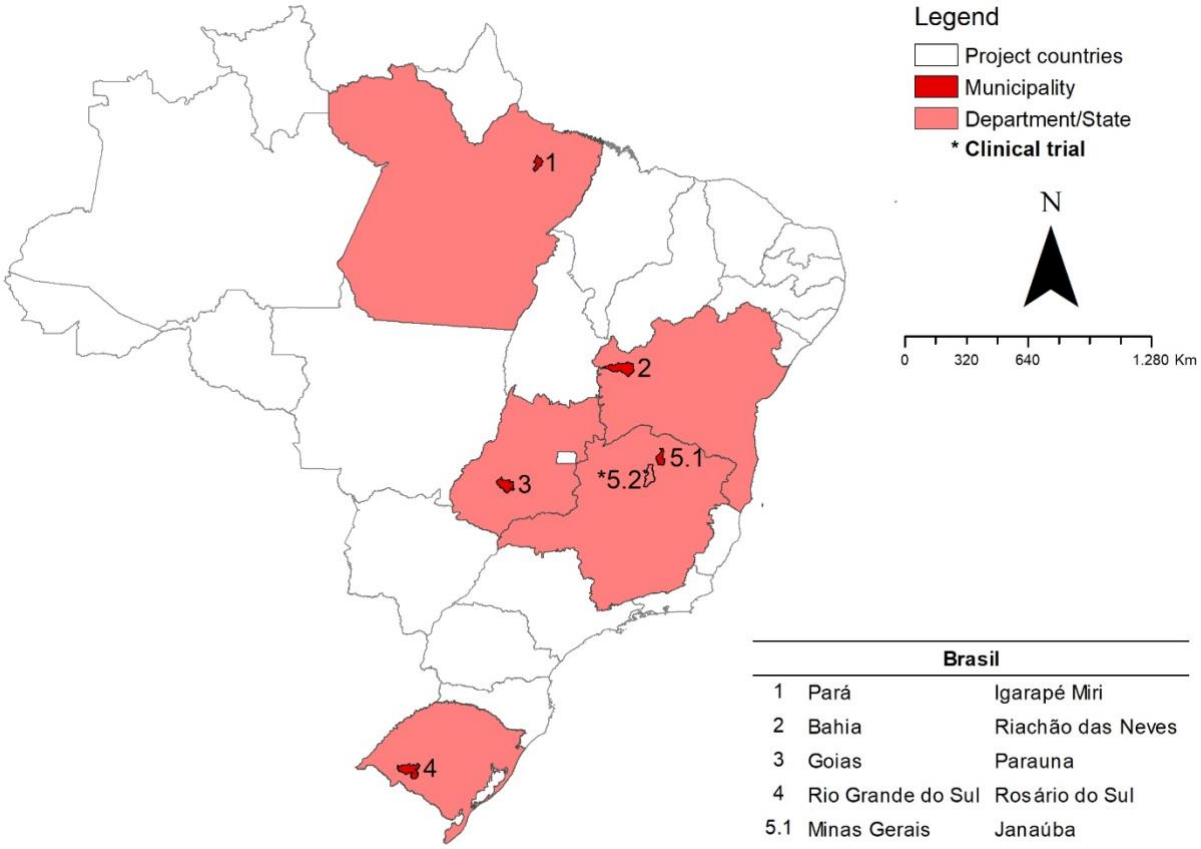
Research locations in Brazil

This research will take place in five municipalities in five states in Brazil, targeting a total of 49,300 people for CD screening. The municipalities have been selected by the Brazilian MoH according to public health priorities, ensuring geographically and epidemiologically diverse contexts, with PHC as the central focus of interventions, integrating with existing initiatives most relevant to the country context, such as health and vaccination campaigns and EMTCT-plus. Table 6 and Figure 10 provide more detailed information on the Brazilian study sites.

Table 6 – Research sites Brazil

Country	Department / State	Municipality	Population	No. of WCBA	Estimated no. of CD cases	Estimated no. of persons to be screened	Estimated no. of persons to be treated	Estimated no. of PHCs	No. of maternity wards (as part of PHC or stand alone)	No. of laboratories	Current CD testing and treatment approach
Brazil	Pará	Igarapé Miri	63,036	19,830	1,169	15,500	140	3	5		Current approach as described in national guidelines
	Bahia	Riachão das Neves	22,334	6,372	414	5,000	45	12	1	2	
	Minas Gerais	Janaúba	72,018	22,674	1,336	18,000	160	16	3	5	
	Rio Grande do Sul	Rosário do Sul	39,314	10,489	729	8,300	75	3	5		
	Goias	Parauna	10,980	3,198	204	2,500	20	7	1	1	
		Totals	**207,682**	**62,563**	**3,853**	**49,300**	**440**	**41**	**15**	**8**	

Figure 10 – Research sites Brazil



Brasil		
1	Pará	Igarapé Miri
2	Bahia	Riachão das Neves
3	Goias	Parauna
4	Rio Grande do Sul	Rosário do Sul
5.1	Minas Gerais	Janaúba
***5.2**	**Minas Gerais**	**Montes Claros**

Colombia

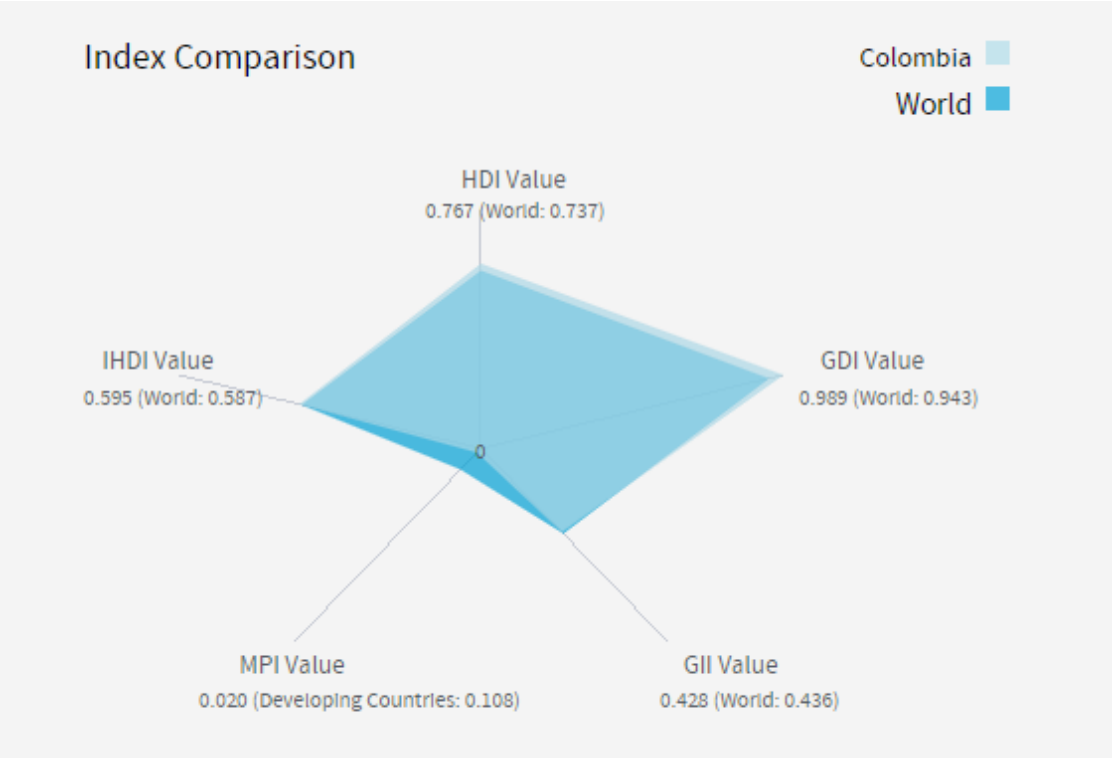
The Republic of Colombia is located in South America's northern Andes, bordering Venezuela, Brazil, Peru, Ecuador and Panama. The country has a land mass of 1,138,910 km^2 and an estimated population of 50,355,650. (51) Ethnic groups in Colombia include Mestizo, White, Afro-Colombian and Amerindian. (51)

Colombia is in the midst of a demographic transition resulting from steady declines in its fertility, mortality, and population growth rates. The birth rate has fallen from more than 6 children per woman in the 1960s to just above replacement level today as a result of increased literacy, family planning services, and urbanization. However, income inequality is among the worst in the world, and more than a third of the population lives below the poverty line. (51)

The 52-year armed conflict between the Revolutionary Armed Forces of Colombia (FARC) and the government officially ended with a peace accord in 2016. Despite an initial overall decline, conflict-related violence has taken new forms and serious abuses continue. In 2019, civilians in affected parts of the country suffered serious abuses at the hands of National Liberation Army (ELN) guerrillas, FARC dissidents, and paramilitary successor groups. Human rights defenders, journalists, indigenous and Afro-Colombian leaders, and other community activists have faced death threats and violence. Violence associated with the conflicts has forcibly displaced more than 8.1 million Colombians since 1985. (52)

According to the UNDP HDR, Colombia ranks as number 83 (out of 189 countries) on the HDI. Figure 11 demonstrates the index comparison between Colombia and the world average. (53)

Figure 11 - Human Development Indicators Colombia



The Colombian health system

Colombia's health system is made up of a social security sector and a private sector. The backbone of the system is the General Social Security Health System, which has two plans, contributory and subsidized. Workers from certain institutions (5.4%) are covered by a third plan. The contributory regime covers salaried workers, pensioners, and independent workers, with the subsidized plan covering anyone who cannot pay. (54) In 2020, 95.07% of Colombians had some form of health care coverage, totaling approximately 47,7 million people. (55)

The National Health Authority's functions under the system include improving the quality of health care and strengthening supervision, surveillance, and control of health insurance. (54) Enrollment in the General Social Security Health System is compulsory and is handled through public or private health promotion agencies (known as EPS). Health care is provided by institutional health service providers, which may or may not be part of the EPS. Those who can afford to purchase health insurance coverage on their own and who can pay for any uncovered fees out-of-pocket use the private sector. (56)

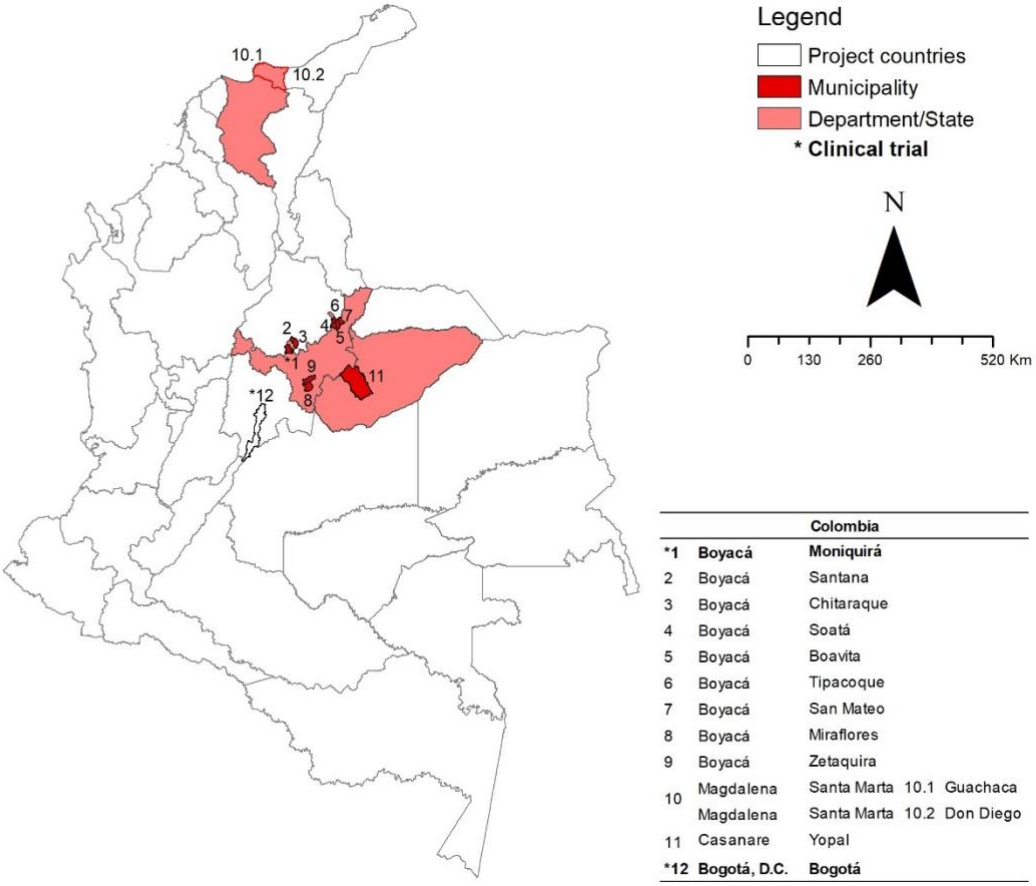
Research locations in Colombia

This research will take place in 12 municipalities in three departments in Colombia, targeting a total of 50,900 people for screening. The municipalities have been selected by the Colombian MoH according to public health priorities, ensuring geographically and epidemiologically diverse contexts, with PHC as the central focus of interventions, integrating with existing initiatives most relevant to the country context, such as health and vaccination campaigns and EMTCT-plus. Table 7 and Figure 12 provide more detailed information on the Colombian study sites.

Table 7 – Research sites Colombia

Country	Department / State	Municipality	Population	No. of WCBA	Estimated no. of CD cases	Estimated no. of persons to be screened	Estimated no. of persons to be treated	Estimated no. of PHCs	No. of maternity wards (as part of PHC or stand alone)	No. of laboratories	Current CD testing and treatment approach
Colombia	Boyacá	Moniquirá	21,182	4,126	1,142	3,600	85	3	1	0	Current approach as described in national guidelines
		Santana	7,605	1,445	410	1,250	30	1	1	0	
		Chitaraque	5,298	1,007	286	850	20	1	1	1	
		Soatá	6,541	1,218	353	1,000	25	3	2	1	
		Boavita	6,442	1,224	347	1,000	25	1	1	0	
		Tipacoque	2,969	564	160	500	10	1	0	0	
		San Mateo	3,304	628	178	550	10	1	0	0	
		Miraflores	9,802	1,893	528	1,600	40	1	2		
		Zetaquira	4,326	822	233	700	15	1	1		
	Magdalena	Guachaca	7,879	1,655	425	1,400	35	1	0		
		Don Diego	1,860	391	100	350	10	0	0		
	Casanare	El Yopal	155,882	43,757	8,402	38,100	910	5	3	1	
Totals			**233,090**	**58,730**	**12,564**	**50,900**	**1,215**	**19**	**12**	**3**	

Figure 12 – Research sites Colombia



		Colombia
***1**	**Boyacá**	**Moniquirá**
2	Boyacá	Santana
3	Boyacá	Chitaraque
4	Boyacá	Soatá
5	Boyacá	Boavita
6	Boyacá	Tipacoque
7	Boyacá	San Mateo
8	Boyacá	Miraflores
9	Boyacá	Zetaquira
10	Magdalena	Santa Marta 10.1 Guachaca
	Magdalena	Santa Marta 10.2 Don Diego
11	Casanare	Yopal
***12**	**Bogotá, D.C.**	**Bogotá**

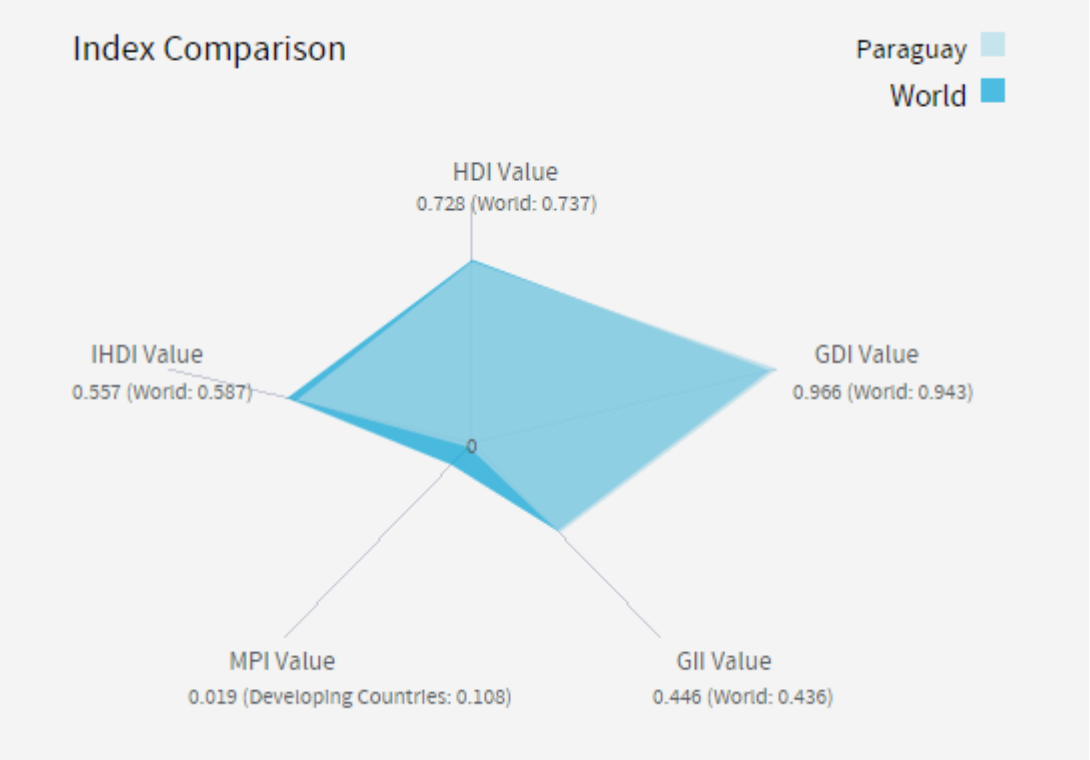
Paraguay

The Republic of Paraguay is a landlocked country in south central South America, bordering Argentina, Brazil and Bolivia. It has a total land mass of 406,752 km^2 and an estimated population of 7,272,639. (57)

Paraguay falls below the Latin American average in several socioeconomic categories, including immunization rates, potable water, sanitation, and secondary school enrollment, and has greater rates of income inequality and child and maternal mortality. Paraguay's poverty rate has declined in recent years but remains high, especially in rural areas, with more than a third of the population below the poverty line. However, the well-being of the poor in many regions has improved in terms of housing quality and access to clean water, telephone service, and electricity. The fertility rate continues to drop, declining sharply from an average 4.3 births per woman in the late 1990s to about 2 in 2013, as a result of the greater educational attainment of women, increased use of contraception, and a desire for smaller families among young women. (57)

According to the UNDP HDR, Paraguay ranks as number 103 (out of 189 countries) on the HDI. Figure 13 demonstrates the index comparison between Paraguay and the world average. (58)

Figure 13 - Human Development Indicators Paraguay



The Paraguayan health system

The Paraguay National Health System created by Law 1032/96 is comprised of the public, private, and mixed sectors, and is characterized by a lack of integration as well as asymmetrical territorial coverage. Although by law, the health authority is the Ministry of Public Health and Social Welfare, leadership is weak and the National Health System operates in an uncoordinated and fragmented manner, with different financing, regulation, enrollment, and service delivery modalities. (59) Historically, human resources for health development in Paraguay was not given high political priority, resulting in an inadequate availability and composition of the health workforce, particularly health workers required

for essential health services. Inequitable geographic distribution of health workers due to lack of infrastructure and incentives to work in rural areas has resulted in a majority of health workers concentrated in the area around Asunción, where approximately 30% of the population lives. (60)

The Ministry of Public Health and the Institute of Social Welfare (IPS) are the two most important health institutions in Paraguay and together cover approximately 92% of the country's population. In 2020 it was reported that the IPS covered 22% of the economically active population, primarily workers in the formal sector and their families. (59,61) Approximately 8% of the population has another type of health insurance, and approximately 70% has no insurance and is covered by the Ministry, which must therefore meet most of the demand for health services. (62)

Paraguay has set ambitious goals to improve the health of its citizens. Rising to the challenge faced by its health system, Paraguay has undertaken a series of reforms that have set the stage for a new approach to health care based on primary care. It has also improved the health of its citizens, especially in relation to communicable, maternal, neonatal and nutritional diseases. Its efforts, however, have not significantly altered the foundations of the health system or its fragmentation into multiple subsystems. According to the Organisation for Economic Co-operation and Development (OECD), in order to achieve its ambitious goals, Paraguay will need to redouble its efforts and strategize to successfully remodel its national health system. (63)

The country has made considerable headway in working towards its objectives, but much stronger policy actions are needed to achieve universal coverage. In 2015, the government defined the National Health Policy for 2015–30, and the state portfolio currently addresses seven key lines of action. For its part IPS has drawn up a series of clear, strategic medium-term objectives. However, the efforts in terms of public finance that sustained progress in the period 2005-15 would be difficult to replicate in the current context. Eventually, without significant reform efforts, Paraguay would fall short of achieving universal coverage. (63)

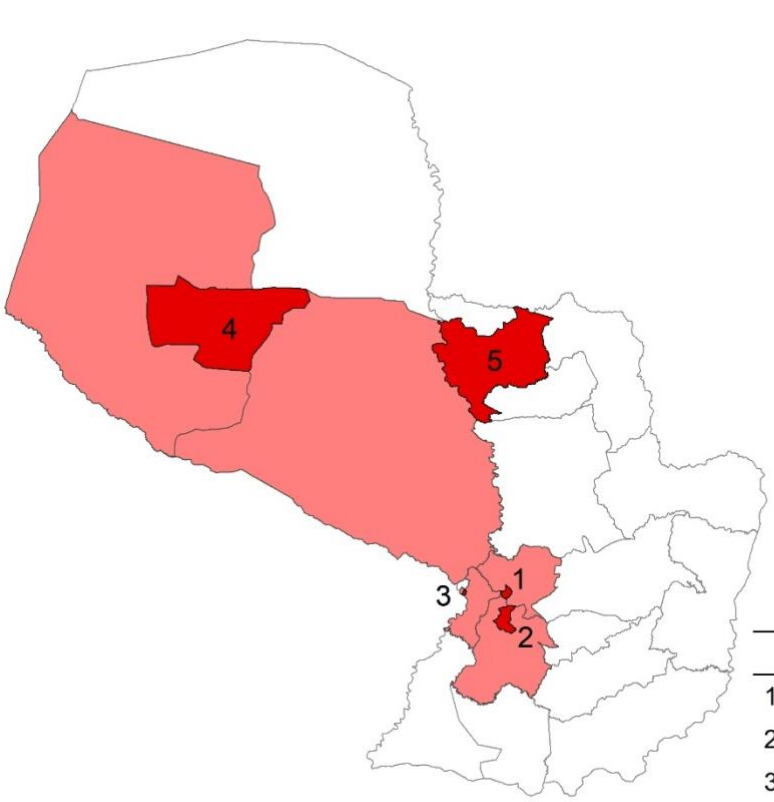
Research locations in Paraguay

This research will take place in 5 municipalities in three departments in Paraguay, targeting a total of 45,500 people for CD screening. The municipalities have been selected by the Paraguayan MoH according to public health priorities, ensuring geographically and epidemiologically diverse contexts, with PHC as the central focus of interventions, integrating with existing initiatives most relevant to the country context, such as health and vaccination campaigns and EMTCT-plus. Table 8 and Figure 14 provide more detailed information on the Paraguayan study sites.

Table 8 – Research sites Paraguay

Country	Department / State	Municipality	Population	No. of WCBA	Estimated no. of CD cases	Estimated no. of persons to be screened	Estimated no. of persons to be treated	Estimated no. of PHCs	No. of maternity wards (as part of PHC or stand alone)	No. of laboratories	Current CD testing and treatment approach
Paraguay	Cordillera	Caacupe	61,616	12,323	1,596	10,000	120	5	1	1	Current approach as described in national guidelines
	Paraguari	Paraguari	24,164	4,833	626	3,900	45	3	1	1	
	Central	Villa Elisa	81,223	16,245	2,104	13,000	160	5	1	1	
	Boquerón	Mariscal Estigarribia	28,348	5,670	734	4,600	55	7	4	0	
	Concepción	Concepción	87,215	17,443	2,259	14,000	175	15	6	1	
Totals			**282,566**	**56,513**	**7,318**	**45,500**	**555**	**35**	**13**	**4**	

Figure 14 – Research sites Paraguay



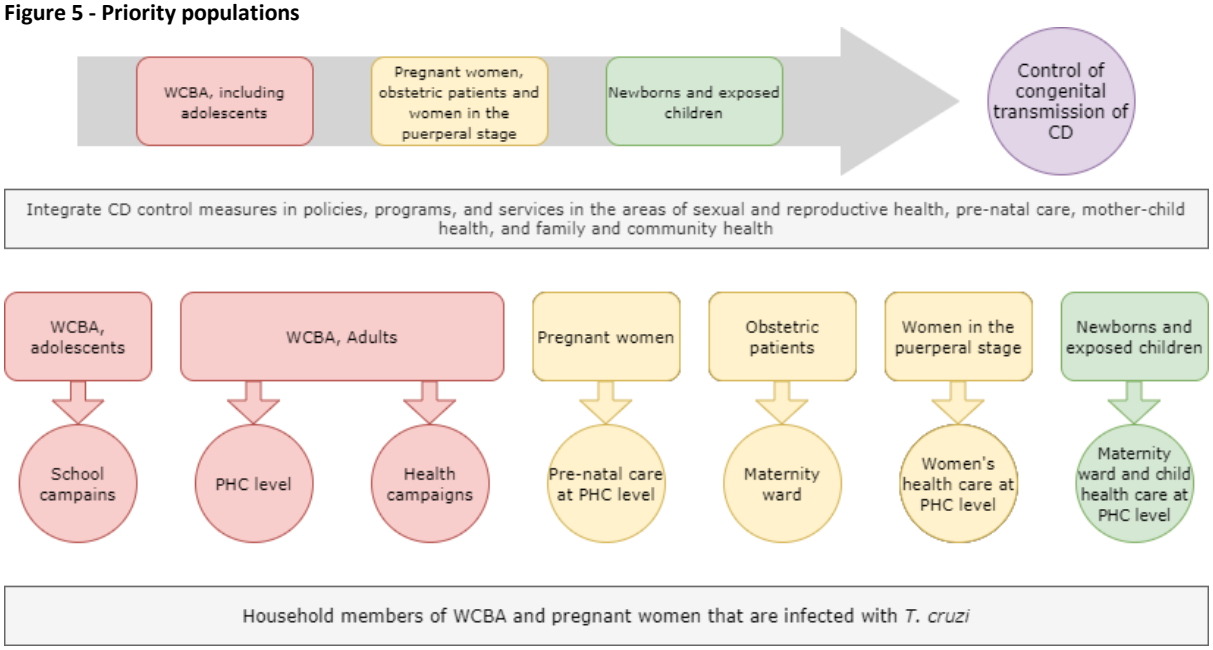
Paraguay	
1 Cordillera	Caacupe
2 Paraguari	Paraguari
3 Central	Villa Elisa
4 Boquerón	Mariscal Estigarribia
5 Concepción	Concepción

Study Population

The populations that will be targeted for this study are WCBA, including pregnant women, their infants and children and their household contacts in a total of 32 municipalities in the four countries. While CD is not considered to be contagious from person to person, this study aims to be inclusive, which is why persons living in the same house (e.g. household contacts) of CD positive WCBA will be included in the screening and treatment efforts. Figure 5 demonstrates the different study populations and where/how they will be recruited.

In addition to the above mentioned target groups, community members and leaders, PHC and community health workers, and health managers will be also targeted for capacity building or formative research purposes. As this study will be conducted through existing primary health and maternity structures, links will be established with initiatives such as health and vaccination campaigns and EMTCT-plus target as many WCBA as possible.

Figure 5 - Priority populations



CD: Chagas disease; PHC: primary health center; *T. cruzi: Trypanosoma cruzi*; WCBA: women in childbearing age.

Study design

Implementation research is a type of research that is concerned with the study of clinical and public health policies, programs, and practices, with the basic intent to understand not only what is and isn't working, but how and why implementation is going right or wrong, and to test approaches to improve implementation. (34) As mentioned, current implementation models for CD in Latin America suffer from a number of significant inadequacies. CD in general is a neglected disease, and congenital CD potentially even more so. CD related services exist but are not routinely integrated into maternal and child health care, diagnostics and therapeutics are available but are not efficiently or effectively used, and there is a general lack of knowledge on the disease, its consequences, and how it should be managed, both on the part of health professionals, as well as the general public. In order to develop solutions that may tackle these barriers, this study will include the following elements:

- Formative research, in order to conduct a context analysis of each of the territories included in the research
- Capacity building of health professionals on relevant topics

- Piloting of test, treat and care service provision, which includes:
    - Screening of adults and children through the use of RDTs
    - Diagnosing newborns
    - Providing antiparasitic treatment and comprehensive care
    - Counseling
    - Self-care
    - Surveillance
- Community and civil society engagement
- Cost-effectiveness study

Formative research

Formative research involves gathering data that is useful for the development and implementation of intervention programs and can be used to make these programs both culturally and geographically appropriate. Formative research has roots in applied anthropology, sociology, social marketing and educational psychology. (64) Formative research occurs either before a program is designed and implemented, or while a program is being implemented in order to refine and improve program activities. (65)

For this study, the specific objective of the formative research is to conduct a situational analysis of each of the territories included in the implementation research in order to better understand the local context considering physical, socioeconomic and cultural environments, health systems, stakeholders and (institutional) culture. These key aspects will guide the development of culturally sensitive and context appropriate information, education and communication (IEC) materials in order to influence behavior change, as well as guide the planning, implementation, monitoring and outcomes of the interventions. Field teams will be recruited, consisting of a site coordinator, a site assistant and interviewers. Training materials and standard operating procedures (SOPs) will be developed in order to train newly recruited fieldwork teams. The training materials will be contextualized to fit each specific context and will be shared with each team during a face-to-face training.

<u>Design</u>

The formative research will be a mixed method research, combining quantitative and qualitative approaches, and will include desk research, open ended semi-structured interviews and focus group discussions (FGD). The desk research will consist of collecting and reviewing national and local level secondary data for each of the countries and municipalities involved in the study. It will focus on demographic and socio-economic data, health systems, CD data, and existing CD policies and programs. The interviews and FGDs will allow the research team to collect primary data on CD. Guides containing suggested questions for each key research area will be used, and they will focus on knowledge of CD, norms concerning CD diagnosis and care-seeking, positive and negative attitudes towards CD, and personal/family experiences related to CD. In addition, primary health units and maternity ward inventories will be conducted to assess the logistical working conditions, the flow of information and the availability of diagnostic and therapeutic supplies. These inventories will be repeated annually.

Formative research typically uses “intentional non-probabilistic sampling", which means that the sample is relatively small, and respondents are selected based on predetermined criteria and study needs. A purposely selected sample is not representative, and the study results cannot be generalized to the entire population. However, the results provide valuable information about how people think and feel, and their reactions to new ideas and practices. The objective is to obtain qualitative information, such as why and how Chagas disease is perceived, diagnosed and treated in these territories.

Target population and intentional sample size for interviews and FGDs

According to the purpose of this study, the number of participants in interviews and FGDs will be different for each group of interest. The target groups of the formative research will be:

- WCBA (according to international standards 15-49 years old), both positive and negative (or status unknown) for CD;
- Household contacts of CD positive WCBA;
- Community members, including leaders who are involved with health-related messages or influence local norms and practices, such as religious leaders and school teachers.
- (Community) health workers and health managers (community health workers, nurses, doctors and other health professionals who provide prevention and care services related to Chagas disease in basic health units and maternity hospitals, and health managers)

For each territory, it is estimated that 19 open, semi-structured interviews will be carried out, divided over the different informant groups. FGDs will have a maximum of eight participants, and will be conducted with CD positive WCBA, household members and health professionals, totaling 3 focus groups per municipality.

The distribution of the target population is described in Table 9.

Table 9 – Number of participants in interviews and focus groups discussions:

Key- informant	**Number of interviews**	**FGD size**	**Number of FGDs**
WCBA (positive for CD)	4	8	1
WCBA (negative or unknown for CD)	3	n/a	0
Household contacts	5	n/a	0
Community members	3	8	1
(Community) health workers and managers	4	4 professionals of PHCs 4 professionals of maternity	1
Total per municipality	19	24	3
Project Total	608	768	96

Since quantifiable issues of knowledge and experience of testing or health education on CD will be addressed, community members, PHC and community health workers, health managers and WCBA with no history of CD will be selected using both a convenience and purposive sampling method. Considering the close contact with local civil society groups and community leaders, informants may be identified using snowball sampling, which is a nonprobability sampling technique where existing study subjects recruit future subjects from among their acquaintances. Thus, the sample group is said to grow like a rolling snowball. WCBA with a history of CD will be recruited from PHC records.

Interviews

The formative research will include face-to-face interviews with WCBA, both positive and negative/status unknown for CD, household contacts, community members and health professionals (see table 9). The main objective of these interviews is to understand the individual perspective of these persons regarding CD, including their knowledge of the disease, how it can/should be diagnosed and how it should be treated. The interviews will be conducted by trained interviewers, who will probe and potentially modify questions, taking local contexts into account, and explore relevant issues identified during the interviews. Interviewers will conduct three to four interviews per day and will return for follow-up interviews where necessary. Each interviewer will be responsible for about 19 interviews, thus requiring about 6 days per site for the study. The interviews will have a semi-structured character, which will allow unanticipated topics to arise, which can be followed up with a question to further explore these. (66) Furthermore, conflicting information can be resolved with a question during the interview to provide more clarity on complex phenomena (67) and emphasis on particular issues that interviewees find important can become part of the data gathered. Interview guides will be used to guide the field workers when conducting the interviews.

Before the interviews, each participant must sign an informed consent form (ICF) including an audio recording authorization, in their native language, which will be understandable to the potential participant. Interview guides and other instruments will be translated into the local language and piloted before use in the study. The interviews with key informants will be conducted at a location that suits them. During the interviews, some questions may have an emotional impact on the participants, and the research team will make sure that there are assistance services available for referral purposes. Interviews will be audio recorded and interviewers will be requested to log additional information such as non-verbal behaviors and specific observations separately. Each afternoon, interviewers will return to a central location where audio recordings and notes will be included in the study's REDCap database.

Focus group discussions

FGDs using participatory techniques will be guided by a moderator from the field team, who will use a number of fixed questions as described in a predetermined and piloted topics list, to guide an otherwise open discussion. The FGDs will be conducted with CD positive WCBA, household members of CD positive WCBA and health workers (see table 9). The main objective of the FGDs will be to gain a better understanding of social issues underlying CD, in a way that would not be feasible through interviews. The perspective of each group regarding the direct (positive WCBA and health professionals) or indirect (households contacts of positive WCBA) contact with Chagas disease presents as a significant variable to investigate the disease's possible impacts on identity and experience. As the discussions may touch upon sensitive issues which could potentially have an emotional impact on the participants, the research team will make sure that there are assistance services available for referral purposes. Before the FGDs, each participant must sign an ICF including an audio recording authorization, in their native language, which will be understandable to the potential participant. FGD guides and other instruments will be translated into the local language and piloted before use in the study.

Data collection will take account of both the dialogue and the interaction that occur within the group, thereby observing both verbal and non-verbal communication. Examples of the non-verbal communication that will be collected are facial expressions of participants, eye contact, gestures, loudness or tone of voice and body language. The FGDs will be conducted by two people: a local researcher, who is familiar with local culture, language and customs, and a national level researcher, who will participate in all the FGDs in their respective country in order to safeguard the methodology that is to be applied to all discussions. FGDs will be audio recorded and the field team will be requested

to log additional information such as observations on non-verbal communication separately. After each FGD, the field team will return to a central point where audio recordings and observations are included in the project's REDCap database.

Inclusion and exclusion criteria

Inclusion criteria

- WCBA;
- Household contacts of CD positive WCBA;
- Community members;
- Health professionals;
- Health managers.
- Informed consent form read and signed by the participant. In case the participant is illiterate, a finger print will also be accepted.
- In case of minors, informed assent form read and signed. In case the participant is illiterate, a finger print will also be accepted.

Exclusion criteria

- Any member of the target population that does not reside in the study municipality.

Data preservation

Qualitative data collected in the supporting studies will be kept confidential. Key informant interviews and focus group data will be reported and securely (electronically) stored in REDCap, only including generic identifying characteristics, (i.e. female, age). Interviewers will be trained on confidentiality procedures and sign for confidentiality as part of their contractual agreements. Standards for storage of data include electronic security (i.e. antivirus/firewalls), daily backups, easy access for authorized users and measures to prevent theft of tablets/laptop computers.

Data analysis

For the analysis of qualitative data, Content Analysis (Bardin, 1977) will be used, a research method which allows the qualitative data collected in research to be analyzed systematically, hoping to gain new insights as well as in-depth understanding of Chagas disease.

The audio of the interviews will be recorded and transcribed verbatim. Times, locations and significant events (e.g. interruptions) will be collected separately. To protect the privacy of the participants, both data collectors as well as interviewees/FGD members will be advised to not use any names that may identify themselves or others. Each participant will get assigned a pseudonym code which will be used instead of their name in the transcript. This practice will be highlighted in the interviewer trainings that will be provided before the start of the interviews. Both the audio recordings as well as quantifiable information will be stored via REDCap. The transcripts from the interviews will be stored, coded and analyzed with NVivo or other computer assisted qualitative data analysis software (CAQDAS). Data analysis will start with vertical analysis. This means that the transcripts will be read repeatedly, to identify patterns, convergence, similarities and differences among themes. The themes will be summarized and organized according to the purpose of the study. Second, thematic coding will be conducted. A coding sheet will be created to analyze the text fragments per theme. Additional codes can be added during this process when unexpected themes emerge.

For the analysis of the focus group discussions constant comparison analysis will be used. This process will consist of identifying, understanding meaning and assigning codes to the data, identifying patterns

and emerging themes and constructing a situational analysis of each territory. (68) During the first stage (i.e., open coding), the data are chunked into small units and a code will be attached to each of the units. Then, during the second stage (i.e., axial coding), these codes are grouped into categories. Finally, in the third and final stage (i.e., selective coding), the one or more themes are developed that express the content of each of the groups. (69) The last step will be data triangulation, where datasets will be compared to see if there are similarities of differences (or a combination). This step has two main objectives: (i) to review the results against those collected using other data collection methods in order to determine the validity or truthfulness of your findings and (ii) review whether routine data sources confirm your findings. Data will be reported in the form of a narrative or frequency tables.

Although supervision will take place on a daily basis, weekly meetings will be organized with the fieldworkers in each country to review findings and to follow up on special issues. Final reports will be developed and disseminated for each country.

For the analysis of quantitative data information from the different databases will be imported to a statistical program. Graphs and tables will be constructed to represent the results. Categorical variables will be expressed by absolute and relative frequencies and continuous variables will be expressed by means and standard deviation or median and interquartile range.

Capacity building and training of health professionals

This study will include capacity building and training of health professionals in the 32 municipalities on different topics relevant to this study: training of health professionals on CD surveillance; CD clinical management and counseling; parasitological diagnosis; and a training of laboratory staff on molecular biology. The initial outline of the trainings will be developed by the researchers, in consultation with local partners, and will be further defined and contextualized based on the outcomes of the formative research. The study will try to align the trainings, where possible, with existing capacity building/training calendars in each country and will advocate for their permanent inclusion in the same. In cases where alignment may not be possible, the study team will ensure that capacity building sessions are organized in such a way that the provision of care is not negatively affected (an example may be the reduction of group sizes so that not all health professionals are in training at the same time) In addition, the training manuals will be made widely available through the study website and collaboration platform. Table 10 describes the courses that will be offered.

Table 10 - Course overview

Topic	Target Population	Contact hours	Modality	Planning
Training of health professionals on CD surveillance and attention in each municipality	Community health workers and other professionals working in the field	Up to 24 hours	If sanitary conditions allow, this course will consist of both in-person sessions as well as remote activities.	Between January and June 2022
Training of health professionals on CD clinical management and counseling in each municipality	General physicians and nurses	Up to 16 hours	If sanitary conditions allow, this course will be conducted in-person	
Training of health professionals on CD parasitological diagnosis in each municipality	General physicians and laboratory technicians	Up to 12 hours	If sanitary conditions allow, this course will be conducted in-person	
Training of laboratory personnel at national level on molecular biology	Laboratory technicians	Up to 8 hours	If sanitary conditions allow, this course will be conducted in-person. In case the pandemic prevents this from happening, the course will be conducted online.	

Pilot test, treat and care service provision

Test, treat and care services will be provided in each of the 32 municipalities included in the study. The reasons for selecting these municipalities and more detailed descriptions will follow in the section on study locations. This test, treat and care service provision will find its basis in countries' existing policies, protocols and directives on CD, and will be incorporated in the daily work practices of primary health care and maternity staff, with the aim of turning existing diagnostic and treatment processes more efficient and effective. More specifically, it will include the deployment of CD screening through the use of RDTs, enhance the diagnosis and counseling of target groups, provide treatment to eligible CD positive patients and provide counseling to improve treatment adherence, conduct patient follow-up, provide aftercare and linkages to social or complementary services, strengthen CD surveillance at the local level and establish information flows to provincial, state and national levels, and establish selfcare/self-help groups for persons affected by CD. The different interventions will be integrated with and accompanied by strong technovigilance, pharmacovigilance and quality control activities, which will be described in further detail in the SOPs related to diagnosis and treatment of CD. Demand for this service provision will be generated through targeted IEC campaigns and community engagement interventions.

Screening of adults and children through the use of RDTs

The use of RDTs for the screening of CD has the potential to reduce the time needed for a diagnosis and will enable the coverage of a larger part of the population. The use of rapid tests for the screening of suspected CD patients is foreseen in the national guidelines in Bolivia, Brazil and Paraguay (though not regularly used as such). For Bolivia and Paraguay, the study will make use of the StatPak® RDT from Chembio Diagnostics, as this test is both registered and currently used, albeit in limited numbers. For Brazil, the project will use the RT-Bio-Manguinhos test, produced by Bio-Manguinhos in Brazil. All the RDTs that will be used in this study have been registered with national regulatory agencies and have a sensitivity and specificity of >95% when used in CD endemic areas. However, Colombia's guidelines do not include this provision, nor is it foreseen in PAHOs regional policies where RDTs are only recommended for epidemiological surveys. It is for this reason that an additional study will be conducted to validate a rapid test for screening, as agreed with the Colombian Ministry of Health (MoH). The first phase of this study comprises a laboratory based, pre-analytical evaluation, comparing all RDTs that are commercially available Colombia, as well as those that are easily available in Latin America, in order to define the best performing test for the purpose of screening. The second phase will be a field validation of one of the RDTs selected in the previous phase, in order to confirm the sensitivity of the test. The necessary sample was calculated based on a target sensitivity of 97% for the RDT (minimum 93%, with a power of 90% and significance of 0.05), resulting in the need for 310 positive cases for CD. The field validation is expected to be carried out in a high prevalence area in Colombia, currently estimated at 15%, which would mean that a total number of 2,070 people will have to be screened. During the validation, all participants will be submitted to the standard diagnostic protocol, with blood collection for two serological tests and concomitant assessment of the RDT. If the sensitivity of the test is confirmed, the use of RDTs for screening will be rolled out in all Colombian territories included in this study, following the diagnostic flows as outlined in this protocol for the other countries.

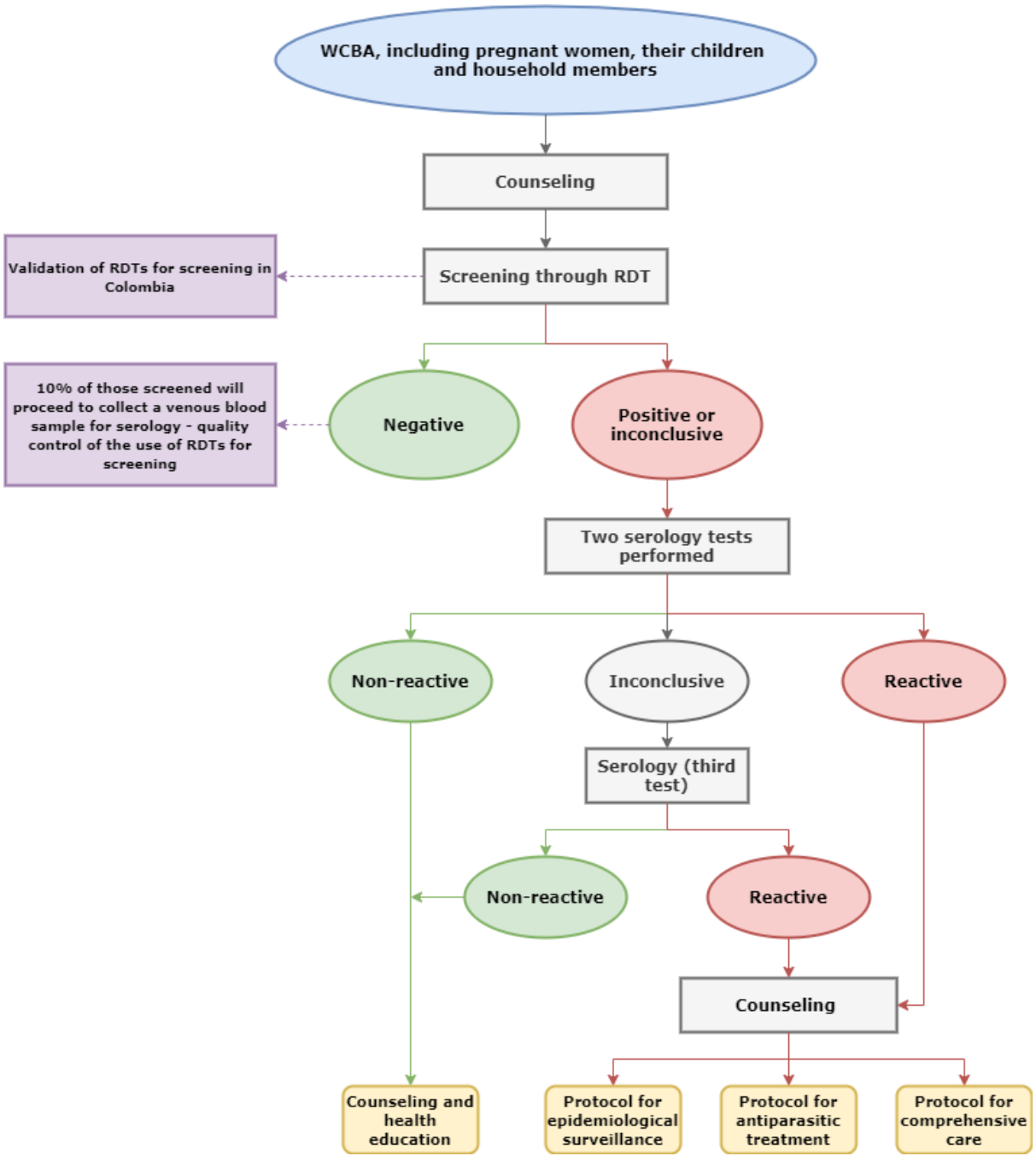
The target population that will undergo screening for CD will be registered at the health post where they are attended to, and will be asked to sign consent forms. In case of the patient being a minor, their parent or legal guardian will be requested to sign a consent form and depending on the age, the minor will be asked to sign an assent form. Before undergoing the RDT, the patient will be offered counseling, which will exist of a brief explanation on CD, why a diagnosis is important, how the RDT will work, and what next steps will be in case of positive, negative or inconclusive results. In case of a

negative result, the news is delivered to the patient along with counseling aimed at health education. For positive or inconclusive results, venous blood samples will be collected, in order to perform serological tests. The collection of blood samples will follow a specific SOP. The sample will be sent to the reference laboratories of each municipality included in the study. The laboratories will perform two serological tests, based on two different methodological principles, as recommended by the national protocols, in agreement with PAHO and WHO guidelines. The samples with divergent/inconclusive results will be submitted to a third serological test to confirm the result. In case of a reactive diagnosis, the patient is again offered counseling, in order to mitigate any fear or negative response that they may have upon receiving the diagnosis. After counseling, additional protocols regarding surveillance, antiparasitic treatment and comprehensive care will take effect.

As a quality control measure, 10% of patients with a negative RDT results will be requested to provide an additional blood sample in order to conduct a serological test. This is included in the ICFs and patients will therefore not be asked to provide additional consent.

Figure 1 shows the flow for the diagnosis of chronic CD in the study's target populations.

Figure 1 - Diagnostic flow for chronic CD patients

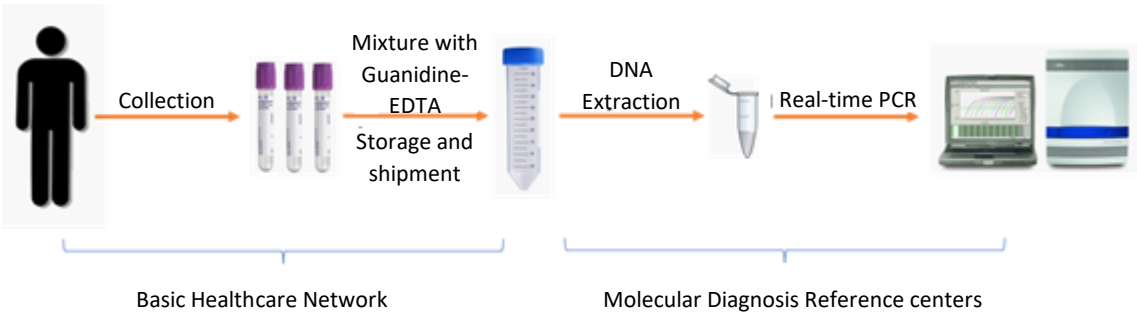


Diagnosing newborns

Currently, diagnosing CD in newborns is complex, as the antibodies of an infected mother to *T. cruzi* can persist in her baby in the first few months of life. Direct parasitological exams are the gold standard for congenital CD diagnosis straight after birth, but these are often not available outside large urban centers and demands trained professionals. A good alternative is the use of molecular biology, PCR, a

method recognized in each of the four countries for other diseases but not accessible in clinical practices related to CD. By including molecular biology in the diagnostic algorithm of congenital CD in newborns, the study may reduce at least six months of the time that is necessary for diagnostic confirmation, thereby decreasing the chances of follow-up losses. The study intends to introduce the collection of a sample of 300-500 µL of venous blood for PCR, which will be added to the same volume of guanidine solution 6M-EDTA 0.2M. The entire collection process will follow the relevant SOP, minimizing the risks inherent to the venous blood collection as much as possible. The invasiveness of the procedure will be mitigated by the use of a winged steel needle, with an extension tube (a butterfly). The mother will be asked to hold the baby, leaving only the extremity of the site of venipuncture exposed after which a trained physician will draw blood. Breastfeeding or oral sucrose will be used as a non-invasive pain management method in infants. (70,71) Figure 2 summarizes the collection process.

Figure 2 - Procedures related to the collection of a blood sample for PCR

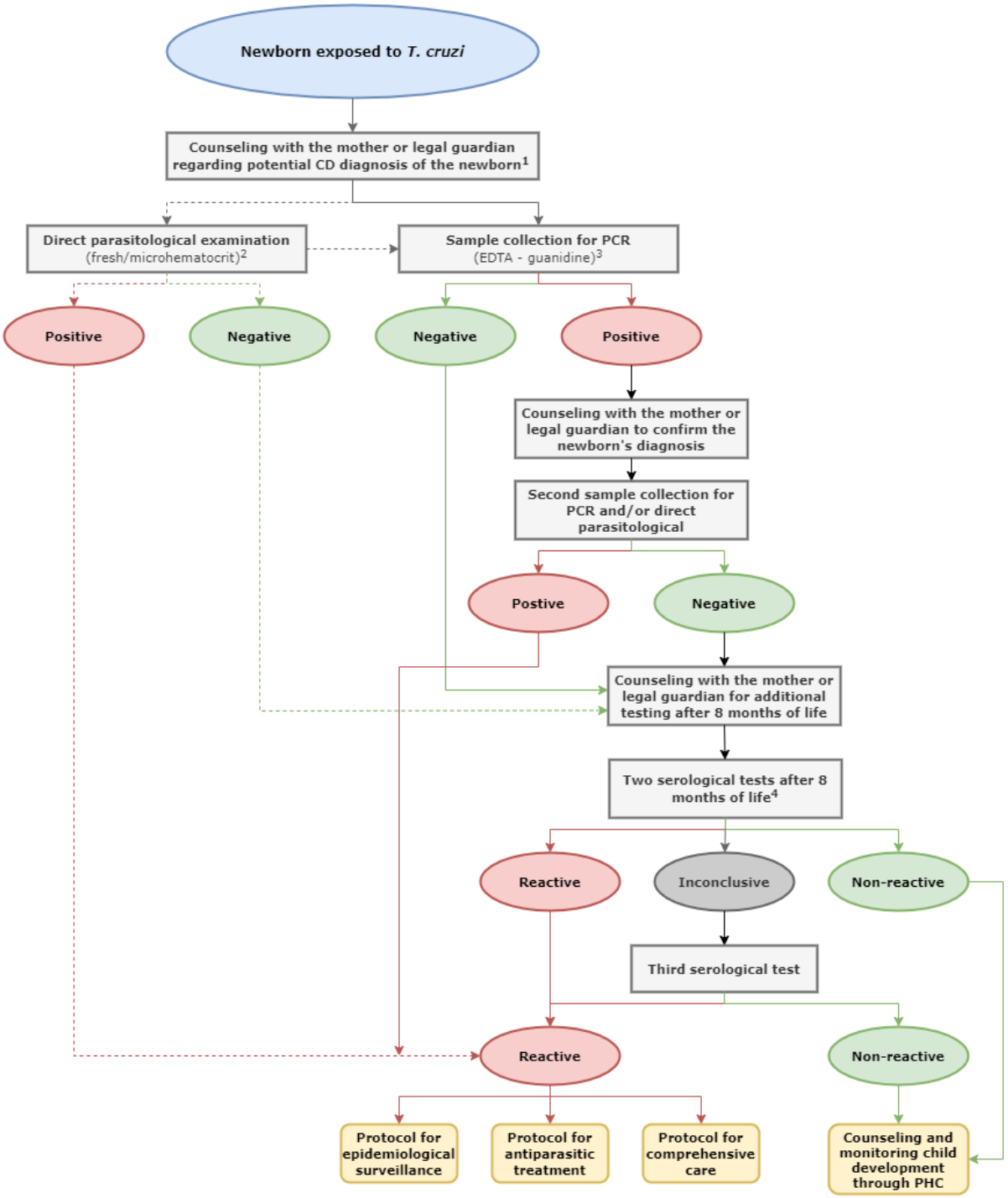


Samples may be transported without any kind of refrigeration or thermal conditioning, as guanidine preserves the integrity of the extracted genetic material even at room temperature.

If a direct parasitological test is performed and the result is positive, blood collection for PCR is not necessary, as this is the current reference standard for diagnosing congenital CD. In this case, the treatment of congenital CD should be started immediately. In case of a positive result from PCR, the collection of a second sample is necessary for the repetition of either technique. Should direct parasitology or the second PCR test also have a positive result, the antiparasitic treatment of the child will start. If the results are negative, the child will need to return after the 8th month of life to collect another sample for two additional serological tests, in order to make a definitive diagnosis. In order to ensure that the mother and child will return to the PHC, counseling will be applied to explain why a return is necessary and what the benefits will be.

Once the case of congenital CD is confirmed, protocols for epidemiological surveillance, antiparasitic treatment and comprehensive care will go in to effect. As for adults, PHC will be the main focus of care, but the patient may be referred to other points of mother and child care, if needed. Figure 3 describes the diagnostic flow for newborns.

Figure 3 - Diagnostic flow for newborns



[1] In case of symptomatic newborns: hospitalization and further investigation - follow the TORCH syndrome investigation protocol (Toxoplasmosis, Other Agents, Rubella, Cytomegalovirus and Herpes Simplex. Other agentes include syphilis, parvovirus, varicella zoster and Chagas disease)
[2] Collection of a biological sample for direct parasitology at the maternity ward or PHC until the 3rd month of life (0.5ml of venous blood, tube with heparin)
[3] Collection of a biological sample at the maternity ward of PHC (in case of non-hospital birth) until the 3rd month of life (0.3 - 0.5 ml of venous blood - EDTA tube - guanidine). Ideally samples should be collected from each newborn for both direct parasitology and PCR.
[4] Collection of biological sample, primarily in PHC - peripheral venous blood.

Antiparasitic treatment and comprehensive care

There are two drugs available for the treatment of CD: BZN and NFX, which are both released for use in all four target countries. Bolivia and Brazil primarily use BZN in their daily clinical practices, while Paraguay primarily uses NFX and Colombia uses both. The choice for NFX is often driven by economic reasons, as it is donated through the PAHO Strategic Fund. For the purpose of this study, we will supply PHC with BZN to treat confirmed CD patients, according to the national protocols and international guidelines. NFX will be available as a second line drug for those cases where BZN may not be used due to contraindications.

Once a positive diagnosis is confirmed, patients will undergo a clinical assessment to verify whether the regular course of treatment may be initiated. The suggested therapeutic recommendations for the antiparasitic treatment of CD according to age range and the clinical form are:

I. Acute phase: treat all age groups;
II. Chronic indeterminate or digestive form: follow national guidelines;
III. Mild chronic cardiac form (absence of heart failure or severe arrhythmias): shared decision for all age groups;
IV. Severe cardiac form: do not treat.

Before initiating treatment, patients will receive counseling to explain the procedure regarding treatment, possible side effects and next steps. For adults, the recommended BZN dose is 5 milligrams (mg) per kilogram (kg) per day, with a suggested maximum of 300mg per day, for a total of 60 days. For children, including newborns, the recommended treatment is 5-10mg per kg per day, for a maximum of 60 days. In newborns weighing <3,200 grams, premature newborns or newborns with co-morbidities, the treatment with BZN will start at a dose of 5mg/kg and will be increased in the second week to 8-10mg/kg/day. The treatment should last at least 30 days, though the full 60 days would be ideal. In general, children under 14 years of age have less adverse effects and better tolerance to the medication than adults. The most recent studies in children show that babies respond well to the treatment, with demonstrable negative *T. cruzi* antibodies and a low risk of AE. Nifurtimox is used as an alternative in cases of non-tolerance to benznidazole or as first choice in some countries, and its dose is 8-10 mg/kg/day in 3 doses and for 90 days; children (<40Kg), 15-20 mg/kg/day, three daily doses for 60 days.

In addition to the real prospect of cure, especially for newborns, children and adolescents, the antiparasitic treatment also enables reduction of the parasitic burden and future chances of congenital transmission. Pregnant women will not receive antiparasitic treatment because of the potential teratogenicity risk. During the clinical assessment, WCBA will be asked about the date of their last menstruation and the possibility of an ongoing pregnancy. A pregnancy test may also be offered in case of doubt. Exceptions to the no-treatment policy are allowed in severe acute cases, where the mother´s life is prioritized. For women in the puerperal stage, treatment may be initiated immediately after the end of the exclusive breastfeeding period and before another pregnancy.

The monitoring of AE, as included in tables 11, 12 and 13, is important when antiparasitic treatment is started. Ideally, the patients are followed up with blood tests and clinical visits 1 to 3 times during the 60-day course of the treatment (baseline, 15-30 and 60 days after the treatment has started). Children need clinical and analytical follow-up during the entire treatment, taking into consideration that the described AE may be manifested in the first weeks and up to 4 weeks after the end of treatment. Clinical and laboratory controls should be routine practice, but do not always happen. However, the controls will be made available periodically (preferably every 15 days) as part of the

study, until the end of treatment. Information on AE will be collected using the study's Case Report Forms (CRFs). In case of a serious adverse event (SAE), the principal investigator in each country is to be informed immediately (within 24 hours of discovery), and they will in turn inform the study's principal investigator. SAE will be collected on the CRFs as well and will be reported to the relevant independent ethics committee.

Follow-up once or twice a year is recommended for all CD patients to monitor clinical progression, control possible visceral complications and discard other risk factors for cardiovascular disease. ECGs should be done during the diagnostic confirmation visit, to define the presence of chronic cardiac complications, and should be repeated annually, while the need for echocardiography will be assessed according to clinical and ECG findings. Children will be monitored every six months in order to detect clinical manifestations related to the infection and assess the criteria for cure, up until the presentation of two consecutive non-reactive tests. The persistence of serology reagent or evidence of positive parasitological tests may indicate therapeutic failure, which will result in the offer of another treatment cycle.

Table 11 - Classification of skin related adverse reactions to BZN

Classification	Type of reaction	Response
Light	Local itching; Macules and papules located in only one part of the body.	**Maintain treatment.** • Review the dosage of BZN according to the person's current weight. • Start antihistamines (chlorpheniramine or loratadine) and/or corticosteroids. • Strengthen family counseling, avoid exposure to the sun and consumption of alcoholic drinks. • Daily control by family members or caretakers.
Moderate	Fever; (severe) itching; Macules, papules, hives and erythema on body or limbs; Itchy skin erosion and/or signs of infection; Symptoms affect more than one part of the body.	**Suspend treatment for three to five days.** • Check BZN dosage (do not exceed 300 mg/day). • Start antihistamines and/or corticosteroids: ◦ Chlorpheniramine administered orally Children < 12 years 0.1 - 0.3 mg/kg/day; Children ≥ 12 years 4 mg/dose every 8 hours, maximum dose: 24 mg/day. ◦ Loratadine administered orally: <30Kg 5 ml/day; ≥30Kg 10ml/day. ◦ Prednisone administered orally, 1mg/kg/day in two doses for three to five days. • Ibuprofen or paracetamol in case of fever. • Assess secondary infection (define need of antibiotics). • If the signs reduce or disappear by the 5th day, keep the treatment until completion. • In case of a new reaction, suspend definitely and consider Nifurtimox as a therapeutic alternative.

Severe	**Stevens-Johnson syndrome** (1 to 2% mortality) Fever, rash, enanthema, Nikolsky's sign (+) affecting <10% of the body surface; **Toxic Epidermal Necrolysis** (6% mortality) Fever, rash, enanthema, Nikolsky's sign (+) affecting more than 30% of the body surface.	**Suspend treatment immediately and urgently refer to other health care levels.** Apply norms for stabilization and transport. Hospitalization at a burn victim or intensive care unit.

Nikolsky sign (+): easy detachment of the skin by applying simple digital pressure.

Table 12 - Classification of digestive adverse reactions to BZN

Classification	Type of reaction	Response
Light	Sporadic or diffused abdominal pain located in the epigastrium of mild intensity.	**Maintain treatment.** • Review the dosage of BZN according to the person's current weight. • Check association with the use of BZN and rule out other pathologies. • Administer symptomatic treatment: **aluminum hydroxide.** • Reinforce family counseling on food and recommend administration of BZN after the meals.
Moderate	Abdominal pain of greater intensity; Decreased and/or loss of appetite; Nausea; Vomiting; Loss of weight <5%.	**Suspend the treatment for three to five days** (or until the symptoms disappear). • Check the BZN dosage. • Check association with the use of BZN and rule out other pathologies. • Administer symptomatic treatment: ◦ Aluminum hydroxide, administered orally every 8-12 hours, 20 mg/Kg/day; ◦ Omeprazole 20 mg/kg/day, adults only. • In case of <5% weight loss: hydration according to the re-hydration protocols. • Reinforce family counseling on food (fractionation of diet). • If the symptoms reduce, maintain the administration of BZN until the therapeutic regimen is completed.
Severe	Intense and continuous abdominal pain; Intense vomiting; Loss of weight > 10%; Choluria;	**Suspend treatment immediately and urgently refer to other health care levels.** • Check the state of hydration and start hydration according to the re-hydration protocol. • Apply norms for stabilization and transport.

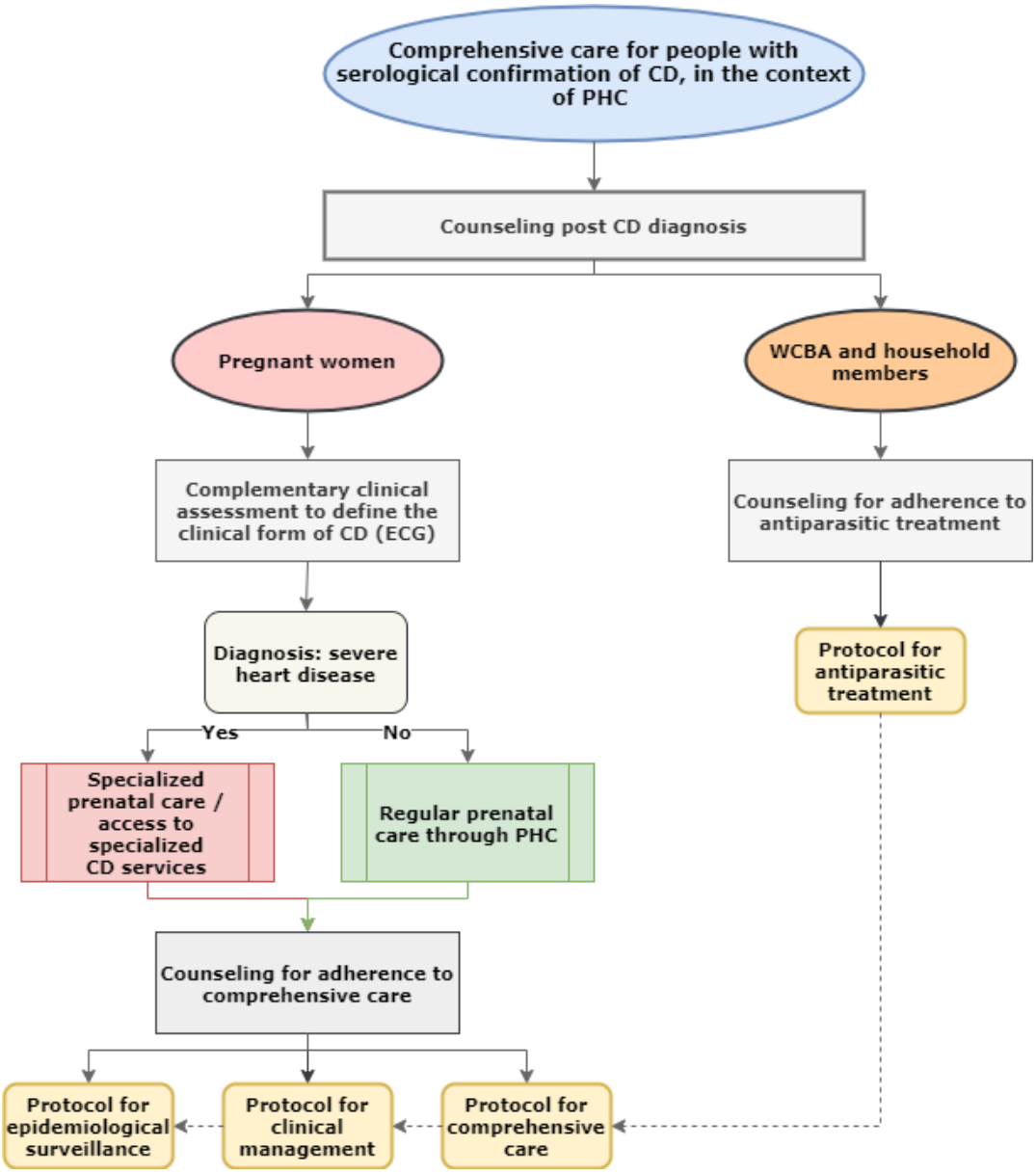
	Hepatomegaly; Jaundice.	• Once at secondary or tertiary care facility, discard other pathologies (hepatitis).

Table 13 - Classification of neuromuscular adverse reactions to BZN

Classification	Type of reaction	Response
Light	Pain in the extremities without functional impotence; localized and sporadic myalgias in upper and/or lower extremities, with or without headache; Tingling sensation.	**Maintain treatment.** • Review the dosage of BZN according to the person's current weight. • Check association with the use of BZN and rule out other pathologies. • Administer ibuprofen or paracetamol. • Reinforce family counseling.
Moderate	Joint and muscle pain with functional potency, located in the upper and/or lower extremities, with or without headache; Tingling sensation.	**Suspend the treatment for three to five days** (or until the symptoms reduce). • Check the BZN dosage • Check association with the use of BZN and rule out other pathologies such as migraine or trauma. • If other causes are ruled out, administer ibuprofen or paracetamol as long as necessary. • Daily clinical control: if the clinical condition worsens in three days, forward to secondary or tertiary health care levels.
Severe	Invalidating functional impotence; Pain in extremities, with or without headache; Tingling sensation; Sensitivity change (hyperesthesia, hypoesthesia, absence); Localized and intermittent arthralgia and myalgia in extremities, with or without functional impotence.	**Suspend treatment immediately and urgently refer to other health care levels.** • Advise family. • Apply norms for stabilization and transport.

Figure 4 describes the comprehensive care for patients diagnosed with CD.

Figure 4 - Comprehensive care for patients diagnosed with CD



Counseling

Counseling is understood as a tool that promotes active, individualized and people-centered listening. This process assumes the ability to establish a relationship of trust and empathy between the main actors: the patient and the health professional. Counseling will be applied at different moments during the process of diagnosis, treatment and aftercare for CD. Three main components have been identified:

I. Emotional support to patients during the establishment of the diagnosis and the therapeutic plan, considering the sensitive nature of the moment, including dimensions of stigma, guilt and potential fear of death. Health professionals should be sensitive to patients' needs and establish a relationship with them along the diagnosis and treatment cycle;

II. Educational support that should consist of an active exchange of information on CD, such as potential clinical manifestations, forms of transmission, prevention and treatment. This is a process that can be carried out individually or in groups;

III. (Self)assessments of values, attitudes and behaviors that can make the contexts of individual and group risk clearer, including the possibility of congenital transmission of CD. It also includes the deconstruction of any ideologies of stigma and discrimination possibly related to the diagnosis of the disease, as well as more general reflections that stimulate the independence and resilience of persons affected or at risk of CD, which is an essential process in order to overcome the vulnerability that is often related to the disease.

The study will develop protocols for counseling on chronic and congenital CD, as well as health education material to be used by professionals to make the information-exchange process easier during individual and collective counseling.

Self-care

Common in diseases like leprosy and lymphatic filariasis, self-care groups have proven effective in preventing aggravation of health conditions. (72,73) Different studies have shown that they also have the potential of catalyzing community solidarity and development. (72,74) Self-care and participation in self-help groups are important strategies for enabling independence, improving self-esteem, addressing depression, anxiety, and fear of death, and overcoming stigma. (75,76)

For patients who are in the chronic stages of the CD, many are already faced with often irreversible physical as well as psychological consequences. This is where self-care groups can provide essential support. They are set up to empower persons affected by CD to build self-care into their daily life. Individual group members are trained to manage their own condition but they are also supported by, and accountable to, the other group members. The study will set-up self-care groups in each municipality and participation will be voluntary. The groups will initially be set up by the research teams, yet always with the aim to quickly link them to PHC and integrate local health professionals so as to make them more sustainable. During the counseling that happens in the test, treat and care service provision, the health professionals will alert CD positive patients to the existence of the groups and provide access for those patients that are interested in joining.

Surveillance

Public health or epidemiological surveillance is the ongoing systematic collection, analysis, and interpretation of data, closely integrated with the timely dissemination of these data to those responsible for preventing and controlling disease and injury. (77) The ongoing monitoring of disease or health trends within populations informs what public health actions are taken and reflects whether those actions are effective. (78) Surveillance data are crucially important to inform policy changes, guide new program interventions, sharpen public communications, and help agencies assess research investments. (79)

Public health surveillance is conducted in many ways, depending on the nature of the health event under surveillance, the nature of healthcare and information infrastructures, the population involved, resources available, and information needs. The widespread and expanding use of the Internet, electronic media, communication technologies, and mobile computing have enabled innovations in public health surveillance that reach far beyond traditional methods. (79)

Epidemiological surveillance for CD tends to focus on controlling the vector. However, as congenital transmission has become proportionately more relevant in the wake of progress in vector transmission control, an increase of surveillance directed towards this type of transmission is necessary. This study will not set up surveillance systems in the target countries. However, the study will conduct different types of surveillance activities. Seropositive WCBA will be requested to identify

their children and household contacts, who in turn will be invited for screening. Seropositive pregnant women will be accompanied throughout their pregnancy and their newborns will be screened and accompanied throughout the first months of life.

Unfortunately, out of the four countries included in the study, only Brazil has recently adopted a mandatory notification system for chronic CD cases, as part of an effort to improve national HMIS. In the other countries, this is not yet a reality. However, the study will record each positive CD case and notify the ministries of health on different administrative levels. The information flows will be established in the first months of the study, together with the different Ministries of Health, health managers and health professionals.

Sample size implementation research

In order to reach the implementation study's sample size, an impact calculation was made determining central, best-case, worst-case and counterfactual scenarios. Table 16 demonstrates the different indicators that were used. The central scenario will be leading for this study, which means that 75% of the WCBA of each municipality will be screened. For the CD positive women, an average of two (2) infants or children per woman will be screened and one (1) household contact. This will result in a total of 234,000 people will be screened for CD, including 181,000 women of childbearing age, 37,000 children and infants, and 16,000 other household members. Based on the prevalence of disease in the different communities participating in the study, this would enable treatment of 8,600 people (after accounting for contraindications and loss to follow-up). The direct impact would be potential avoidance of 2,000 cases of cardiomyopathy and 290 congenital infections, with a reduction in healthcare costs of US$ 15 million and a decrease in annual disability adjusted life years of 3,500.

Table 16 - Indicators and scenarios

Indicators	Central scenario	Best-case scenario	Worst-case scenario	Counter-factual scenario	Comments
Expected prevalence	Bolivia: 25% Brazil: 1.86% Colombia: 5.39% Paraguay: 2.59%	Bolivia: 36% Brazil: 2.65% Colombia: 7.70% Paraguay: 3.70%	Bolivia: 14% Brazil: 1.06% Colombia: 3.08% Paraguay: 1.48%	Bolivia: 25% Brazil: 1.86% Colombia: 5.39% Paraguay: 2.59%	General prevalence in pooled data provided by countries for project communities. Central scenario reduces this by 30%, and worst case scenario by 60%. Counter-factual scenario defaults to central scenario.
Percentage of *T. cruzi* positive women eligible for treatment	70%	80%	60%	60%	Some women not eligible due to contraindications including cardiomyopathy, comorbidities, or pregnancy, breastfeeding during project period. Counter-factual assumes worst case.
Percentage of eligible seropositive women treated	50%	70%	35%	35%	Number of eligible women who are able to actually start treatment, accounting for patient and system level barriers. Counter-factual and worst-case are based on current data.
Expected fertility rate in seropositive women	Bolivia: 0.29 Brazil: 0.1776 Colombia: 0.2284 Paraguay: 0.2224	Bolivia: 0.29 Brazil: 0.1776 Colombia: 0.2284 Paraguay: 0.2224	Bolivia: 0.29 Brazil: 0.1776 Colombia: 0.2284 Paraguay: 0.2224	Bolivia: 0.29 Brazil: 0.1776 Colombia: 0.2284 Paraguay: 0.2224	Annual fertility rate based on WHO data, multiplied by 4 for the project lifetime. (80)
Percentage of births to seropositive mothers screened	90%	100%	80%	65%	Source for counter-factual scenario is Picado et al. 2018 (18)
Expected congenital transmission rate	5%	5%	5%	5%	Zulantay et al 2013. (81)
Percentage of congenital cases treated	85%	100%	70%	50%	Alonso-Vega et al 2013 report 70% of seropositive infants returned for treatment in Bolivia, but this program is no longer in place. (82)
Number of household contacts screened per seropositive woman	1	2	0.5	0.3	No data available on these rates currently.

Expected prevalence in household contacts	20%	30%	10%	20%	A range of 9->40% is reported in limited studies in the literature. (81,83,84)
Percentage of seropositive household contact eligible for treatment	65%	65%	65%	65%	65% of adult patients testing positive are typically eligible for treatment. (85)
Percentage of seropositive household contacts treated	60%	80%	33%	1%	For counterfactual scenario, WHO data, unpublished.
Number of other children per seropositive woman screened	2	3	1	0.5	Estimate based on demographic data from each country.
Expected prevalence in other children	5%	5%	5%	5%	17.5% prevalence was reported in a study in Chile (81), however, this study is taking a more conservative percentage.
Percentage of other children treated	80%	100%	60%	40%	Estimation.
Efficacy of treatment	80%	90%	60%	80%	>80% antiparasitic efficacy for treatment. (86)
Rate of cardiomyopathy	30%	30%	30%	30%	Rassi et al 2010. (2)
Percentage of future congenital infections avoided	100%	100%	100%	100%	Assuming each treated woman has one additional child, and 100% efficacy of treatment in preventing congenital transmission. (12,87,88)
Average healthcare costs for *T. cruzi* infected individuals in USD	2,888	2,888	2,888	2,888	Based on Lee et al 2013 (6), adjusted to 2020 dollars.
DALYs accrued per person annually	0.51	0.51	0.51	0.51	Lee et al 2013. (6)

Tables 17 and 18 describe, respectively, the number of people estimated for screening and treatment.

Table 17 - Total estimated number of people to be screened

Country	Screening for CD - RDT			Molecular Biology	Totals per country
	Estimated number of WCBA	Estimated number of children born to *T.cruzi* infected WCBA	Household members of *T. cruzi* infected WCBA	Estimated number of newborns exposed to *T.cruzi*	
Bolivia	48,500	24,600	12,000	3,200	88,300
Brazil	46,600	1,700	870	130	49,300
Colombia	43,800	4,550	2,100	450	50,900
Paraguay	42,100	2,150	1,030	220	45,500
Totals	181,000	33,000	16,000	4,000	234,000

Table 18 - Total estimated number of people to be treated

Country	Estimated number of people to be treated				
	Estimated number of WCBA	Estimated number of children of *T.cruzi* infected mothers	Household members of *T. cruzi* infected WCBA	Estimated number of newborns exposed to *T.cruzi*	Totals per country
Bolivia	4,300	990	965	135	6,390
Brazil	300	70	65	5	440
Colombia	825	185	185	20	1,215
Paraguay	375	85	85	10	555
Totals	5,800	1,330	1,300	170	8,600

Recruitment

Due to the nature of the implementation research and the importance of developing and maintaining adequate control actions related to congenital CD, strategies for the recruitment of the study's target population should be widely discussed with health professionals, (health) managers, civil society and patient representation. This discussion will allow for the implementation of feasible and sustainable strategies in different contexts. The different priority populations will be recruited as follows:

- **Adolescent Women of Childbearing Age:** carrying out information and educational campaigns in the school environment; invitation to adolescents who attend primary care units for other reasons; conduct counseling and testing in primary health facilities (only in the presence of a parent or a legal guardian).
- **Women of Childbearing Age**: carrying out information and educational campaigns in the community and at the health unit; invitation to women who attend the PHC health units for other reasons (family planning, vaccination, clinical care, prenatal care, etc.); conduct counseling and testing in primary health facilities.
- **Pregnant women:** for those who undergo prenatal care, inclusion of counseling-testing in one of the consultations, preferably at the beginning of pregnancy; conducting information and educational campaigns in the community and at the health unit; counseling-testing in maternity

hospitals, for those who were not tested during pregnancy; during postpartum care for those who have not been tested previously.

- **Newborns and babies up to 3 months of age:** offer molecular biology tests at birth, for deliveries in a hospital environment; active search for children in the community who were born at home to be tested at the primary health unit; screening during the performance of the heel prick test or follow-up consultation of exposed children who did not undergo testing; counseling the mother and/or other legal guardians about congenital Chagas disease.
- **Other children of women of childbearing age with *T. cruzi* infection**: advising the mother and/or other legal guardians about congenital Chagas disease; home visit for active search.
- **Household contacts**: advising women with *T. cruzi* infection on surveillance of contacts; home visit for active search.

Recruitment will be done by researchers, research staff and PHC staff. The recruitment process will be described in more detail in a separate SOP. In addition, it is important to note that investigators responsible for the protocol will permanently evaluate the recruitment strategies, in accordance with ethical principles and good clinical practices that govern clinical research, both nationally and internationally.

Inclusion and exclusion criteria

Inclusion criteria implementation research

- WCBA;
- Newborns and children exposed to CD through a CD infected WCBA;
- Household contacts of CD infected WCBA.
- Informed consent form read and signed by the participant. In case the participant is illiterate, a fingerprint will also be accepted.
- In case of minors, a parent or legal guardian will be requested to sign a consent form and depending on the age, the minor will be asked to sign an assent form. Should the minor be a female adolescent, they should be asked first about their willingness to participate in the study before approaching a parent. Only when the female adolescent agrees to participate, should the consent of a parent or legal guardian be sought. In case the participant is illiterate, a fingerprint will also be accepted.

Exclusion criteria implementation research

- Non applicable.

Community and Civil Society Engagement and regional collaboration

The engagement of communities and civil society is central in any public health intervention, but potentially even more so in settings where there is evidence of inequalities in health. Due to the nature of Chagas disease, the people it affects most, and its classification as an NTD, the focus on community and civil society engagement becomes even more important. Currently, the number of CSOs working on CD is limited, especially compared to diseases such as HIV, as is the meaningful engagement of communities and their leaders (traditional, religious, etc.). This study will therefore place special emphasis on: understanding the existing CS that is either working on CD or has potential links with CD (such as NTDs, HIV, or RMNCH) in each of the countries (as part of the formative research); understanding the international CS that this study can potentially link up with; promote CS networking and set-up or strengthen existing platforms for this purpose; facilitate meetings between CS and local leaders and policy/decision makers; develop joint CS advocacy campaigns in each territory/country,

including campaigns for the world Chagas disease day on the 14th of April each year; develop social behavior change communication (SBCC) strategies and campaigns in each country that are contextualized to different territories and target groups and where possible, and include the broader group of EMTCT-plus communicable diseases in these strategies; train local leaders on CD signs and symptoms, potential adverse reactions of treatment and the need for referral to primary care posts; and conduct a leadership training with community leaders and CSO representatives on interrelated modules with the aim of strengthening their capacity to represent their communities and influence policies.

In addition to the community and CS engagement, special emphasis will be placed on regional collaboration, in an effort to promote further sustainability. We will invite countries are either endemic for CD or that face public health problems related to CD to receive additional attention to participate in specific activities that are included in the broader CUIDA Chagas project. The specific activities that will be organized to further promote regional collaboration are peer exchanges between government officials, health professionals or civil society organizations in order to exchange experiences and learn from the project's initiatives; (virtual) linking and learning events on topics relevant to congenital CD; the set-up of technical working groups that aim to improve collaboration between actors conducting different CD related studies in the region; set-up of technical working groups that aim to improve the integration of CD into the EMTCT-plus initiatives; regional conferences on CD, bringing together important stakeholders, increasing CD visibility, both in the mother and child health care realms as well as the NTD world; and organizing collective advocacy campaigns.

Conduct a cost-effectiveness study

An additional component of this study will be a cost-effectiveness analysis that will compare the interventions that are part of the implementation research with the status quo in each country, taking into account both health effects (disability adjusted life years (DALYs) and quality adjusted life years (QALYs)) as well as costs related to the expansion of access to diagnosis and treatment through primary health care. The cost-effectiveness study will make use of data collected during the implementation research (real-world data), in addition to modelling exercises commonly used in cost-effectiveness analyses. The goal is to understand the cost-effectiveness of the interventions that are implemented through the test, treat and care strategy aimed at WCBA, their newborns and children. These interventions will also include the foreseen capacity building courses targeting health professionals, and communication campaigns to target the priority populations. Table 14 and 15 describe the cost and outcome data that will be collected, calculated or estimated, recorded in a specific instrument (Annex 2).

Table 14 – Cost data to be collected, calculated, or estimated for the cost-effectiveness analysis

Data	Description
Costs related to expanding access to diagnosis, treatment and care for WCBA and their newborns and children, through PHC	*Costs related to counseling in PHC or any other locations; school campaign costs for adolescent WCBA; costs related to PHC testing activities; costs related to PHC campaigns.*
	Costs related to RDTs in PHC, including in prenatal care, to reduce the time to complete the investigation of suspected cases and increase population coverage, especially of more vulnerable groups. They include the costs to make rapid screening available in each location, costs related to the application of the tests, such as individual protection materials, cleaning and disposal of materials,

	transportation, refrigeration and storage of samples, as well as the costs related to the personnel involved in the application of the tests.
	Costs associated with molecular biology (standardized and validated commercial kits), which is a tool to be added to diagnostic protocols for CD in newborns. These costs will be compared to the costs of conventional serological strategies performed at nine months of age or the direct parasitological examination at birth. The objective is to reduce the time needed for diagnostic confirmation, thus decreasing the chance of losses to follow up of new cases.
	Costs related to counseling to increase adherence to treatment - Once the diagnosis of CD has been obtained, adequate counseling should be carried out so that the treatment is initiated timely (in a PHC setting) and adhered to.
	Costs related to parasitological treatment - costs related to conventional parasitological treatment of CD for diagnosed WCBA and newborns. These will include costs related to drugs, professional care and other inputs used when providing treatment.
	Costs incurred to the patients. These data will be obtained using a questionnaire to obtain indirect costs - time lost by patients in seeking testing and treatment and other costs they bear, such as transportation to obtain testing/ counseling and/or treatment. The *'Questionário para Levantamento de Custos Indiretos Associados Ao Tratamento da Doença de Chagas em Adultos'* will be used to obtain these data.
(Existing) Costs related to national or local access to diagnosis and treatment strategies in each country targeted at WCBA and their newborns and children, through PHC	Existing strategies or policies at the national or local level related to the diagnosis and/or treatment of WCBA and their newborns and children in PHC will be identified. Cost estimations will be performed information provided by existing datasets, reports, government personnel, interviews with informants or indirect estimation (whenever information is limited or very limited) based on components of strategies identified. In the event of non-existing strategies, will compare the new costs to the cost of doing nothing.

Table 15. Outcome data to be collected, calculated, or estimated for the cost-effectiveness analysis

Data	Description
Outcomes	Proportion of pregnant women with a positive diagnosis of Chagas disease (before and after the implementation).
	Proportion of newborns diagnosed and treated (before and after).

	Quality-adjusted life years (QALYs) – by using the EQ-5D-3L in a representative sample of WCBA (16 years and older) in each region of the implementation study. Participants will be asked to respond to EQ-5D-3L instrument in four different stages of the study: before any Chagas diagnosis (i.e.: in schools, lectures, primary health care settings, etc.); immediately after the diagnosis, preferably immediately after counseling; during treatment and in the last encounter (once treatment is finished). The goal is to have an assessment of quality of life and estimate utilities for QALY calculation in four important stages of the strategy: general population of WCBA before any diagnosis; upon receiving diagnosis and before starting treatment; during treatment and after treatment.
(Existing) Outcomes related to national or local access to diagnosis and treatment strategies in each country targeted at WCBA and their newborns and children, through PHC	Epidemiological indicators will also be needed in the cost-effectiveness modelling such as disease prevalence, accuracy of tests used, side effects associated with different types of treatment, mortality associated with the disease in each country and sub-region, mortality associated with other causes in each country and sub-region, etc. These indicators will be obtained from a variety of sources, such as government repositories, literature, consultation of country experts or indirect estimation whenever data is not available from any other source.

Effectiveness measurement

Effectiveness (outcome) data will be collected throughout the study. It will include the change in the proportion of pregnant women with a positive diagnosis of Chagas disease and the proportion of newborns diagnosed and treated, before and after the implementation. The study's hypothesis is that these proportions will increase with the intervention, given the higher levels of screening throughout implementation. The second component of effectiveness measurement is obtaining preference-based quality of life data using the EQ-5D-3L instrument.

Preference-based data using EQ-5D-3L

The use of preference-base instruments is strongly recommended in health economic analyses to adjust the study outcomes by the health-related quality of life. This study will use the instrument EQ-5D-3L to allow for the calculation of quality adjusted life years (QALYs). EQ-5D-3L is a standardized tool for quality of life assessment and estimation of utility scores for health status consisting of only five questions, with each question having three alternative answers. The questionnaire covers five dimensions: mobility, personal care, habitual activities, pain/illness and anxiety/depression and a visual analogue scale (VAS). (89)

Preference-based instruments like EQ-5D-3L can be used to estimate utility after the use of algorithms with local value sets, also known as preference or utility weights. Utility reflects the preference of a specific health state (contained in one of the five dimensions of the EQ-5D-3L) and is a number generally between 0 and 1 (with "0" representing dead and "1" representing full health).

The main measurements of the cost-effectiveness study will be the cost per QALY gained and cost per case detected. The QALYs will be obtained after the multiplication of years lived after each intervention by the utility values of each health state lived within the study duration.

Authorization to use EQ-5D-3L within our specific research was requested and obtained (registration ID: 38274). The study will use the EQ-5D-3L self-complete paper versions in all sites. After data collection, EQ-5D-3L data will be entered in electronic datasets. Utility estimates will be calculated for each study visit at patient-level, using local value sets. When patients come from a study site with no availability of value sets, we will use the algorithms of the nearest or most similar country in respect of culture.

Costs

Direct medical costs will be collected using micro costing techniques whenever possible. Estimation or indirect measurements will be used whenever micro costing is not possible. Indirect costs will also be measured in order to capture costs incurred by patients. This assessment will be made using "Questionário para Levantamento de Custos Indiretos Associados Ao Tratamento da Doença de Chagas em Adultos". The costs will be expressed in the local currency of each country and converted to purchasing power parity (PPP) in American dollars to allow comparison between countries.

Steps in the development of the cost-effectiveness analysis

Once costs and outcome data are collected for the implementation as described in detail in Tables 14 and 15, these data will be contrasted with costs and outcome data for the existing scenarios in each country (*status quo* in terms of strategies to diagnose and treat WCBA, their newborns and infants in each country or sub-region where the study will take place). These data will allow calculation of the following summary measures:

1. Incremental cost-effectiveness ratios between interventions (implementation vs *status quo* for each country). These ratios will be obtained by dividing the incremental cost by the incremental health benefit/outcome. The data will be analyzed with the aid of modelling strategies using decision trees. The difference in effectiveness between the two scenarios will be calculated, as well as the difference of average costs associated with each of them. The results will be presented as the cost-effectiveness ratio of each intervention, incremental cost-effectiveness ratio and the incremental net monetary benefit.
2. Detailed sensitivity analyses will be presented, including probabilistic ones to take into account the uncertainty around the parameters of the cost-effectiveness analysis models.
3. After that, as standard in all cost-effectiveness publications, acceptability curves will be drawn, which characterize the likelihood that the new treatment will be deemed cost-effective based on incremental costs and outcomes and an established cost-effectiveness threshold. If the ratio is below the threshold, it is deemed cost-effective, if it is established above it, it is considered not cost-effective. For cost-effectiveness analyses, the threshold ranges estimated for each country will be considered (90), using the value adjusted by the PPP.

Study duration

This study will last a total of 48 months. Table 19 demonstrates a detailed activity overview for the duration of the study.

Table 19 – detailed activity overview

	Year	**Y1**				**Y2**				**Y3**				**Y4**			
	Quarter	*Q1*	*Q2*	*Q3*	*Q4*	*Q1*	*Q2*	*Q3*	*Q4*	*Q1*	*Q2*	*Q3*	*Q4*	*Q1*	*Q2*	*Q3*	*Q4*
Outcome	**Increased access to and demand for effective diagnostics, treatment, and care for CD.**																
Activity 1	**Preparation of the implementation research**																
A1.2	Acquire Ethical Review Board (ERB) approval at all levels (national and WHO)	♦															
A1.3	Site preparation, including elaboration of study documentation (standard operating procedures (SOP), forms, data system, etc.)		♦														
A1.4	Training of team members	♦	♦														
A1.5	Formative research - desk research	♦	♦														
A1.6	Stakeholder mapping	♦	♦														
A1.7	Preparation of the monitoring and evaluation protocols	♦	♦														
Activity 2	**Execution of implementation research**																
A2.1	Formative research - rapid assessment			♦	♦												
A2.1.1	Revision and contextualization of the formative research plans for each country			♦													
A2.1.2	Revision and contextualization of tools and instruments			♦													
A2.1.3	Conduct a training of trainers			♦													
A2.1.4	Training of local research teams in each municipality			♦													
A2.1.5	Data gathering (desk review, site visits, interviews)			♦	♦												
A2.1.6	Focus group discussions			♦	♦												
A2.1.7	Analysis of data gathered				♦												

A2.1.8	Monitoring of research activities by country level central staff and project level staff (through distance meetings and possible site visits)			♦	♦														
A2.1.9	Production of final report and recommendations for each site				♦														
A2.2	Workshop to establish diagnostic confirmation flows, references and clinical management			♦															
A2.3	Capacity building and training of health professionals					♦	♦	♦	♦										
A2.3.1	Conduct trainings of trainers in each country					♦													
A2.3.2	Training of health professionals on CD surveillance in each municipality						♦	♦											
A2.3.3	Training of health professionals on Clinical Management and Counseling in each municipality						♦	♦											
A2.3.4	Training of health professionals on Parasitological Diagnosis in each municipality							♦	♦										
A2.3.5	Training of laboratory personnel at national level on molecular biology							♦	♦										
A2.4	Pilot test, treat and care service provision			♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦				
A2.4.1	Preliminary study to select RDT for screening in Colombia			♦	♦	♦	♦												
A2.4.2	Develop information, education and communication materials and campaigns			♦	♦				♦				♦						
A2.4.3	Deploy screening and diagnosis of target groups			♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦				
A2.4.4	Provide treatment for eligible infected persons			♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦				
A2.4.5	Follow-up of treated patients at 3, 9 and 12 months			♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦				
A2.4.6	Provide aftercare and linkage to social/complementary services			♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦				
A2.4.7	Technovigilance, quality control and pharmacovigilance			♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦				

A2.4.8	Establish selfcare/selfhelp groups for persons affected by CD			♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦		
A2.5	Monitoring and Evaluation - execution			♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	
A2.5.1	Technical meeting in each country on M&E plans and procedures			♦													
A2.5.2	Data collection			♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	
A2.6	Conduct a cost-effectiveness study												♦	♦	♦		
Activity 3	**Community and civil society engagement and demand creation**																
A3.1	Civil society mapping (local, national, regional and international)	♦	♦														
A3.2	Develop social behaviour change communication (SBCC) strategies in each country for different regions, populations and stakeholders		♦	♦													
A3.3	Develop IEC campaigns and materials for different target groups			♦													
A3.4	Training of local leaders (traditional, religious, etc.) on signs and symptoms of CD, potential adverse reactions of treatment and the need to refer patients to primary health posts				♦								♦				
A3.5	Training of Trainers in each country on Leadership training				♦												
A3.6	Leadership training of CSO and local leaders					♦	♦	♦									
Activity 4	**Civil society advocacy and policy influencing**																
A4.1	Civil society networking consolidation		♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦		
A4.2	Develop and deploy campaigns for the international Chagas disease day		♦				♦				♦				♦		
A4.3	Civil society advocacy campaigns developed and launched			♦				♦				♦				♦	

A4.4	Facilitation of meetings between civil society organizations, local leaders and local policy/decision makers			♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦	♦		
Activity 5	**Analysis and dissemination of research results**																
A5.1	Analysis of research data				♦		♦		♦		♦		♦		♦	♦	
A5.2	Dissemination of results, reports, implementation resources and lessons learnt												♦	♦	♦	♦	♦
A5.3	Share data with normative bodies				♦				♦				♦				♦
A5.4	Publication of scientific articles														♦	♦	♦

COVID-19

The COVID-19 pandemic has had an enormous impact on Latin America, not only in terms of cases and related deaths, but also in terms of economic and social impact. (91–93) CD patients are at a greater risk of complications due to COVID-19, and time and resources that have been shifted away from primary health care to attend to the effects of the pandemic has meant an even further decrease in attention for NTDs such as CD. (94) COVID-19 precautions will be taken throughout every phase of the study, and will be monitored and revised according to changing circumstances in each of the countries involved. Overall, the study execution will be adapted to local conditions and regulations for COVID-19 and until the numbers of cases according to each locality are at an acceptable control figure, international recommendations will be followed for the reduction of exposure and transmission, as well as the local standards that regulate the mobility of people.

Additional recommendations regarding COVID-19 that will be taken into account:

- Choose COVID-19 appropriate locations for activities, meaning enough space for physical distancing and easily accessible.
- Promote and safeguard physical distancing: at least 1.5 meters (this can be marked on the floor with chalk or tape, e.g., for queuing), but if national guidelines advise a greater distance, national guidelines should be followed. Allocated time slots and invitations can be used to avoid crowding.
- Handshakes should ALWAYS be avoided.
- All persons involved in the study should be given the opportunity to clean their hands with an alcohol-based hand lotion or wash them with soap and water and dry them with disposable towels before, during and after participation.
- Medical professionals should wear personal protection equipment such as gloves, facemask, gown and if needed protective glasses. For participants a face mask will be mandatory.
- In case of in-person training sessions, both facilitators and participants should wear facemasks and an alcohol-based hand lotion should be available to all.
- All persons involved in the study should be informed of the additional risks of participation caused by COVID-19. IEC materials will be made available.
- Any person involved in the study has the right to refuse engagement in the study activities if they are not willing to take the risk.
- More information is available in the different SOPs.

Data Management and (statistical & qualitative) analysis

The following information is included in the data management plan, included as Annex 3.

Data collection and storage

Data from different collection instruments (Case Report Forms (CRFs), questionnaires, interviews, FGDs) will be recorded in predefined data collection forms. Paper data collection forms and a centralized database were developed for the study at Fiocruz in Brazil. CRFs may be found in Annex 4.

CRFs were developed specifically for this research protocol on the Research Electronic Data Capture (REDCap) platform, used for the collection, management and dissemination of research data. The platform is free to use, not for profit. All data collected from this project will be anchored on the Fiocruz server, and a dedicated team will provide data management support.

REDCap is a secure web application for building and managing online databases and was developed by an informatics core group at Vanderbilt University. While REDCap can be used to collect virtually any type of data, it is specifically generated to support online or offline data capture for research studies and operations. The REDCap Consortium has a vast support network of collaborators and is composed of thousands of active institutional partners, including Fiocruz.

In each municipality included in the research, a team made up of a coordinator and a research assistant will be in charge of reviewing and entering the data into REDCap. Paper CRFs completed by healthcare professionals responsible for screening and follow-up procedures will be reviewed and signed by the research coordinator or assistant. The coordinators or assistants will transfer the data on paper into the electronic CRF in REDCap. This transfer of data will be done at the different health posts, and the CRFs will be stored in a locked cabinet before and after typing (uploading) into the electronic database. Electronic CRFs will be electronically monitored for accuracy (verification of source document), protocol adherence, and compliance with good clinical practice. Data manipulation practices for this study will be documented in the data manipulation manual. This document is under development and will be updated throughout the study as practices are refined and new situations arise. Specifically, this document will address issues such as data manipulation conventions, obvious fixes, data query practice, and will also serve to log data manipulation exceptions.

REDCap forms will be developed from “near-final” drafts of paper forms. Form and field names will be assigned in a way that makes it obvious what they are. Form names will be kept to less than 20 to 25 characters. For example, variable/field names will follow convention: a prefix will be used to indicate which form the variable is in (eg 'bas_' for baseline data, 'scr_' for data screening; suffixes 'd will be used ', 'dt' and 'tm' for date, time and time variable names respectively (eg 'visitd' for the date of visit (date), 'visitdt' for time and date of visit (datetime), ' visittm' by visit time.) During export, REDCap will present the code lists based on the variable name. Wherever possible, text fields will be restricted through the use of “validation”. The same will be true for the verification of numerical ranges. Field type validations will be performed automatically by REDCap. Whenever necessary, descriptive text fields will be used above the REDCap variable and the smaller variable label will be kept. Team members not involved in creating the database will test the forms as they are developed in REDCap and before putting the database into production.

The study data will be produced by the countries and belong to them. A document detailing agreements on data sharing and intellectual property has been signed with each of the country

partners. All analyses of pooled data will be conducted with the explicit consent of each of these centers.

Table 20 will demonstrate the type of data that will be collected during the study.

Table 20. Description of the data to be collected in the study

Purpose	Collection Instrument			N° of variables	Type of data	Source	Volume		Frequency	Total estimated number of forms	Storage
	N°	Código	Nome								
Formative research	1	CCHIFRE01	Demographic and socioeconomic information on municipalities	40	Secondary / quantitative	Data collection form	32	1 per municipality	1 x	32	Physical: in a safe and secure place, such as a locked cabinet inside the health facility Digital: In REDCap
	2	CCHIFRE02	Instrument 2a: National level Chagas disease policy and context (Methodology: desk research)	36			4	1 per country	1 x	4	
	3	CCHIFRE03	Instrument 2b: Chagas disease policy and context at municipal level (Methodology: desk research)	30			32	1 per municipality	1 x	32	
	4	CCHIFRE04	Instrument 3: Local health systems (formal and informal - Methodology: desk research)	64			4	1 per country	1 x	4	
	5	CCHIFRE05	Checklist for basic health units	34	Primary / quantitative	Data collection form	281	1 per health unit	1 x	281	
	6	CCHIFRE06	Checklist for maternity wards	37	Primary / quantitative	Data collection form	61	1 per maternity ward	4x	244	
	7	CCHIFRE07	Annual inventory of health posts	Tbd	Primary / quantitative	Data collection form	220	1 per health unit, per year	4x	880	
	8	CCHIFRE08	Semi-structured instrument for interviews with WCBA	Tbd	Primary / qualitative	Audio recordings and interviewers notes	608	19 interviews per municipality	1 x	608	Physical: in a safe and secure place, such as a locked cabinet inside the health facility Digital: On Digital
	9	CCHIFRE09	Semi-structured instrument for interviews with household contacts	Tbd							
	10	CCHIFRE10	Semi-structured instrument for interviews with community members	Tbd							
	11	CCHIFRE11	Semi-structured instrument for interviews with health workers and managers	Tbd							
	12	CCHIFRE12	Semi-structured instrument for focus groups with WCBA	Tbd	Primary / qualitative	Audio recordings	32	1 per municipality	1x	32	

Purpose	Collection Instrument			N° of variables	Type of data	Source	Volume		Frequency	Total estimated number of forms	Storage
	N°	Código	Nome								
						and field team notes					Recorder/ In REDCap
	13	CCHIFRE13	Semi-structured instrument for focus groups with community members and leaders	12			32	1 per municipality	1x	32	
	14	CCHIFRE14	Semi-structured instrument for focus groups with health professionals	10			32	1 per municipality	1x	32	
Screening of adults and children using RDTs	13	CCHICRF01	Single Screening Form for Chagas disease, CUIDA Chagas	29	Primary / quantitative	Data collection form	234,000	Total estimated number of persons to be tested	1 x	234,000	Physical: in a safe and secure place, such as a locked cabinet inside the health facility Digital: In REDCap
	14	CCHICRF02	CRF Outcome for Chagas disease (CHD) - Adults and Children (except newborns and children under 3 months)	17			21,500	Estimated of the total number of positive people with RDT	1 x	21,500	
	15	CCHICRF03	CRF Follow-up of adults and children (over 3 months) with Chagas disease	37			21,500	Estimated of the total number of positive people with RDT	1 x	21,500	
	16	CCHICRF05	Instrument for the quality control of rapid diagnostic tests (RDT)	9			1,560	Estimated number of forms to be filled out	1 x	1,560	
Diagnosing newborns	17	CCHICRF06	CRF newborns and children up to 3 months of age, born to mothers who tested positive for Chagas disease	57	Primary / quantitative	Data collection form	4,000	Estimated of the total of NB/with up to 3 months to test	1 x	4,000	

Purpose	Collection Instrument			N° of variables	Type of data	Source	Volume		Frequency	Total estimated number of forms	Storage
	N°	Código	Nome								
Cost-effectiveness	18	CCHICEI01	Questionnaire to survey direct costs for activities associated with the treatment of Chagas disease in adults	22	Primary / quantitative	Data collection form	281	1 per health unit/ maternity	1 x	281	
	19	CCHICEI02	Questionnaire to survey indirect costs associated with the treatment of Chagas disease in adults	Tbd						0	
Totais				**475**	-	-	**284,179**	-	-	**285,022**	-

Once the data is captured in REDCap, the study database will be preserved as a project file in Fiocruz's REDCap Database Management System (DBMS), and may be exported by authorized personnel in the following formats: (1) Comma-separated value, .csv, for Microsoft Excel software (Labels or Raw); (2) Pathway Mapper (.bat), .sps and .csv, for SPSS software; (3) .bat, .sas, and .csv for SAS software; (4) .R and .csv for R software; and, (5) .do and .csv for the STATA software.

During the study, the study team will define whether qualitative data will be stored through a metadata standard. For qualitative studies, NVivo or another computer-assisted qualitative data analysis software (CAQDAS) will be used. The raw data of the research, assuming an n-sample of 230 thousand participants, with different follow-ups and with an expected average number of 3,000 columns of information in .csv format, will occupy 5 Gb. Audio files of interviews and FGD discussions, as well as interviewer and field team notes will occupy around 30 Gb. The data will be stored on the an IT server of Fiocruz. This server has a much larger storage capacity than the demands of this research and contains large unused storage capacity.

Data documentation

The metadata that will accompany the study database (.csv and .rda format) containing the long-term value data (listed in 1.2) is a Word file with a summary of the research protocol, the data dictionary and the script of statistical analysis in R software.

The file with the abstract of the research protocol must contain the research title, the name of the main researcher, the rationale for the study design, the target population with the inclusion and exclusion criteria, the objectives, the outcomes and confounders, the exposure or intervention groups, schedule of procedures, the statistical methodology used and a brief summary of the main results obtained. This file can be replaced by the main publication of the research published in a magazine in the area or preprint versions that contain this information previously listed.

Data dictionaries (code books) will be created and updated immediately when any changes are made to each of the data sets. These will minimally have the following descriptions for each field in the dataset: (1) Field name (variable name); (2) Description: a label or descriptor that clearly identifies the content of the field; (3) Type: The data will be numeric, characters, a date or something else. If numeric, it will be an integer or real number. If a date, it will be accompanied by the date format (e.g. MM/DD/YYYY, etc.). Also, key metadata (data about data) such as the coding scheme, the metric used (e.g., micrograms) or the values represented (e.g., 1 = male, 2 = female), or a standard representation of the measurement. Wherever possible it will be documented how the measurement was obtained, or the algorithm for a derived value (e.g. age). Where present, the upper and lower limits of continuous numeric data will also be documented. Missing data will be consistently represented by NA = field not entered or not applicable.

The analysis files from the R software will include: scripts in .R format, which will allow, at any time, the reproduction of results published in academic journal articles; files containing anonymized data processed in .rda format.

The data dictionary and other metadata will accompany the data in a text file named "readme.txt.

Long-term digital preservation will utilize widely accepted open and non-proprietary file formats. The file formats adopted for the database will be .xml and/or .csv.

The metadata standard established by Fiocruz will be adopted. Descriptive metadata will be based on the Dublin Core (DC) standard and the minimum set of long-term preservation metadata will conform to the PREMIS (Preservation Metadata Implementation Strategies) standard and will be tailored to meet specific database requirements, as established by Fiocruz's Digital Collection Preservation Program (2020) (Fiocruz. https://www.arca.fiocruz.br/bitstream/icict/44220/4/prog_preservacao_digital_acervos_fiocruz.pdf)

Data quality

Data quality will be enhanced by:

- Careful translation, back-translation and pilot-testing the research tools, e.g. forms and questionnaires (95). Translation will be done by pre-approved translation services, and will be checked by native speakers on the research team;
- Training and supervision of the data collectors on the use of tools, interviewing skills, etc.;
- Establishing clear guidelines and protocols for training and supervision;
- Cross-checking of findings at field level;
- Triangulation of data sources to enhance the validity of the results.

The main risks to the confidentiality and security of information related to human participants, include:

- A possible violation of confidentiality by data collectors (addressed through proper training of all that are involved in the data collection);
- A loss of data through computer errors (addressed through the use of reliable equipment);
- Theft (prevented by making anti-theft methods available);
- A violation of internet security (resolved by allowing only limited and audited access, applying password protection, firewalls and antivirus software).

Electronic data collection forms will be programmed into REDCap with online validation checks (also known as edit checks). Based on an understanding of the ways in which data is collected, these checks will: (1) Alert data entry users to missing data; (2) Verify that numeric variables and dates are within reasonable ranges; (3) Check consistency in data. Authorized staff will analyze data quality using REDCap's data quality module. In addition, the data entered will be subject to visual and electronic validation by the data management team in accordance with an approved data validation plan (which is under development). Data issues will be entered into the data capture system in the form of queries/discrepancies. Persons responsible for data entry will respond to queries directly in the data collection system. Query responses will be reviewed by the data management team and closed when the issue is resolved.

Data curation and preservation

Printed forms will be kept in secure locations at study sites.

Once the CRF information is transcribed from paper (source document) to the REDCap platform, regular automated "backups" will be performed (1x a day) to protect against accidental loss of the INI computer service server. Following best practices, data will be stored and backed up in standard non-proprietary or open formats for long-term software readability. All research data generated in REDCap will be preserved indefinitely on the Fiocruz institutional server. Digital data storage rooms are structurally sound and free from the risk of flooding and fire. In addition, the Fiocruz IT service REDCap

server is password-only accessed, contains a physical firewall and power surge protection through power surge protection systems by interactive and uninterruptible power supply (UPS) systems.

Data collected on printed forms will be stored in a locked cabinet before and after inclusion into the electronic database. Once the complete data of the participants has been entered through the transcription of the CRFs on paper, the data can only be exported in anonymized format.

Data security will be managed by the security modes of the REDCap system. This platform has a comprehensive user rights module that allows for a very granular approach. In this way, one can define user roles with specific rights to access forms and functionality and then assign users to each role. This approach makes it easier for the study team to be consistent in defining end-user roles and responsibilities. In addition to user roles, each user can be assigned a data access group (DAG). Once assigned to a DAG, the user can only interact with records belonging to that DAG. Users who are not assigned to a DAG can interact with all participant records and also reassign participants from one DAG to another.

The following describes how some user roles and permissions for each user group will be configured for the study, and these will be customized according to the attributions by levels (local, municipal, national and central at Fiocruz):
(1) Data entry: Create records (data entry users cannot delete or rename participants); locks/unlocks disabled, and data entry rights (view and edit); statistics and graphs; calendar; file repository;
(2) Supervisor/Coordinator: Usage rights; add/edit reports; statistics and graphs; calendar; export of anonymized data; run data quality rules; file repository; create, rename and delete records; respond only to open queries;
(3) Administrator/Researcher: Project design and configuration; usage rights; data access groups; data exports - complete dataset; add/edit reports; statistics and graphs; calendar; data comparison tool; file repository; run data quality rules; open, close and respond to queries for data resolution workflow; lock/unlock with electronic signature authority; data entry rights (view);
(4) Monitor: Export of anonymized data; add/edit reports; statistics and graphs; open, close and respond to queries.
(5) Data manager: Data exports - complete dataset; add/edit reports; statistics and graphs; calendar; exploration, file repository; create, edit and execute data quality rules; open, close and respond to queries; create, rename and delete records; data entry rights (view and edit).
Persons responsible for data entry will only have access to their own data, with only the ability to enter new records, without authorization to delete or edit data that have been finalized. Monitoring and data management will be carried out by teams from each country. A dedicated data manager in each country will only have access to view and monitor data from their respective country. The Fiocruz central team has viewing privileges and editing permission in case of inconsistencies proven by the national teams. All data transfer between the sites will be done in a secure way and with an internal record (log file) by REDCap.

The team of each center will have access to their own data, only with the ability to enter new records, without authorization to delete or edit data that have been finalized. Monitoring and data management will be carried out by teams from each country. The data manager in each country only has access to viewing and monitoring data in their own country. Fiocruz's central team has viewing privileges and editing permission in case of consistencies proven by the national teams. All data transfer between the sites will be done in a secure way and with an internal record (log file) by REDCap.

In accordance with local legislation in the study countries, data will be kept up to five years upon completion of the study, whether for adults, children or adolescents. After this period, the data may be destroyed with prior permission from Fiocruz. The data contained in documents in printed format will be disposed of using paper shredders or by contracting an incineration service for this purpose. The data in electronic format will be destroyed by requesting Fiocruz that the research protocol project developed in REDCap be deleted.

Risks and mitigation measures related to data management

The main risks to the confidentiality and security of information related to human participants include:

- A possible breach of confidentiality by those responsible for data collection (addressed through training);
- An Internet security breach (resolved by allowing only limited and audited access, applying password protection, firewalls and antivirus software).

The study will comply with the Data Protection Legislation of each country (for example, in Brazil, the General Personal Data Protection Law (LGPD) - Law nº 13.709/2018). Participants will be informed how research data will be stored, preserved and used in the long term, how confidentiality will be maintained (anonymization process), and informed consent for data sharing will be obtained. To ensure informed consent, it should be given freely, without any form of payment, and with sufficient information provided on all aspects of data participation and use. Patients who have not explicitly given their consent through the specific consent forms approved by the ethical review committee will not be included.

The study foresees the pseudo-anonymization of the research database, that is, both the removal of direct personal identifiers, which can lead to the identification of an individual, and the processing of personal data in such a way that the data can no longer be assigned to a specific data individual without the use of additional information, provided that such additional information is kept separately and subject to organizational measures to ensure that it is not assigned to an identified or identifiable individual. There will be no direct identifiers, for example: first name, middle name, last name, address, full postal code, telephone number, fax number, mobile number, email address, social security number, insurance card number social or SUS card, other identifying reference number, names of relatives, friends or colleagues, photographs/video showing face or other distinguishing features. The probability of being able to identify an individual by combining any indirect identifiers (Demographic data: marital status, gender, ethnicity, occupation, salary; geographic data; date of birth, date of death, date of admission; and/or medical identifiers: height, weight, unusual medical condition) will be very low.

Fields where users can enter free text (annotation boxes) and other potential Identifiers will be minimized. Two approaches will be employed to reduce probability or re-identification using indirect identifiers: (1) Increase the granularity of the data to be shared: ranges rather than exact values for items such as salary, height or weight; age at baseline instead of date of birth; month and year or year for other dates; the first three characters of a zip code instead of the full zip code; and, (2) Link IDs (randomly assigned unique ParticipantID) will be kept securely and in a separate location (spreadsheet or database) from REDCap data.

Access to REDCap will only be limited to those authorized by the Principal Investigators in each country. Standards for data storage include security (ie antivirus/firewalls), regular backups, and easy access for authorized users.

Qualitative data collected in the supporting studies will be kept confidential. Interviews with key informants and discussions with FGD will be numbered to safeguard participants' identification data. Data will be reported by generic identifying characteristics (ie, gender, age) only. The files of interviews with key informants and of discussions with the FGDs will be uploaded to REDCap, from where the researcher responsible for the qualitative analysis of the data will have access. Interviewers will be trained in confidentiality procedures and will sign for confidentiality as part of their employment contract.

Data monitoring

Preliminary analyses will be performed periodically after the start of the study. Study specific indicators will be analyzed every six months while process indicators will be monitored during three-monthly reflection meetings with the implementation team and stakeholders at the municipal levels.

Publication of findings and data sharing

The study team will ensure that all data generated by the study will be made available to WHO as needed for the development of guidelines and policies. All data generated by the study will be made available to the public health community in terms of adequate open access.

Fiocruz, through its Open Data Plan (PDA-Fiocruz), establishes actions for the implementation and promotion of open data under its responsibility. (Fiocruz. https://www.arca.fiocruz.br/bitstream/icict/42561/2/plano_de_dados_abertos_fiocruz_2018.pdf).

Data and results will be communicated through international peer-reviewed scientific publications, congress presentations, meetings, etc. Publications and sharing of data will follow Fiocruz guidelines. The posting of publications will be announced through mailing lists, e-mails to partners, and through the use of social media. Special emphasis will be placed on the dissemination of findings and results with the communities that will participate in the study. The dissemination strategy will be part and parcel of the communication and community engagement strategy that will be developed in the first six months of the study. This strategy may include activities such as the development of appropriate, tailored communication materials and the organization of town meetings where study results will be disseminated.

Data may be kept for up to five (5) years after publication of the study results in a journal in the field (estimated time to complete analysis of all data and publication). The database will be shared without restrictions for reuse by third parties under an "open data commons" license (eg Public Domain Dedication and License (PPDL); Attribution License (ODC-BY); and/or Open Database License (ODC) - ODBL).

A detailed dissemination plan will be developed at the start of the study, however, a preliminary plan may be found in table 23.

Table 23. Products for dissemination

Type of Data	Result/finding	Recipients	Approximate timeline
Evidence generated on effective test, treat and care approaches through implementation research			
Clinical database	*Presentations, informal reports*	*WHO, general stakeholders*	*Q3 2022*
	Peer-reviewed published reports & related datasets	*General (consumers of open access journals)*	*Q1 2023*
	Individual patient dataset	Curated data repository, partners under consortium agreement	*2024-2025*
Programmatic data	*Presentations, informal reports, implementation resources*	*WHO, national governments, general stakeholders*	*2024-2025*
Formative Research	*Presentations, informal reports*	*WHO, general stakeholders*	Q4 2022
	Peer-reviewed published reports & related datasets	*General (consumers of open access journals)*	Q2 2023
Primary analysis	*Peer-reviewed published reports & related datasets*	*General (consumers of open access journals)*	*2024-2025*
Community and civil society engaged at local, national and regional levels to increase demand for services and advocate for integration of recommended approaches for Chagas disease in policies, strategies and plans			
Clinical database	*Presentations, informal reports*	*WHO, general stakeholders*	*Q3 2022*
	Peer-reviewed published reports & related datasets	*General (consumers of open access journals)*	*Q1 2023*
Programmatic data	*Presentations, informal reports, SBCC and advocacy resources*	*WHO, general stakeholders*	*2024-2025*
Formative Research	*Presentations, informal reports*	*WHO, general stakeholders*	Q4 2022
	Peer-reviewed published reports & related datasets	*General (consumers of open access journals)*	Q2 2023

Potential risks

Potential risks that may impact the study:

1. Reduced access to people and territories included in this study;
2. The COVID-19 pandemic has depleted the national resources on health (financial, human, material);
3. COVID-19 restrictions remain in place well beyond the start of the project;
4. High turnover of health professionals;
5. Health professionals lack time to participate in the capacity building sessions;
6. The project generates a demand that the local PHC centres cannot attend to;
7. Scientific, financial or other types of misconduct;
8. There are instances of civil unrest in the territories that are included in this study;
9. There are issues with the supply of drugs or diagnostic tools that are central to this study;
10. Natural disasters.

Mitigation plan:

1. Through the work with local partners in each country, access to territories will be facilitated. In addition, strategies will be adopted that will allow the project to gain access to the target population, building trust and relationships that will facilitate access. These strategies include setting up local teams, consiting of a site coordinator and a site assistant that will be continuously present in each of the municipalities;
2. All essential products for the execution of the project have been included in the budget. As the project will aim to find a connection to existing health services, the additional burden on health staff will be minimal;
3. The study has been designed in such a way that a number of in-person activities can also be done online. Throughout implementation, the situation regarding the COVID-19 pandemic will continuously be assessed. If the study cannot enter into the field in a safe and responsible way, alternatives will be sought. In case in-person activities are possible, the budget includes a provision for PPE materials;
4. Unfortunately, high PHC staff turnover is something the study has no control over. However, the consortium will ensure that training materials are easily available so that crucial training elements may be facilitated. In addition, the project will try to build institutional memory at the health centers which should facilitate in building the capacity of new staff coming in;
5. The project will try to be as efficient as possible in the capacity building activities, making use of distance techniques, on-the-job learning and trying to keep in-person sessions as concise as possible, while at the same time maintaining high quality standards;
6. As the research will take a total of 48 months, demand will be generated over time, meaning that the flow of patients to the clinics will be mitigated. This will allow health professionals to participate in training activities and become acquainted with the updated work processes. The assumption is that the training and updated work processes will reduce the work load, rather than increase it.
7. Several mitigating measure have been put into place to reduce the risks related to scientific, financial and other types of misconduct. Researchers will be trained on GCP, all researchers and staff will be requested to sign a code of conduct, and there are structures in place to deal with the financial aspects of the study (please see the project plan in Annex 5 for more details).
8. The potential effects of civil unrest will be mitigated due to the fact that this research will work through local structures and with local health staff.
9. The budget that was developed and approved for this research includes all the different health products that will be needed for the diagnosis and treatment of CD. The broader project that

this study is part of includes a procurement strategy that will mitigate the risk of potential stock-outs.

10. Together with local partners and staff, the potential for natural disasters will be assessed, and mitigation plans will be put in place.

Project Management

As mentioned, this research is part of the larger CUIDA Chagas project, which will be implemented in the four countries through a consortium consisting of governmental and non-governmental organizations. The CUIDA Chagas project has its own structure and (support) staff, who will also contribute to the implementation research. Please see Annex 6 for a full overview of the project, including the governance structure and organogram. As part of the governance structure, it is important to note that the project will establish a community advisory board (CAB), amongst others. The purpose of this CAB is to include the perspective of the community and persons affected by CD into project interventions and activities.

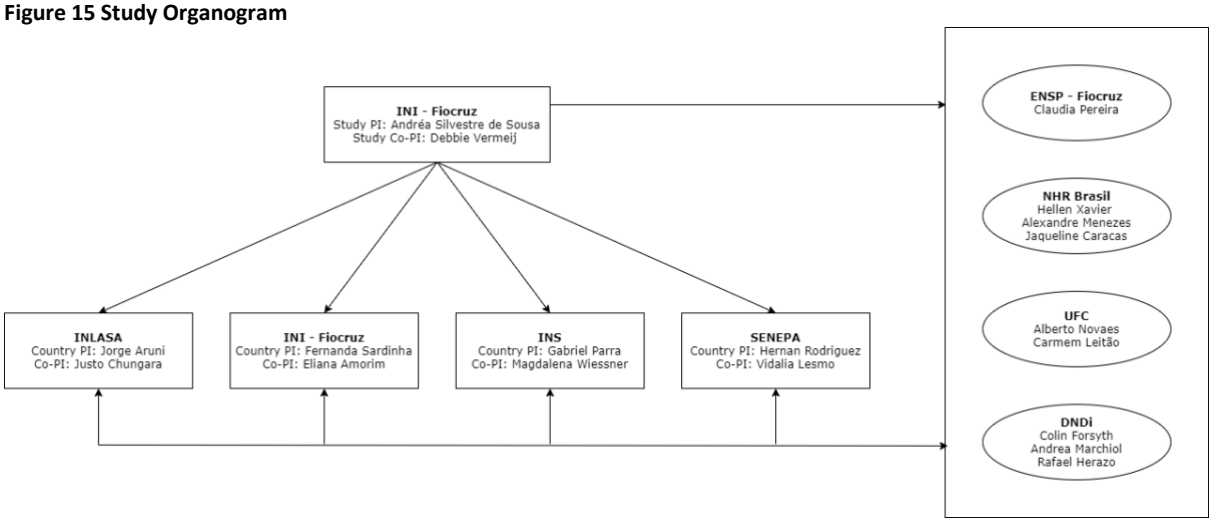
All researchers and staff will sign a code of conduct that will be developed specifically for the project. In addition, all researchers will take part in a Good Clinical Practice course before the start of the implantation research. Table 24 demonstrates the project management team, both on overall study as well as country level and figure 15 demonstrates the study's organogram.

Table 24 – Project management team implementation research

Researcher	Institution	Role and responsibility
Andréa Silvestre de Sousa	Instituto Nacional de Infectologia Evandro Chagas	Principal Investigator (PI), overall coordination of study in the four countries
Debbie Vermeij	Instituto Nacional de Infectologia Evandro Chagas	Co-PI, implementation of the study in the four countries
Jorge Aruni	Instituto Nacional de Laboratorios de Salud	Country PI Bolivia, coordination of the study in Bolivia
Justo Chungara	Instituto Nacional de Laboratorios de Salud	Co-PI Bolivia, implementation of the study in Bolivia
Fernanda Sardinha	Instituto Nacional de Infectologia Evandro Chagas	Country PI Brazil, coordination of the study in Brazil
Eliana Amorim de Souza	Universidade Federal da Bahia	Co-PI Brazil, implementation of the study in Brazil
Gabriel Parra	Instituto Nacional de Salud	Country PI Colombia, coordination of the study in Colombia
Magdalena Wiessner	Instituto Nacional de Salud	Co-PI Colombia, implementation of the study in Colombia
Vidalia Lesmo	Servicio Nacional de Erradición del Paludismo	Country PI Paraguay, coordination and implementation of the study in Paraguay

Claudia Pereira	Escola Nacional de Saúde Pública	Collaborating researcher
Alberto Novaes Ramos Jr.	Universidade de Ceará	Collaborating researcher
Carmem Leitão Araújo	Universidade de Ceará	Collaborating researcher
Hellen Xavier Oliveira	NHR Brasil	Collaborating researcher
Alexandre Menezes	NHR Brasil	Collaborating researcher
Jaqueline Caracas	NHR Brasil	Collaborating researcher
Colin Forsyth	Drugs for Neglected Diseases *initiative*	Collaborating researcher
Andrea Marchiol	Drugs for Neglected Diseases *initiative*	Collaborating researcher
Rafael Herazo	Drugs for Neglected Diseases *initiative*	Collaborating researcher

Figure 15 Study Organogram



The study PI and Co-PI will oversee the execution of the research as a whole. Each country will have their own research team, which will consist of a country PI and a Co-PI. In addition, the different collaborators will assist in specific elements, such as the formative research, the capacity building sessions or the cost-effectiveness study. As mentioned, this research is part of a broader project, with an elaborate staff structure. The project structure fits seamlessly into the broader project, and will receive all assistance necessary to ensure its success.

Ethics

This study will be carried out in accordance with the principles set out in the Declaration of Helsinki. Principles of Good Clinical Practice (ICH 1996) and other applicable regulations and guidelines will be used to guide procedures and considerations.

This protocol will be reviewed and approved by the WHO Ethics Review Committee (ERC), National Research Ethics Commissions and local research ethics committees. Patients will not be included until all approvals have been obtained for the applicable center.

As this study will be executed within the context of local health systems' routine activities, i.e. not a controlled environment, some specific ethical dilemmas are anticipated:

- The autonomy and understanding of study participants may be limited given that CD generally affects territories and populations with greater social vulnerability, precarious living and working conditions, as well as greater limitation of access to the health care network.
- WCBA may feel pressured to participate in the study or to allow their children to participate in the study.
- Interviews and focus group discussions may trigger personal discomfort or psychological distress for persons affected by CD. The process of diagnosis, treatment and care may equally trigger some degree of personal discomfort or psychological distress when sensitive issues are presented, discussed or recorded, and patients may feel that there is a risk of the study violating doctor-patient confidentiality.
- Ethical issues associated with the implementation research may generate discussions, influencing the results of quantitative and qualitative research, with inherent ethical risks. Loss of privacy, fear of the possibility of CD influencing your life and that of your family, time spent on interviews and diagnostic tests and treatment, or possible adverse psychological effects with the diagnosis of CD are some examples of these issues.

These risks will be mitigated through the design of the SOPs and the different instruments that will be used. The ICFs will stress the fact that participation is voluntary and that the decision to participate or not participate will not affect the quality of the health care that people will receive. In addition, the counseling component of the different interventions will assist in mitigating the risks related to psychological distress.

This study also presents some specific ethical challenges related to the collection of data from a large number of people in different contexts, involving a wide range of interested people. To mitigate this risk, strict data management plans will be set up, following Fiocruz' guidelines.

The different focus group discussion may present ethical challenges. In certain cases anonymity and confidentiality may be difficult to ensure. In addition, the spontaneous and at least partially unpredictable nature of the dialogue between participants may give rise to problems to do with harm or confidentiality, and also limits the extent to which such problems can be identified in advance during the consent process. (96)

The research team will monitor ethical issues such as power relations in the different socio-cultural contexts, gender relations, illiteracy, possible interruption of routine health services, perceived unfair selection of study participants, increased expectations of participants with the project and a potential overload of local health care systems teams throughout the execution of the study. Health workers and researchers will be trained on gender sensitive approaches towards patients and study subjects

and gender sensitive HR policies are applied when recruiting staff for this study. In addition, the study will place special emphasis to ensure that congenital CD control actions are integrated in already existing health care programs and procedures, thereby ensuring that the study does not overload health professionals, but rather provides them with tools to make the process of diagnosing and treating CD more effective and efficient.

Informed consent forms

Informed consent will be obtained from all individuals that participate in the study. The ICFs will be translated into the local languages of the different study sites and may be found in Annex 7-11. Informed consent should be obtained in writing, and with the option of providing a finger print if the study participant is unable to provide a signature. In case the patient is a minor, their parent or legal guardian will be requested to sign a consent form and depending on the age, the minor will be asked to sign an assent form. Should the minor be a female adolescent, they should be asked first about their willingness to participate in the study before approaching a parent. Only when the female adolescent agrees to participate, should the consent of a parent or legal guardian be sought. The informed assent form may be found in Annex 12.

In the case of adult participants who are unable to provide free and informed consent due to their mental or physical state, the participant's wishes can be stated by a guardian, in accordance with the center's policy on obtaining consent for medical procedures. If, during the course of the study, the participant's state changes so that he can consider consent independently, free and informed consent should be discussed and obtained.

A copy of the ICF will be given to the person providing the consent.

Budget, financing and insurance

The total budget for the implementation research is USD 11 million, which will be financed by Unitaid and the Brazilian Ministry of Health.

Expense group	**Total amount**		Y1	Y2	Y3	Y4
Health commodities and health equipment	3,233,208		808,302	808,302	808,302	808,302
Procurement and supply chain	431,568		107,892	107,892	107,892	107,892
Travel related	1,574,717		393,679	393,679	393,679	393,679
External professional services	424,177		106,044	106,044	106,044	106,044
Equipment other than health related	65,843		16,461	16,461	16,461	16,461
Communication materials and publications	840,288		210,072	210,072	210,072	210,072
Project staff	3,892,207		973,052	973,052	973,052	973,052
Other project expenses	10,000		2,500	2,500	2,500	2,500
Grant financial audit	80,000		20,000	20,000	20,000	20,000
Administrative expenses	569,014		142,254	142,254	142,254	142,254
Total	**11,121,022**		2,780,255	2,780,255	2,780,255	2,780,255

Collaboration with scientists or research institutions

The study will seek collaboration with a number of research institutions to ensure the quality of the research. Table 25 demonstrates this list of institutions.

Table 25 - Collaborating institutions

Institution	Abbreviation
Instituto Nacional de Infectologia - Fundação Oswaldo Cruz	INI - Fiocruz
Escola Nacional de Saúde Pública - Fundação Oswaldo Cruz	ENSP - Fiocruz
Instituto de Comunicação e Informação Científica e Tecnológica em Saúde - Fundação Oswaldo Cruz	ICICT - Fiocruz
Institute Gonçalo Muniz - Fundação Oswaldo Cruz	IGM - Fiocruz
Institute Oswaldo Cruz - Fundação Oswaldo Cruz	IOC - Fiocruz
Casa de Oswaldo Cruz - Fundação Oswaldo Cruz	COC - Fiocruz
Instituto Rene Rachou – Fundação Oswaldo Cruz Minas Gerais	IRR - Fiocruz
Instituto Nacional de Laboratorios de Salud	INLASA
Instituto Nacional de Salud	INS
Servicio Nacional de Erradicación del Paludismo	SENEPA
Drugs for Neglected Diseases *initiative*	DND*i*
Foundation for Innovative New Diagnostics	FIND
Universidade Federal do Rio de Janeiro	UFRJ
Universidade Estadual do Rio de Janeiro	UERJ
Universidade Federal Fluminense	UFF
Universidade Federal de Goiás	UFG
Universidade Federal da Bahia	UFBA
Universidade Federal do Ceará	UFC
NHR Brasil	NHR

Links to other projects or studies

CUIDA Chagas

This study is part of a broader project, CUIDA Chagas, aimed at contributing to the elimination of congenital transmission of Chagas disease in Latin America. This project's main goal is 'to contribute to the elimination of congenital transmission of Chagas disease by scaling up and enhancing access to diagnosis, treatment and comprehensive care, through innovative and sustainable approaches in Bolivia, Brazil, Colombia and Paraguay'. In addition to a number of initiatives related to equitable market access, CS engagement, and advocacy, the project includes three studies:

I. Implementation research;
II. The validation of an RDT based diagnostic algorithm;
III. A double-blind, phase III clinical trail to evaluate a shorter BZN treatment regimen.

A full description of the project may be found in Annex 6.

IntegraChagas Brasil

In addition to CUIDA Chagas, this study has a clear link to the project IntegraChagas Brasil, a strategic project demanded and financed by the Brazilian MoH, initially planned as a pilot for CUIDA Chagas. IntegraChagas' general objective is to expand access to the detection and treatment of chronic CD in PHC, integrating care and surveillance of chronic CD cases in five Brazilian municipalities. The project is coordinated by INI - Fiocruz, in partnership with UFC. This project will generate intervention models that may be replicated in different scenarios and on a larger scale.

A full description of this project may be found in Annex 13.

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