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REVIEW

Worldwide Control and Management of Chagas Disease in a New Era of Globalization: a Close Look at Congenital *Trypanosoma cruzi* Infection

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SUMMARY Population movements have turned Chagas disease (CD) into a global public health problem. Despite the successful implementation of subregional initiatives to control vectorial and transfusional *Trypanosoma cruzi* transmission in Latin American settings where the disease is endemic, congenital CD (cCD) remains a significant challenge. In countries where the disease is not endemic, vertical transmission plays a key role in CD expansion and is the main focus of its control. Although several health organizations provide general protocols for cCD control, its management in each geopolitical region depends on local authorities, which has resulted in a multitude of approaches. The aims of this review are to (i) describe the current global situation in CD management, with emphasis on congenital infection, and (ii) summarize the spectrum of available strategies, both official and unofficial, for cCD prevention and control in countries of endemicity and nonendemicity. From an economic point of view, the early detection and treatment of cCD are cost-effective. However, in countries where the disease is not endemic, national health policies for cCD control are nonexistent, and official regional protocols are scarce and restricted to Europe. Countries of endemicity have more protocols in place, but the implementation of diagnostic methods is hampered by economic constraints. Moreover, most protocols in both countries where the disease is endemic and those where it is not endemic have yet to incorporate recently developed technologies. The wide methodological diversity in cCD diagnostic algorithms reflects the lack of a consensus. This review may represent a first step toward the development of a common strategy, which will require the collaboration of health organizations, governments, and experts in the field.

KEYWORDS congenital Chagas disease, *Trypanosoma cruzi*, diagnosis, health policies, endemic, nonendemic

INTRODUCTION

Chagas disease (CD), caused by the protozoan parasite *Trypanosoma cruzi*, was diagnosed for the first time in humans in 1909 (1). For a long time, CD was mainly confined to poor rural areas of Latin America, being vectorially transmitted by blood-sucking triatomine bugs (2). However, CD has gradually changed its epidemiological pattern, becoming more urban and international, and is now a global health care issue (3). Apart from vector-borne transmission (classical and oral routes), *T. cruzi* infection can occur through other pathways: blood transfusion, organ transplantation, mother to child (congenital), and laboratory accidents (2). Among them, congenital transmission is of particular concern, and congenital CD (cCD) has emerged as a growing threat in both areas where the disease is endemic (where the vector is present) and areas where the disease is not endemic (in the absence of the vector) (4, 5). Indeed, mother-to-child transmission represents the main challenge for CD control in countries where the disease is not endemic (6, 7).

In this context, the aims of this review are to (i) describe the current global situation in the management of CD, emphasizing congenital infection, and (ii) summarize the spectrum of available strategies, both official and unofficial, for cCD prevention and control in countries of endemicity and nonendemicity.

TRANSMISSION OF *TRYPANOSOMA CRUZI*

Vector-Borne Transmission

The vectorial transmission of *T. cruzi* takes place via blood-feeding insects belonging to the subfamily Triatominae (Hemiptera: Reduviidae), commonly known as blood-sucking bugs or kissing bugs, with around 150 described species (8). However, only about 20

triatomine species from the genera *Triatoma*, *Rhodnius*, and *Panstrongylus* are responsible for *T. cruzi* transmission to humans, with the most important vectors being *Triatoma infestans* (Southern Cone), *Triatoma dimidiata* (northern South America), *Triatoma brasiliensis* (northeastern Brazil), *Rhodnius prolixus* (northern South America), and *Panstrongylus megistus* (central and eastern Brazil) (2, 9, 10). The multihost parasite *T. cruzi* is able to infect all tissues of more than 150 reservoir mammal species, including both domestic (e.g., dogs, cats, and pigs) and wild (e.g., marsupials, primates, rodents, armadillos, and bats) animals (2, 10–12).

Humans act as an accidental host for *T. cruzi*, and CD is a zoonosis associated mainly (but not exclusively) with vectorial transmission (13), which takes place by means of three interrelated cycles: wild, peridomestic, and domestic. The wild cycle involves wild animals, with transmission occurring via triatomines and/or the food chain. In the domestic cycle, triatomines transmit *T. cruzi* infection from domestic animals to humans and between humans. The peridomestic cycle, which originates from the wild cycle, is maintained by domestic animals living around human dwellings and peridomestic triatomines (14). Adding to the complexity of this scenario, characterized by a high diversity of vectors and hosts, is the heterogeneity of the parasite lineage (15). Natural populations of *T. cruzi* are currently divided into seven genetic subdivisions, the discrete typing units (DTUs) TcI to TcVI (16) and Tcbat, first isolated from bats in Brazil (17). *Trypanosoma cruzi* genotypes have a variable distribution in regions of endemicity as well as different transmission cycles (18, 19).

Two modalities can be distinguished within the vectorial pathway, the transcutaneous and oral routes (20). The former is a direct consequence of vector activity. The nocturnal blood-sucking bugs habitually defecate during or soon after feeding on the host skin, usually in the facial area. Parasite transmission occurs when an infected insect releases *T. cruzi* metacyclic trypomastigotes in the stool, which penetrates the skin of the vertebrate host through the bite wound or mucous membranes (eye, nose, or mouth) (10, 13). Once in the bloodstream of the mammalian host, trypomastigotes invade host cells and gradually differentiate into intermediate epimastigotes and then replicative amastigotes. After successive divisions, the amastigotes transform again into bloodstream-form trypomastigotes and leave the infected cell to invade other cell types (21, 22). In turn, bloodstream trypomastigotes can be ingested by the insect vector, differentiate into epimastigotes, multiply in the gut, and transform into metacyclic trypomastigotes in the rectum, which are released through the feces of the vector during blood meals (23). Alternatively, the oral route is related to the consumption of food and beverages contaminated with feces of *T. cruzi*-infected triatomine bugs (20). The first oral case was documented in Brazil in 1967 (24), after which other outbreaks have been reported in Brazil (25), Colombia (26), Venezuela (27), French Guyana (28), and Bolivia (29).

Nonvectorial Routes

Blood transfusion. The blood transfusion route was first suggested by Mazza et al. in 1936 (30), but it was not until 1952 that Pedreira de Freitas et al. (31) described the first cases in Brazil. The first report of blood transfusion transmission in the United States was in 1987 (32), and the first report in Canada was in 1989 (33), with more cases subsequently being reported in both countries (34, 35). After the implementation of a national screening program, two probable transfusion-transmitted cases were detected in the United States through a lookback analysis of blood component recipients performed from 2007 to 2011 (see "Blood Transfusion and Organ Transplant Control," below) (36). Cases of transfusion-transmitted CD have also been detected in Spain (34, 37–39), and an unconfirmed case has been reported in Switzerland (40).

The probability of acquiring a *T. cruzi* infection after receiving a blood transfusion from an infected donor is between 12% and 20% (10). The key point is that due to the nature of the disease, a considerable number of infected individuals are unaware of their status, which enhances the risk of transmission (38, 41). The probability of transmission depends on several factors, such as the amount of transfused blood, the number of parasites inoculated (directly related to parasitemia), the recipient's immune status, and the component transfused (the risk is higher for platelets than for other blood components) (2, 38).

Transplantation of organs and tissues. Transmission of *T. cruzi* from infected donors to naive recipients may occur but not as a general rule. Thus, the allocation of organs from

infected donors is allowed under certain circumstances based on risk-benefit assessments (42). The risk of *T. cruzi* transmission with liver or kidney transplants is lower (13 to 22%) than with heart transplantation (75 to 100%) and is not recommended for the latter. Furthermore, all recipients of organs from *T. cruzi*-infected donors require exhaustive monitoring posttransplantation (43) (see "Blood Transfusion and Organ Transplant Control," below). In 1981, Chocair et al. (44) reported the first case of CD in a kidney recipient, which was followed by more transmission events resulting from solid-organ transplants (45). In the United States, the first known cases of *T. cruzi* transmission through organ transplantation occurred in 2001 in three patients who had received a kidney-pancreas, liver, and kidney, respectively, from the same donor (46). More recent cases in the United States have been reported. For instance, Kun et al. (47) reported two cases of *T. cruzi* transmission by heart transplantation in California in 2009, which resulted in the death of both recipients. Huprikar et al. (48) reported nine cases of CD transmitted via transplantation of different organs (including heart, liver, and kidney) from 2001 to 2011. In 2017, Corey et al. (49) described *T. cruzi* transmission in a lung transplant recipient. In the literature, there are also cases reported in Europe (50).

Congenital transmission. Congenital CD occurs in around 5 to 10% of births from *T. cruzi*-infected mothers (51). Furthermore, vertical transmission is possible at any phase of maternal infection (acute or chronic), in each pregnancy of an infected woman, and even in successive generations (4). Thus, this pattern of transmission contributes to the uncontrolled expansion of the disease (52). Four main factors are involved in the transmission and potential development of cCD: parasite, mother, placenta, and fetus (53). Indeed, Carlier and Truyens (54) proposed cCD as an ecological model of multiple and complex interactions among the above-mentioned factors. The hematogenous transplacental route is the most feasible for vertical transmission (4), being more likely in pregnancies with high parasitemia (54). However, to date, there is no way to predict whether a *T. cruzi*-infected woman will transmit the parasite to her children (55).

Congenital transmission was first reported in Venezuela in 1949 by Dao (56). In Europe, where the first case was reported in 1981 (57), around 70 years after the discovery of CD, a new chapter in the history of the disease was opened (58). The case involved an asymptomatic 5-year-old child born in Romania of Chilean parents and residing in Sweden who had never visited a country where CD is endemic (57). Since then, numerous cases of cCD in Europe have been reported (59–64). In the United States, the first known case of cCD was reported in 2012 (65), with subsequent reported cases, all of them in children of Bolivian mothers (66, 67). Several potential cCD cases have been reported in Canada but without clear confirmation (68, 69). Congenital *T. cruzi* transmission has also been reported in Japan (70, 71).

Laboratory accidents. Laboratory accidents represent the least common way of transmission due to the biosecurity protocols established in laboratories (72). Laboratory workers can become infected through the manipulation of blood, tissues, and/or cultures containing *T. cruzi* parasites or exposure to the feces of infected triatomine bugs (73). The first such case, reported in 1938, was a consequence of ocular mucosal contact with triatomine feces (74).

CLINICAL MANIFESTATIONS OF CHAGAS DISEASE

Acute Phase

The CD incubation period may be quite variable depending on the route of transmission. Incubation in vectorial pathways is relatively short, 1 to 2 weeks when transcutaneous and 3 to 22 days for the oral route. In contrast, in congenital, transfusional, and transplant transmissions, the duration is more variable; it can last for several weeks after birth in congenital infection and 8 to 160 days in the other cases (75). The acute stage lasts approximately 4 to 8 weeks (75, 76) and is usually asymptomatic or characterized by mild and nonspecific symptoms such as fever, malaise, and enlargement of the liver, spleen, and lymph nodes (2, 77). In vectorial transcutaneous transmission, if the parasite penetrates the body through the skin, a nodule called a chagoma may appear. Alternatively, if the portal of entry is located in the ocular mucous membranes, a painless prolonged eyelid edema, the so-called Romaña sign, may be observed (2, 77, 78). Severe manifestations such as myocarditis or

meningoencephalitis are uncommon but could lead to fatal disorders in fewer than 5 to 10% of cases, especially in children (2, 10). Oral transmission is related to more severe symptomology and higher mortality rates than the transcutaneous route (79).

Congenital Chagas disease. Congenital Chagas disease is defined as acute-stage infection of the newborn (76). Although most cases are asymptomatic or mild, the clinical presentation of cCD is highly diverse and includes abortion, neonatal death, prematurity, low birth weight, fever, hepatosplenomegaly, respiratory distress, anemia, myocarditis, meningoencephalitis, and megaviscera (2). Degrees of severity may be related to the stage of pregnancy when transmission occurs (childbirth included), with a higher probability of spontaneous abortion in early transmissions during the first weeks of gestation (80). High parasitemia also seems to be positively associated with increased morbidity and mortality (81).

Chronic Phase

In immunocompetent individuals, the acute phase usually resolves successfully and spontaneously and, in the absence of treatment, leads to a chronic stage (75). Most individuals never develop symptoms and stay in the asymptomatic indeterminate phase for life (10). However, approximately 10 to 30 years after acquiring the infection, around 10 to 15% of chronically infected patients will develop gastrointestinal dysfunction (megacolon, megaesophagus, or both), and 20 to 30% will develop heart disease, mainly cardiomyopathy (2, 78). Heart disease is the most severe type of organ involvement (75).

Chagas Disease and Immunosuppression

In the immunocompromised host, the natural history of *T. cruzi* infection might be modified (82). Transplant recipients and individuals infected with human immunodeficiency virus (HIV) are at risk of reactivation of previously acquired CD. Reactivation usually implies the recurrence of acute symptomatology due to the inability of the immune system of the chronically infected host to control the infection (83). The parasite can also be transferred from infected organ donors to noninfected recipients (82). Individuals with immunosuppression are more likely to develop severe acute infection, including meningoencephalitis and/or myocarditis (84). Furthermore, in the context of the current coronavirus disease 2019 (COVID-19) pandemic, there is concern that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) acquisition may trigger a reactivation of CD due to possible induced immunosuppression caused by the virus or some COVID-19 treatments (85).

DIAGNOSIS

Diagnostic procedures used to confirm *T. cruzi* infection depend on the phase of the disease (86). During the acute phase of CD, parasitemia tends to be high and detectable by parasitological methods, whereas the chronic phase is characterized by low and intermittent parasitemia, and serological methods are the best option for diagnosis (87).

Parasitological Methods (Microscopy-Based Diagnosis)

Direct parasitological tests are the tests most used for the diagnosis of acute CD, including in newborns, and are based on the microscopic observation of *T. cruzi* trypomastigotes in the blood (88). The basic procedure consists of a fresh drop of peripheral blood being placed onto a glass slide where the parasite can be observed due to its motility. Other options are thin and thick blood smears stained with Giemsa or other blood stains (89).

When the level of parasitemia is low, sensitivity can be increased by concentration techniques such as the Strout test, the microhematocrit or micromethod (87), and the triple-centrifugation method (90) (mainly used for African trypanosomes but also applicable for *T. cruzi*). In the Strout method (91), around 5 mL of blood is collected without an anticoagulant and left at 37°C or room temperature to coagulate. Once the clot is formed, the serum is transferred to another tube, and after two centrifugation cycles, trypomastigotes can be observed in the sediment under a microscope (87). Microstrout is a variant of the technique that requires a lower volume of blood (approximately 500 μL) and can be carried out in microtubes (92). The microhematocrit method (93) consists of the microscopic examination of the buffy coat from centrifuged heparinized capillary tubes, which allows the

detection of live trypomastigotes in blood (94, 95). These micromethods are particularly useful in the diagnosis of cCD when only a small amount of blood can be collected. Venous blood of the newborn is preferable to that of the umbilical cord, as the latter can be contaminated with maternal blood, but if the sample is extracted carefully, avoiding the mixture of blood, umbilical cord blood is the least invasive option (86). The triple-centrifugation method consists of a first step of centrifugation of anticoagulated blood to obtain the supernatant, followed by two more centrifugation steps and the subsequent observation of the sediment (90).

Another possibility is to perform parasitological tests based on the multiplication of parasites (xenodiagnosis and hemoculture), although they require specific laboratory conditions (87), are slower and more laborious than direct tests, and are insufficiently sensitive for low levels of parasitemia (89).

Molecular Diagnosis

The presence of *T. cruzi* trypomastigotes in the bloodstream can also be detected by the amplification of its genetic material. Molecular methods are more sensitive than parasitological techniques and are also useful in the chronic phase to detect therapeutic failure in treated patients and CD reactivation in immunosuppressed individuals (96). The molecular tool of choice for *T. cruzi* detection is polymerase chain reaction (PCR), which is based on the replication of specific DNA sequences by the use of a *Thermus aquaticus* (*Taq*) DNA polymerase (97). A later variation of this method, known as real-time PCR, allows the amplification product to be quantified by the use of intercalating dyes or labeled probes (98, 99). Its advantages over the conventional method include faster determination and less carryover contamination (100); moreover, the parasitic load in the sample can also be quantified by adding standard curves for a known amount of cultured *T. cruzi* parasites (quantitative real-time PCR) (101, 102). The most common targets are nuclear repetitive sequences of satellite DNA (SatDNA) and the minicircle kinetoplast DNA (kDNA), and numerous strategies have been developed for both variants (18, 103–111). Commercial real-time PCR kits, mostly based on SatDNA, are available, e.g., TcruziDNA.CE (Diagnostic Bioprobes SRL, Sesto Giovanni, Italy) (112), RealCycler Chag (Progenie Molecular, Valencia, Spain) (99, 113), the Viasure *Trypanosoma cruzi* real-time PCR detection kit (CerTest Biotec, Zaragoza, Spain), and RealStar Chagas PCR kit 1.0 (Altona Diagnostics, Hamburg, Germany). All these kits have been designed to prevent contamination by minimizing the handling and providing easy-to-interpret results. They usually provide a ready-prepared mix of reagents with all of the components necessary to set up the real-time PCR assay, in which the user has only to pipette the required amount of mix, add the sample (nucleic acid extraction), load it into the thermocycler, and run the protocol. Some of them are available in alternative testing types. For instance, RealCycler Chag can also be processed in a monotest format when used in combination with the SmartCycler automated real-time PCR system (Cepheid, Sunnyvale, CA), and the Viasure *Trypanosoma cruzi* real-time PCR detection kit comes in individual 8-well strips or 96-well plates containing in each well everything needed for the reaction. The application of commercial real-time PCR kits may contribute to a wider application of molecular methods to health centers and to the harmonization of protocols between laboratories (99). In congenital cases, the use of PCR methods is growing, but it seems to be more effective 1 month after birth than at birth, as the parasite burden reaches a peak in the newborn (114, 115), and there is a lower risk of detecting DNA of maternal origin (53). The latter could be explained by the increasing frequency of blood parasite transmission in mid- and late pregnancy (and also during labor) compared to the first trimester, when the intervillous space is still closed (4). Another molecular alternative, especially in congenital cases, is loop-mediated isothermal amplification (LAMP), a technique first developed by Notomi et al. (116). Based on highly specific DNA isothermal amplification (65°C) for 60 min, the method uses a *Bacillus stearothermophilus* (*Bst*) DNA polymerase and a set of 4 to 6 primers. LAMP was first applied for *T. cruzi* DNA detection by Thekisoe and coworkers using a primer set designed from 18S rRNA (117, 118). Eiken Chemical Co. Ltd. (Tokyo, Japan) has developed a *T. cruzi* prototype LAMP kit, which uses the repetitive

SatDNA sequence as a molecular target and does not need sophisticated laboratory devices (119). The results reported in countries of both endemicity and nonendemicity suggest that LAMP is a useful tool for the early detection of cCD (120–122).

Serological Diagnosis

After 90 days of infection, when parasitemia tends to decrease (75), serological diagnosis or immunodiagnosis is based on the detection of anti-*T. cruzi* IgG antibodies, which are predominant in the chronic phase (86). In children of seropositive mothers, serology becomes necessary when parasites are not detected during the first weeks of life, although it should not be used before 8 months of age to avoid the detection of maternal IgG antibodies (5, 89, 123). The analysis of IgG kinetics is also useful in the diagnosis of congenital infection (86). However, the detection of IgM antibodies to diagnose cCD has limited efficiency. False-positive results could appear as a response to trypomastigote excreted-secreted antigens (TESAs) that have passed the placenta (86, 124) or due to the presence of rheumatoid factor antibodies (92). Additionally, in perinatal infection, there is a window period until IgM antibodies appear in detectable amounts, which could also lead to false-negative results (92).

Despite the range of serological methods available, to date, no single test is considered the reference standard for CD diagnosis. Furthermore, it is difficult to make accurate comparisons between serological tests, as genetic variability in human populations and circulating *T. cruzi* genotypes between different geographical areas may contribute to discrepant results (125, 126). There are two groups of serological methods: (i) conventional tests that employ a complex mixture of parasite antigens or the entire parasite and (ii) recombinant or nonconventional tests based on recombinant antigens or synthetic peptides (127). In 2002, the World Health Organization (WHO) (89) established the diagnostic gold standard for CD in which the confirmation of infection requires positive results by at least two conventional tests and a third test, either conventional or nonconventional, if the results do not coincide. The WHO protocol (89) refers to three types of conventional serological tests: the indirect hemagglutination assay (IHA), the indirect immunofluorescence (IIF) assay, and the enzyme-linked immunosorbent assay (ELISA). The IHA is simple, easy to interpret, and inexpensive but with low sensitivity (128); variants such as particle agglutination or direct agglutination can also be used. Alternatively, IIF is more sensitive but has the disadvantage of requiring skilled personnel and a special UV light microscope (89). The most recent technique is based on the ELISA, described in 1975 (129), which is the method of choice in most laboratories to diagnose *T. cruzi* infection. The ELISA has good sensitivity and specificity (89) and, unlike the IHA and IIF, may be performed with automatic equipment (128). Commercial kits are currently available for all these tests. On the other hand, Western blotting (WB) has been proposed as a confirmatory technique for the serological diagnosis of CD. WB is based on the separation of the target proteins by electrophoresis, their transfer to a hydrophobic membrane, and subsequent antibody detection. It has proven useful in the chronic phase (130) and also for congenital infections (7, 131). Ldbio Diagnostics (Lyon, France) distributes a commercial WB assay known as Chagas Western blot IgG. Another available confirmatory technique is the radioimmunoprecipitation assay (RIPA), which has been used in blood donors in the United States (132) (see "Blood Transfusion and Organ Transplant Control," below).

Although conventional serology is characterized by high sensitivity, between patients infected with *T. cruzi* and those infected with *Leishmania* sp. or other trypanosomatids (133). In response, specificity has been enhanced by the use of recombinant antigens or mixtures of antigens (134). As in conventional serology, many commercial kits based on recombinant antigens are available, also including confirmatory tests such as the enzyme strip assay (ESA) or WB. For example, in the Abbott ESA Chagas assay, distributed by Abbott Diagnostics (Abbott Park, IL) (see "Blood Transfusion and Organ Transplant Control," below), individually prepared recombinant antigens are applied separately as discrete lines across nitrocellulose strips laminated onto a plastic support, similar to WB (135). A WB assay based on TESA, known as TESA-blot, is marketed in Latin America (bioMérieux, Rio de Janeiro, Brazil) (130). Specifically, for cCD diagnosis, the shed acute-phase antigen (SAPA) present in TESA

stimulates the production of antibodies, mainly during the acute phase (136), and is also useful as a serological marker of early infection (88).

New-generation techniques such as chemiluminescence immunoassays (ChLIAs), including chemiluminescence microparticle assays (CMIAs), have gone a step further and combine the use of a large mixture of recombinant antigens with powerful detection systems such as chemiluminescence (137, 138) (see Special Remarks Related to Congenital Infection, below). Finally, rapid diagnostic tests (RDTs) have been proposed as a good option for epidemiological surveillance and diagnosis in areas of endemicity that are difficult to access because they do not require a cold chain, work with finger-pricked whole blood, and provide fast results (139–141). The combination of at least two RDTs has been pointed out as a valid option for the conclusive diagnosis of chronic CD in these areas (142). These tests essentially rely on the detection of antibodies against different *T. cruzi* antigens and are based on different principles: immunochromatography, particle agglutination, immunofiltration, and immunodotting (143, 144).

TREATMENT INDICATION

Since the early 1970s, only two drugs have been approved and are available for human CD treatment, benznidazole and nifurtimox (145). Benznidazole is the most commonly used drug due to its better tolerability and efficiency than nifurtimox (75). However, both drugs require prolonged administration and can cause serious side effects, including allergies, dermatitis, pruritus, and gastrointestinal intolerance (146–148). This treatment has higher toxicity in adults, whereas it is generally well tolerated in children (149). Antiparasitic treatment for CD is recommended universally for acute cases of all ages, cases of reactivated infection, and chronically infected children up to 18 years of age (75). In the acute phase, treatment is efficient, with up to 80% cure rates, which increase to almost 100% in the first postnatal year (150). Although treatment in the chronic phase remains controversial (151), most experts believe that there is a potential benefit, particularly in young adults (152). Otherwise, treatment should not be given in advanced stages of cardiomyopathy (152). Treatment is not recommended during pregnancy due to the genotoxic properties of both drugs, and infected mothers should be treated after breastfeeding ends (5). Treating women of childbearing age in the chronic phase before they become pregnant prevents the transmission of *T. cruzi* to their offspring (153–155). Current clinical trials are focused on reducing regimens of benznidazole, new drugs, and drug combination strategies (156, 157).

EPIDEMIOLOGY

Latin America and the Global Situation

An estimated 6 million to 7 million people are infected with *T. cruzi* worldwide (3). The Americas, where CD originated, is the region most affected by the disease (2) (Table 1). CD is endemic in 21 Latin American countries, where it is estimated that around 6 million people are infected, with an annual incidence of 28,000 cases and 12,000 deaths (123). Bolivia is the country with the highest prevalence of CD (158). According to WHO estimations (159), 1,125,000 women of fertile age have CD in Latin America, with a transmission rate averaging 5% but with high differences between countries (160). Additionally, an estimated 8,000 to 15,000 babies are born with cCD every year (161).

In the last decades, globalization has brought a new dimension to CD. Human migration, both regional and international, has enabled the disease to expand, first from rural areas to cities within countries of endemicity and then from Latin American countries of endemicity to the rest of the world (162). The most affected countries outside Latin America are the United States, Canada, Europe, Australia, and Japan (163), which have approximately 26 million Latin American residents and an estimated 400,000 CD-infected individuals (164). However, as migrant health and neglected tropical diseases (NTDs) such as CD are not a priority for countries where the disease is not endemic, the number of cases is clearly underreported, reaching very high levels of underdiagnosis (94 to 96%), as indicated by Basile et al. (165). Colombo et al. (166) reported a CD prevalence of 4.2% in pregnant Latin American

TABLE 1 Comparative prevalence and number of people affected by Chagas disease in countries of endemicity (excluding the United States) according to 2005 and 2010 estimates[a]

	Prevalence per 100 habitants in yr		No. of infections in yr	
Country(ies)	2005	2010	2005	2010
Argentina	4.129	3.640	1,600,000	1,505,235
Belize	0.741	0.330	2,000	1,040
Bolivia	6.752	6.104	620,000	607,186
Brazil	1.019	0.03	1,900,000	1,156,821
Chile	0.985	0.699	160,200	119,660
Colombia	0.956	0.956	436,000	437,960
Costa Rica	0.532	0.169	23,000	7,667
Ecuador	1.739	1.379	230,000	199,872
El Salvador	3.372	1.297	232,000	90,222
Guatemala	1.984	1.230	250,000	166,667
Guyana, French Guyana, and Suriname	1.288	0.838	18,000	12,600
Honduras	3.053	0.917	220,000	73,333
Mexico	1.028	0.779	1,100,000	876,458
Nicaragua	1.140	0.552	58,600	29,300
Panama	0.006	0.515	21,000	18,337
Paraguay	2.543	2.130	150,000	184,639
Perú	0.686	0.439	192,000	127,282
Uruguay	0.656	0.237	21,700	7,852
Venezuela	1.159	0.710	310,000	193,339
Total	1.448	1.055	7,694,500	5,742,167

[a]See references 159 and 194.

women living in countries where the disease is not endemic and a vertical transmission rate of 3.5%. Bolivia is the country of endemicity most represented by migrants in countries of nonendemicity, especially in Europe, and therefore has the highest impact on cCD in settings of nonendemicity (167).

The United States and Canada

The United States is the main destination for Latin American migration and has approximately 20 million residents from countries where CD is endemic (168). According to estimates, in 2010, there were 240,000 to 350,000 people with CD living in the United States (76), with the states with the highest burdens being California, Texas, Florida, and New York (169). In strict terms, the United States cannot be considered an area where the disease is not endemic given that 11 species of triatomine bugs have been detected there, and autochthonous vector-borne *T. cruzi* transmission in humans has been reported (170). Such cases are rare and occur with a far lower incidence than chronic CD imported by migrants (76). However, triatomine species are becoming progressively more tolerant to modified habitats, and therefore, their contact with humans is increasing in both rural and urban environments (171). The geographical distribution of their vectors can also be affected by climate change, thus altering the epidemiological pattern of vector-borne diseases such as CD. Consequently, the incidence of CD could increase in the next decades and expand northward to areas of North America where the disease is currently not endemic, as pointed out by Garza et al. (172) in 2014. Congenital transmission has gained epidemiological relevance in the United States (173), where it is now an important concern. Around 40,000 women of childbearing age with chronic CD are living in the country (174, 175), and an estimated 60 to >600 babies are born with cCD in the United States each year (80, 173, 174). Canada also receives migration from Latin America although to a lesser extent (2). It was estimated that in 2015, around 100,000 Latin American migrants resided in Canada, of whom approximately 2,000 had CD (164).

Europe

Europe is estimated to have more than 4 million residents from countries where CD is endemic (176) and 68,000 to 123,000 individuals with CD (177). Countries of southern Europe, i.e., Spain, Italy, and Portugal, are the most affected by migration flows from Latin

America, probably due to the strong historical, cultural, and even religious ties existing between home and host countries (176); the other main recipient countries are Belgium, France, Germany, the Netherlands, Switzerland, and the United Kingdom (165, 178). The 2009 WHO report for CD control and prevention in Europe also included the following countries as having cases of infection: Austria, Croatia, Denmark, Germany, Romania, Sweden, and Northern Ireland (179). Regarding cCD, Basile et al. (165) estimated that 20 to 183 infected newborns are born each year in Europe.

Spain deserves special mention as the European country with the highest rate of migration from Latin America, being home to half of the continent's migrants from this part of the world (176). Approximately 52,000 individuals with CD are estimated to live in Spain (180). The second-largest recipient country in Europe of Latin American migrants is Italy, with around 600,000 residents (176, 181) and 5,000 to 7,000 estimated CD cases (164). The Portuguese Republic hosts approximately 160,000 migrants from countries where CD is endemic, mostly from Brazil (176), and according to the WHO, an estimated 850 individuals were infected with *T. cruzi* in 2009 (179). However, very little is known about the real situation of CD in Portugal (182, 183).

Western Pacific Region

In Japan, among a Latin American population of about 250,000, over 3,000 were estimated to be infected with *T. cruzi* in 2010 (71, 184). Regarding Australia, data from 2011 indicate that among approximately 80,000 Latin American residents, an estimated 1,928 were infected. Finally, in 2006, New Zealand hosted more than 6,000 people from countries of endemicity, with 82 being estimated to have CD (185).

CONTROL

In 2010, the WHO included CD in its list of NTDs (186), and more recently, the roadmap for NTDs, 2021 to 2030, adopted in the Seventy-Third World Health Assembly, targets CD for elimination as a public health problem (187). The management of NTDs has always been a challenge, especially in countries of nonendemicity, mainly because of the low visibility and awareness of the disease and the lack of specific guidance on diagnosis and treatment. The problem has worsened due to the redirection of resources to the fight against the COVID-19 pandemic (188). This, coupled with overworked health personnel and the traditionally difficult access of vulnerable groups to health care systems, may set back progress toward 2030 NTD targets (188, 189). Although it is not possible to foretell how long this situation will last or how deep its consequences will be, what is certain is that the pandemic will have long-term implications far beyond the health care field. Control efforts focus primarily on vector control and screening, as there is still no vaccine for CD. Indeed, the immunological complexity and long-term nature of CD greatly hamper progress toward a possible future vaccine against *T. cruzi* (190).

Vector Control

From 1991 to 2004, Latin American governments coordinated by the Pan American Health Organization (PAHO) and the WHO established four subregional initiatives to control the vector-borne and transfusional transmission of CD in countries where the disease is endemic (191, 192). Vector control includes spraying with insecticides in infested homes, improvements in houses to prevent vector infestation, and informative community education about CD and other vector-borne diseases (193). These intergovernmental control programs resulted in significant decreases in the prevalence and burden of CD (159, 194) (Table 1). Some countries belong to multiple initiatives due to their geographical situation and/or dimension, as is the case for Bolivia, Brazil, Colombia, Ecuador, Peru, and Venezuela.

The Southern Cone Initiative. The Southern Cone Initiative (INCOSUR) was created in 1991 when the governments of Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay came together to eliminate the main vector, *T. infestans*, from domestic and peridomestic areas and to introduce large-scale screening of blood donors (195, 196). Consequently, the elimination of *T. infestans* was certified in Uruguay (1997), Chile (1999), Brazil (2006), Paraguay

(intradomiciliary transmission) (2018) (197), eight provinces of Argentina (2001 to 2013), and the Departments of La Paz and Potosí of Bolivia (2011 to 2013) (193).

The Andean Initiative. The Andean Initiative (IPA) was created in 1997 by the governments of Colombia, Ecuador, Peru, and Venezuela (191). It represents an area of high complexity because of its ecological diversity and the heterogenicity of triatomine species (196). The most important domestic vectors are *Rhodnius prolixus* in Colombia and Venezuela and *T. dimidiata* and *T. infestans* in Ecuador and Peru, respectively (196). The interruption of the vectorial transmission of CD by *T. infestans* was certified in the Departments of Tacna and Moquegua of Peru, and that by *R. prolixus* was certified in 10 municipalities in the Departments of Casanare, Boyacá, Santander, and Arauca of Colombia (2013) (193).

The Central America and Mexico Initiative. The Central America and Mexico Initiative (IPCAM) was created in 1997 after an agreement between the governments of Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, and Panama (191). After Mexico joined the initiative in 2012, the acronym was changed from IPCA to IPCAM (198). The objectives imposed were to interrupt and reduce transmissions by *R. prolixus* and *T. dimidiata*, respectively, and to interrupt transmission through blood transfusions (196). Vectorial transmission by *R. prolixus* was successfully halted in Guatemala (2008), El Salvador (2010), Honduras (2010), Nicaragua (2010), Costa Rica (2011) (193, 196, 199), Belize (2012) (200), as well as Chiapas and Oaxaca in Mexico (193).

The Amazon Initiative. Created in 2004, the Amazon Initiative (AMCHA) involves Bolivia, Brazil, Colombia, Ecuador, Guyana, French Guyana, Peru, Suriname, and Venezuela (191). This surveillance and prevention network is mainly focused on foodborne outbreaks (193).

Blood Transfusion and Organ Transplant Control

Latin America. In the 1980s, with the onset of the HIV pandemic, blood control programs began to be implemented in most Latin American countries (38, 201). However, it was not until 1991, with the implementation of INCOSUR and subsequent control programs, that serological blood screening progressively expanded to the whole of Latin America, now being mandatory in all countries of endemicity (38, 202), with Mexico being the most recent country to join the system. Consequently, the risk of infection by transfusion has been dramatically reduced (38, 203).

Organ transplantation in individuals with chronic CD as well as the use of organs from infected donors have been a controversial issue in areas where the disease is endemic (42). Screening for *T. cruzi* in solid-organ transplantation is extremely important in countries of endemicity due to the high prevalence of CD and its ease of transmissibility. Countries that perform heart transplants (e.g., Argentina, Brazil, Colombia, Chile, Ecuador, Mexico, Paraguay, Peru, and Uruguay) have a particularly high risk of transmission (43) and routinely conduct pretransplant evaluation of donors and recipients by serotesting (82). In 2010, the Chagas disease Argentine Collaborative Transplant Consortium (42) issued the following series of guidelines: (i) Latin American transplant candidates should be tested for *T. cruzi* infection by two serological assays, and in the case of conflicting results, a third test is needed; (ii) infected candidates with proven parasitemia may receive trypanocidal treatment before transplantation; (iii) all infected transplant recipients should be monitored for reactivation by parasite identification in the bloodstream, preferably by a Strout test or PCR, and receive treatment if necessary; (iv) infected donors should receive trypanocide treatment for 30 days prior to the donation; (v) organs from deceased infected donors should not be accepted for heart transplantation; and (vi) all uninfected recipients of organs from infected donors need to be strictly monitored (and treated) in the same way as infected recipients. Furthermore, Latin American recommendations for the management of endemic diseases and travel medicine in solid-organ transplant recipients and donors published in 2018 (204) and PAHO recommendations in 2021 (205) support the use of kidneys and livers from chronically infected donors and contraindicate the use of hearts and intestines as well as organs from donors with acute infection.

The United States and Canada. In 2007, universal blood donation screening for *T. cruzi* antibodies was implemented in the United States (132, 206) after the Food and Drug Administration (FDA) licensed the Ortho *T. cruzi* ELISA system (Ortho-Clinical Diagnostics,

Inc., Raritan, NJ) for anti-*T. cruzi* antibodies in blood, tissue, and organ donors (36, 207). The confirmatory test used was a RIPA. Subsequently, in 2010, the FDA approved the ChLIA Abbott Prism Chagas assay (Abbott Diagnostics, Abbott Park, IL), and in 2011, the Abbott ESA Chagas assay (Abbott Diagnostics) was licensed as a confirmatory test for positive results in the first screening (207). The FDA also approved the use of the CMIA Alinity s Chagas assay (Abbott Diagnostics) to screen blood and organ donors (208).

Blood donor screening has become a useful tool to identify autochthonous cases among *T. cruzi*-infected blood donors who have not lived outside the United States (209). In 2010, universal screening was replaced by a one-time serological testing approach for all donors (210, 211). In 2011, the Chagas in Transplant Working Group in the United States recommended serological screening of organ donors and recipients who have lived in countries where CD is endemic and advised against heart transplantation from *T. cruzi*-infected donors because of the high risk of transmission (75% or higher) (212, 213). They also contraindicated the transplantation of organs from donors with acute CD and recommended close monitoring by PCR and microscopy of recipients of organs from chronically infected donors (43, 212). In Canada, in 2010, the Canadian Blood Services began selective antibody testing of all at-risk donors, previously identified by a questionnaire (implemented in 2009) (214).

Europe. Most European countries follow European directives 2004/33/CE and 2006/17/CE (215, 216) on the donation and control of human blood, tissues, and cells (217). This legislation refers specifically to CD, recommending the screening of at-risk donors based on a questionnaire of their history and travels. However, several countries also have their own legislation. In the United Kingdom, Spain (RD1088/2005) (218), France, Switzerland, Portugal, and Italy, donors at risk of *T. cruzi* infection are serologically tested, whereas in Sweden, at-risk donors are systematically excluded from donation (217, 219).

In Europe, the CE mark is the official marking required by the European community for all *in vitro* diagnostic (IVD) devices. The marking indicates that the IVD device complies with directive 98/79/EC (220) and is legally commercialized. Europe is currently in a transitional period from IVD directive 98/79/EC (220) to IVD regulation 2017/746 (221), which will be applied in May 2022, with more stringent regulatory requirements. Thus, any commercially available test used for diagnostic/screening purposes in Europe needs the CE IVD mark in compliance with European legislation.

European Parliament directive 2010/45/EU (222) on the quality and safety of human organs for transplantation does not specifically address CD. However, national transplant organizations of individual countries such as Italy, Spain, and the United Kingdom have official recommendations concerning *T. cruzi* infection and routinely screen organ donors (217, 223). In the specific case of Spain, recommendations in place are very similar to those issued by the U.S. Chagas in Transplant Working Group (224, 225). However, in addition to heart transplants from donors with chronic CD, intestinal transplants are also contraindicated (225). Spanish official recommendations also indicate screening for *T. cruzi* infection in umbilical cord blood donations (226).

Western Pacific region. In 2013, Japan's Ministry of Health and the Japanese Red Cross Society implemented selective serological screening for blood donors at risk of *T. cruzi* infection, which were permanently deferred (38, 210). In Australia, selective testing of blood donors based on a risk questionnaire is carried out (38). Similarly, in New Zealand, blood donors are screened with a questionnaire every time they donate blood, but routine testing is not performed (185).

Congenital Transmission Control

Although real progress has been achieved in CD prevention, control programs in countries where the disease is endemic have been based mainly on vector elimination and blood bank control (199). As most cCD cases are asymptomatic, they may go undetected and progress to chronic CD later in life, which, in turn, can lead to new congenital infections (53). Treating women of childbearing age and infected children would help to break the cycle (15, 155). Thus, early diagnosis and treatment of cCD, so-called tertiary prevention, are essential in managing the disease and should be included in newborn screening programs (80).

General recommendations for congenital Chagas disease screening. In general terms, the first criteria for cCD diagnosis were published by the WHO in 2002, recommending the screening of both at-risk pregnant women and newborns (89). The term "at-risk women" refers to those who were born, live, or have lived in countries where CD is endemic or are daughters of Latin American mothers (5). In brief, the mother receives a conventional serological test (IHA, IIF, or ELISA), which if positive should be confirmed by another conventional test; conflicting results require a third test, either conventional or nonconventional (see "Serological Diagnosis," above). In children of seropositive mothers, conventional IgG tests are recommended 8 months after birth, and parasitological testing can be conducted at any time. Finally, the 2002 WHO guide includes a PCR assay in the diagnostic algorithm but without specifying a particular time for testing and advocating its use only in specialized laboratories (89).

In 2018, the PAHO (123) reported new guidelines in Spanish for CD diagnosis and treatment (translated into English in 2019) but maintained the old diagnostic standard previously proposed by the WHO (227). It thus specified (i) two positive serological tests (IHA, IIF, or ELISA), rather than a single isolated test, and potentially a third test in the case of discrepant results for at-risk women and (ii) direct parasitological tests (microhematocrit or direct observation) and subsequent serological follow-up (IHA, IIF, or ELISA) for children of CD-infected mothers, starting at 8 months of age. The cCD diagnostic guide makes no reference to PCR (228).

The most recent updates in the field are from 2019, when the WHO technical group reported their recommendations for cCD management (5) in which they still recommend an IFA, IIF assay, or ELISA as the test of choice for CD detection in women but with some important highlights. They specifically indicate that tests can be based on crude or recombinant antigens. In the case of discrepancies, serology should be repeated in a new sample; if results remain inconclusive, WB is proposed as the tiebreaker test. Another key difference from the previous WHO criteria is the postponement of serological testing of infants until 10 months of age due to the high sensitivity of new techniques (see Special Remarks Related to Congenital Infection, below) (229). The report highlights parasitological tests as the gold standard (microhematocrit or microstrout methods), indicating that molecular methods need more validation to be considered as such (5).

In addition, the information about cCD available on the CDC website (last update in June 2021) includes a diagnostic algorithm for CD in pregnant women and two other algorithms to diagnose cCD in infants $<$3 months and $\geq$3 months of age (175, 230). In this case, infant diagnosis relies on parasite detection at birth (repeated 4 to 6 weeks later in the case of negative results) by microscopic examination and/or a PCR assay for blood samples and serological testing with commercially available assays at 9 to 12 months of age (230).

The strategies outlined by each health organization differ considerably, which reflects the lack of a consensus (141). Moreover, as they are only general recommendations, each ministry of health or regional department of health applies its own criteria, resulting in a multitude of different approaches for the diagnosis of the same disease. More specifically, following the progress in controlling the two main pathways of infection (vectors and blood transfusion), strategies to detect and reduce mother-to-child transmission have been implemented (20, 231). In 2017, the PAHO member states included CD in an initiative entitled *EMTCT Plus*: *Framework for Elimination of Mother-to-Child Transmission of HIV*, *Syphilis*, *Hepatitis B*, *and Chagas*, taking on the challenge of eliminating the congenital transmission of these preventable communicable diseases in the Americas (3). The specific Chagas-related goals were to increase the testing of pregnant women and neonates with seropositive mothers and to increase the rate of treatment of seropositive mothers to 90% or higher (3). Within the same framework of the EMTCT Plus initiative and the WHO roadmap for NTDs from 2021 to 2030 (187), the Ibero-American program for cCD *Ningún Bebé con Chagas: Hacia Nuevas Generaciones Libres de Chagas* was approved at the XXVII Ibero-American Summit held in Andorra in 2020 (232). The aims of this initiative are to work toward the elimination of vertical transmission by promoting close collaboration among countries and to extend it to countries where the disease is not endemic.

TABLE 2 Guidelines for congenital Chagas disease prevention and control implemented in Latin America by country[b]

Country	Type of document	Title of publication	Yr (reference)	Type of guideline[a]
Argentina	Official guide	*Enfermedad de Chagas. Guía Para la Atención al Paciente Infectado con* Trypanosoma cruzi	2018 (233)	Diagnostic algorithm for newborns
		Prevención, Diagnóstico y Tratamiento de la Transmisión Vertical del T. cruzi	2021 (234)	
Bolivia	Official guide	*Manual de Normas para el Diagnóstico y Tratamiento de Chagas Congénito*	2011 (235)	Diagnostic algorithm for newborns
Brazil	Consensus document	*2nd Brazilian Consensus on Chagas Disease, 2015*	2016 (236)	Diagnostic algorithm for newborns
Chile	Official guide	*Norma General Técnica. Control y Prevención Nacional de la Enfermedad de Chagas*	2014 (237)	Diagnostic algorithm for newborns
		Manual de Procedimiento para la Atención de Pacientes con Enfermedad de Chagas	2017 (238)	
Colombia	Official guide	*Guía para la Vigilancia por Laboratorio del* Trypanosoma cruzi	2017 (239)	Diagnostic algorithm for newborns
Costa Rica	Official guide	*Norma de Atención Integral de la Enfermedad de Chagas*	2012 (240)	Diagnostic algorithm for newborns
Ecuador	Official guide	*Manual de Vigilancia y Control de la Enfermedad de Chagas en el Ecuador*	2020 (241)	Recommendation of antenatal screening
El Salvador	Official guide	*Lineamientos Técnicos del Sistema Nacional de Vigilancia Epidemiológica en el Salvador (VIGEPES)*	2019 (242)	Diagnostic algorithm for newborns
Guatemala	Official guide	*Protocolos de Vigilancia Epidemiológica. Enfermedades Vectoriales de Origen Parasitario*	2018 (243)	Diagnostic algorithm for newborns
Honduras	Official guide	*Manual de Normas y Procedimientos para la Prevención y Control de la Enfermedad de Chagas*	2006 (244)	Recommendation of antenatal screening
Mexico	Official guide	*Manual de Procedimientos para la Enfermedad de Chagas en México*	2019 (245)	Diagnostic algorithm for newborns
Nicaragua	Official guide	*Manual de Procedimientos para el Abordaje de la Prevención, Control y Atención de la Enfermedad de Chagas (Tripanosomiasis Americana)*	2013 (246)	Diagnostic algorithm for newborns
Panama	Official guide	*Guía para el Abordaje Integral de la Enfermedad de Chagas en la República de Panamá*	2012 (247)	Diagnostic algorithm for newborns
Paraguay	Official guide	*Guía de Manejo de Pacientes Adultos con Enfermedad de Chagas*	2021 (248)	Diagnostic algorithm for newborns
		Guía Práctica para el Manejo de Transmisión Congénita de Chagas y Chagas Crónico Reciente Infantil	2021 (249)	
Peru	Official guide	*Manual de Procedimientos de Laboratorio para el Diagnóstico de la Trypanosomiosis Americana (Enfermedad de Chagas)*	2005 (250)	Diagnostic algorithm for newborns
Uruguay	Technical report	*Enfermedad de Chagas*	2012 (251)	Diagnostic algorithm for newborns
Venezuela	Official guide	*Guía para el Diagnóstico, Atención y Manejo Clínico de la Enfermedad de Chagas en Venezuela*	2014 (252)	Diagnostic algorithm for newborns

[a]For a diagnostic algorithm for newborns, the guide includes a specific algorithm to diagnose congenital Chagas disease. Diagnostic algorithms are detailed in Fig. 2. For a recommendation of antenatal screening, the guide does not include a specific diagnostic algorithm for congenital CD.
[b]Countries are listed alphabetically. Data were not found for Belize, French Guyana, Guyana, and Suriname.

In the following sections, we provide a more detailed description of the country-specific guidelines for the detection of cCD.

Latin America. Despite the recent publication of guidelines for cCD diagnosis and treatment by the PAHO (123), the strategies adopted in Latin America vary by country (Table 2 and Fig. 1) (233–252). No documents related to cCD prevention and control could be found for Belize, French Guyana, Guyana, and Suriname. In the case of Paraguay, a new guide for cCD management and control was recently issued by the Ministry of Health (249). However, a universal system for CD diagnosis during prenatal monitoring was already being implemented in certain areas of the country, such as the Cordillera and Paraguarí Departments (253). Fifteen out of the 21 countries where CD is endemic incorporate diagnostic algorithms in their protocols, which are detailed in Fig. 2.

The United States. Although there is no maternal screening policy for CD in the United States (254), the U.S. Diagnostic Working Group recently developed and reported recommendations for the screening and diagnosis of CD in the United States (255), in which the

FIG 1 Latin American policies to control congenital Chagas disease (cCD). Countries in blue recommend antenatal screening, but their guides do not include a specific diagnostic algorithm. Countries in green have an official guide that includes a diagnostic algorithm for cCD. Diagnostic algorithms are detailed in Fig. 2. No data were found for countries in gray.

following specifications regarding cCD were made. First, women of childbearing age who have lived in a region of Mexico or South or Central America where CD is endemic should be screened for *T. cruzi* infection, with positive results by at least two tests to confirm the diagnosis being necessary. There are currently four IgG-based serological assays cleared by the FDA for the diagnosis of chronic CD, three ELISAs, the Ortho *T. cruzi* ELISA system (also licensed for blood donor screening [see "Blood Transfusion and Organ Transplant Control," above]), the Hemagen Chagas' kit (Hemagen Diagnostics, Inc., Columbia, MD), and the Wiener Chagatest Recombinante v3.0 ELISA (Wiener Laboratories, Rosario, Argentina), and a rapid immunochromatographic strip test, the InBios Chagas Detect Plus rapid test (InBios International, Seattle, WA) (76, 255, 256). Most commercial laboratories perform a single assay, and positive samples are then forwarded to the CDC in Atlanta, GA, for confirmation (255). Second, infants in whom congenital CD is suspected should undergo evaluation using an existing CDC-based algorithm (see "General recommendations for congenital Chagas disease screening," above) (175, 230).

Europe. Congenital CD screening programs are rare outside Latin America and restricted to Europe. To our knowledge, these programs are implemented in only a few regions of Spain, Italy, and Switzerland (178, 257). After a detailed search, we could find only four European regions with an official screening protocol including a specific algorithm to diagnose cCD (Fig. 3). There are also local initiatives promoted by health centers, but no official guide at a national or European level has been published (223, 258).

Spain has a territorial organization based on regions, dividing the country into 17 autonomous communities (Andalusia, Aragon, Balearic Islands, Basque Country, Canary Islands, Cantabria, Castile and Leon, Castile-La Mancha, Catalonia, Chartered Community of Navarre, Community of Madrid, Extremadura, Galicia, La Rioja, Principality of Asturias, Region of Murcia, and Valencian Community) and 2 autonomous cities (Ceuta and Melilla). Each region has its own health authority, so in the absence of a plan at the state level, cCD

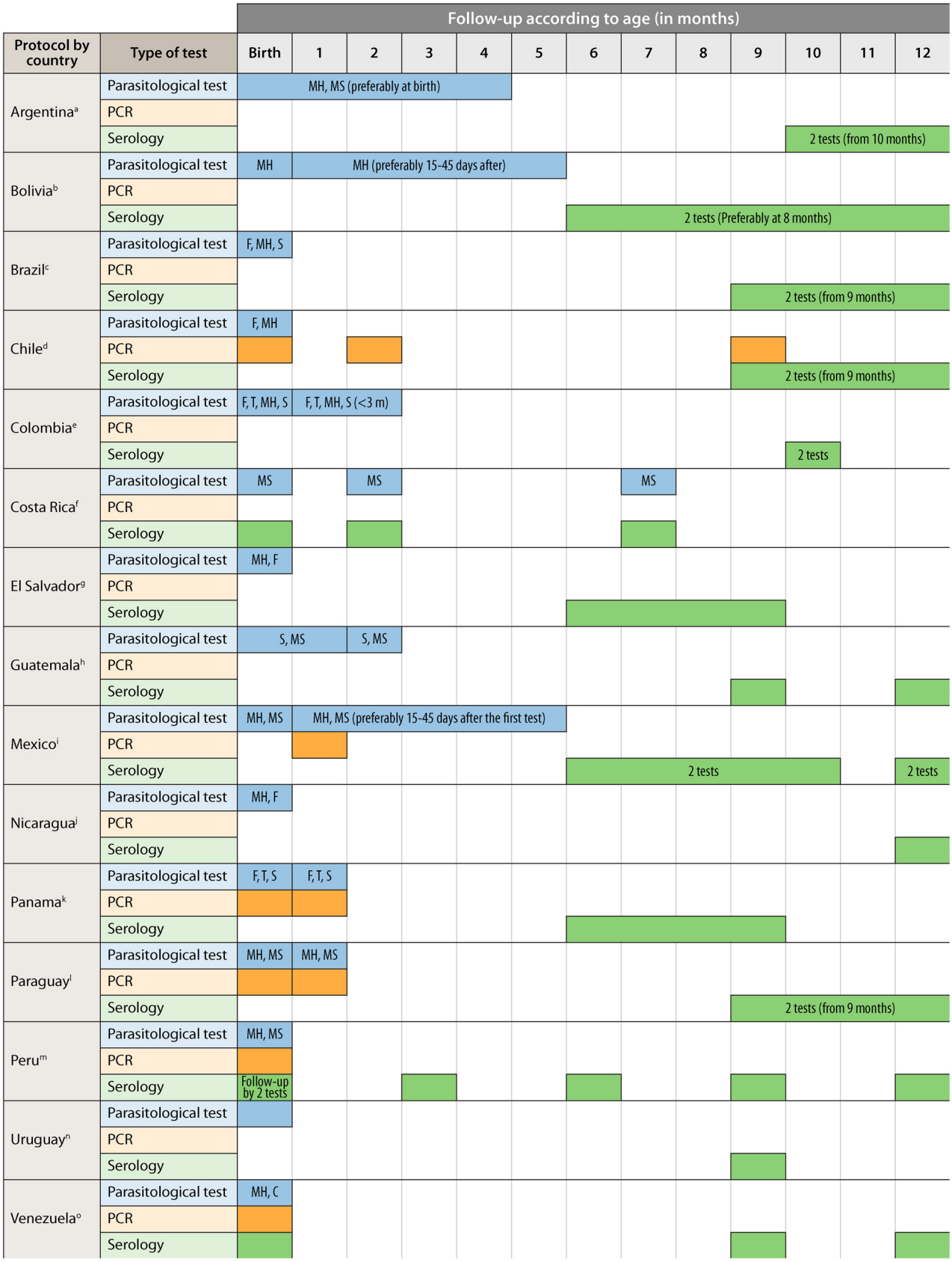

FIG 2 Timeline of the methodologies used in the algorithms for the diagnosis of congenital Chagas disease implemented in Latin America. Blue, parasitological tests; green, serological tests; orange, molecular diagnosis. MH, microhematocrit; S, Strout; F, fresh-blood observation; T, thin and/or

(Continued on next page)

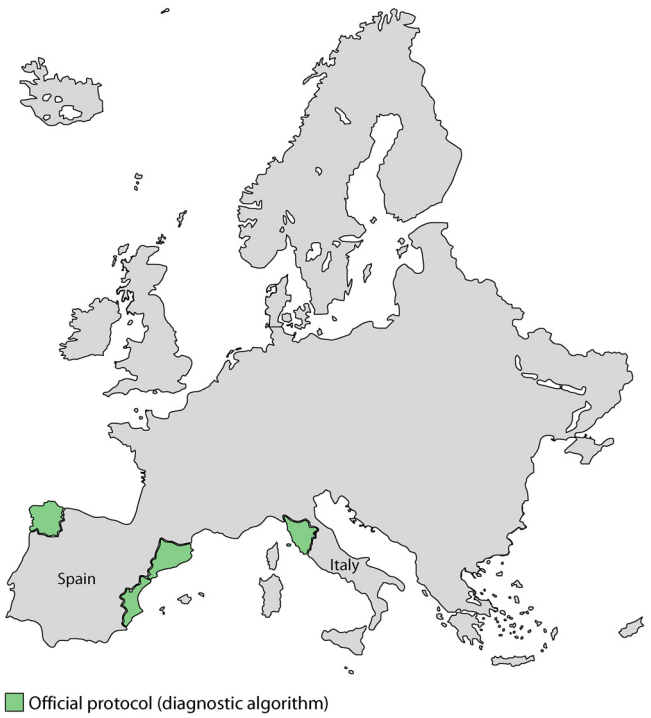

FIG 3 European regions with an official screening protocol including a specific algorithm to diagnose congenital Chagas disease. Diagnostic algorithms are included in Fig. 5.

management differs significantly across the country (Table 3 and Fig. 4) (259–273). Briefly, antenatal screening is recommended in most regions but only the guidelines of the Valencian Community, Catalonia, and Galicia include an algorithm for cCD diagnosis (Fig. 5). Diagnostic schemes are also available for the Community of Madrid and Extremadura, but the former is an unofficial initiative, and the latter refers to an algorithm already published (274) (Fig. 5). In the region of Murcia, besides the guide listed in Table 3, a study published

FIG 2 Legend (Continued)
thick blood smears; MS, microstrout; C, culture; m, months. [a]For Argentina (233, 234), serological techniques that can be used are an enzyme-linked immunosorbent assay (ELISA), an indirect immunofluorescence (IIF) assay, an indirect hemagglutination assay (IHA), and particle agglutination. [b]For Bolivia (235), umbilical cord blood can be used for parasitological testing. Serological techniques that can be used are an ELISA, an IIF assay, and an IHA. A positive result must be confirmed by an ELISA or IHA. In the case of a weak positive result when using the IHA, repeat testing should be performed 3 months later. [c]For Brazil (236), parasitological analysis should be repeated 1 week after birth. Serological techniques that can be used are an ELISA, IIF, and IHA. Serological confirmation requires coincident positive results by two tests based on different principles. [d]For Chile (237, 238), both parasitological testing and PCR should be carried out at birth. Two positive PCRs are needed to confirm the infection. In the case of a positive result by PCR at 2 months, a new sample should be requested immediately instead of waiting until 9 months. A positive PCR result at 9 months needs to be confirmed by a detectable level of antibodies by serology. Serological techniques that can be used are an ELISA, IIF, IHA, and Western blotting (WB). [e]For Colombia (239), serological techniques that can be used are an ELISA, an IIF assay, an IHA, WB, and a chemiluminescence immunoassay (ChLIA). Serological confirmation requires coincident positive results by two tests based on different principles. [f]For Costa Rica (240), it is not clear which is the serological technique (or techniques) of choice. A positive serological result at birth and/or at 3 months without a positive parasitological result requires serological and/or parasitological confirmation at 7 months. [g]For El Salvador (242), serological techniques that can be used are an ELISA, IIF, IHA. [h]For Guatemala (243), serological follow-up continues until 18 months of age. The ELISA is indicated as the serological technique of choice. [i]For Mexico (245), umbilical cord blood can be used for parasitological testing. According to the text, serological control should be conducted from 6 to 10 months of age, whereas the diagnostic algorithm in the same guide recommends serology at 12 months. Similarly, the text indicates the potential diagnostic use of PCR 1 month after birth, but it does not appear in the diagnostic algorithm. Serological techniques that can be used are an ELISA, IIF, and IHA. Serological confirmation requires coincident positive results by two tests based on different principles. [j]For Nicaragua (246), a positive parasitological result at birth requires confirmation by a second parasitological test. Umbilical cord blood can be used for parasitological testing. Serological confirmation requires coincident positive results by two ELISAs: first, a conventional ELISA and, second, an ELISA based on recombinant antigens. [k]For Panama (247), at birth, parasitological testing and/or PCR can be carried out. In the case of negative results at birth, repeat testing every week during 1 month should be performed. [l]For Paraguay (249), at birth and 1 month afterward, parasitological testing and/or PCR can be carried out. Umbilical cord blood can be used for parasitological and molecular testing. It is not clear which is the serological technique (or techniques) of choice. Serological techniques that can be used are an ELISA, IHA, and ChLIA. [m]For Peru (250), parasitological testing or PCR can be carried out at birth. Serological follow-up by an ELISA and an IIF assay is carried out only in cases of a positive result at birth in order to compare IgG titers. [n]For Uruguay (251), parasitological and serological tests that can be used are not specified. [o]For Venezuela (252), parasitological testing and PCR (if possible) can be carried out at birth. Umbilical cord blood is used for parasitological testing. Serological testing by IgG and IgM determination can be performed. Serological techniques that can be used are an ELISA, IIF, IHA, and direct agglutination. Serological confirmation requires coincident positive results by two tests.

TABLE 3 Guidelines for congenital Chagas disease prevention and control implemented in Spain by region[b]

Region	Type of document	Title of publication	Yr (reference)	Type of guideline[a]
Andalusia	Official regional guide	*Documento de Salud de la Embarazada*	2020 (259)	Recommendation of antenatal screening
Balearic Islands	Official regional guide	*Guía de Buenas Prácticas en la Atención del Embarazo, del Puerperio y del Periodo Neonatal en el Área de Salud Maternoinfantil de las Islas Baleares*	2017 (first version in 2014) (260)	Recommendation of antenatal screening
Basque Country	Official regional guide	*Recomendaciones para la Asistencia Médica al Adulto Inmigrante. Plan Vasco de Inmigración*	2008 (261)	Recommendation of antenatal screening
		Recomendaciones para la Asistencia Médica al Niño Inmigrante. Plan Vasco de Inmigración	2008 (262)	
Canary Islands	Official regional guide	*Embarazo. Programa de Atención a la Salud Afecto-Sexual y Reproductiva* (*PASAR*)	2018 (263)	Recommendation of antenatal screening
Cantabria	Official regional guide	*Protocolo de Atención al Embarazo y Puerperio*	2017 (first version in 2007) (264)	Recommendation of antenatal screening
Castile-La Mancha	Official regional guide	*Proceso Asistencial Integrado*: *Atención al Embarazo Normal y Puerperio*	2015 (265)	Recommendation of antenatal screening
Catalonia	Official regional guide	*Protocolo de Cribado, Diagnóstico y Tratamiento de la Enfermedad de Chagas en Mujeres Embarazadas Latinoamericanas y en Sus Hijos*	2018 (first version in 2010) (266)	Diagnostic algorithm for newborns
Community of Madrid	Unofficial initiative/ consensus document	*Control de la Infección por* Trypanosoma cruzi*/ Enfermedad de Chagas en Gestantes Latinoamericanas y Sus Hijos*	2013 (267)	Diagnostic algorithm for newborns
Extremadura	Official regional technical document	*Protocolo de Vigilancia Epidemiológica de la Infección por* Trypanosoma cruzi*/Enfermedad de Chagas*	2019 (268)	Diagnostic algorithm for newborns[c]
Galicia	Official regional guide	*Protocolo de Cribado da Enfermidade de Chagas en Mulleres Embarazadas*	2014 (first version in 2012) (269)	Diagnostic algorithm for newborns
Principality of Asturias	Official regional technical document	*Cribado Prenatal de Enfermedad de Chagas. Memoria Técnica*	2017 (270)	Recommendation of antenatal screening
Region of Murcia	Official regional guide	*Programa Integral de Atención a la Mujer de la Región de Murcia* (*PIAM*)	2012 (271)	Recommendation of antenatal screening
Valencian Community	Official regional guide	*Enfermedad de Chagas Importada. Protocolo de Actuación en la Comunitat Valenciana*	2009 (272)	Diagnostic algorithm for newborns
Tuscany, Italy	Official regional guide	*Programma Regionale per la Prevenzione e il Controllo della Malattia di Chagas Congenita*: *Indicazioni per l'Assistenza in Gravidanza*	2012 (273)	Diagnostic algorithm for newborns

[a]For a diagnostic algorithm for newborns, the guide includes a specific algorithm to diagnose congenital Chagas disease. Diagnostic algorithms are detailed in Fig. 5. For a recommendation of antenatal screening, the guide does not include a specific diagnostic algorithm for congenital CD.
[b]Spanish regions are listed alphabetically. Data were not found for Castile and Leon, Ceuta Autonomous City, Chartered Community of Navarre, La Rioja, and Melilla Autonomous City. Aragon follows the recommendations of the Spanish Society of Gynecology and Obstetrics (278).
[c]Algorithm previously reported by the Working Group on Chagas Disease of the Community of Madrid (274).

in 2017 proposes an algorithm to diagnose cCD in a local hospital (155). In addition, in April 2021, the Murcian Health Service announced the imminent implementation of systematic screening for CD in all pregnant women (275). In the case of Castile and Leon, antenatal screening for CD is recommended in the area of Salamanca (276). In the rest of Spain, screening tests are also generally carried out in pregnant women and migrants at risk of CD but only in accordance with the decision and criteria of individual health professionals. The Spanish guide for care in pregnancy (277) and the Spanish Society of Gynecology and Obstetrics (278) recommend screening for *T. cruzi* infection in pregnant Latin American women. It should be mentioned that the regulations for cCD control in Spain are currently being revised with the aim of establishing a national plan in the coming years.

Italy is divided into 20 regions (Abruzzo, Basilicata, Calabria, Campania, Emilia-Romagna, Friuli-Venezia Giulia, Lazio, Liguria, Lombardy, Marche, Molise, Piedmont, Puglia, Sardinia, Sicily, Tuscany, Trentino-Alto Adige, Umbria, Valle d'Aosta, and Veneto). To date, Tuscany is the only Italian region that implements a cCD control program, in place since 2012 (Table 3 and Fig. 5), which has also been taken up by the province of Bergamo (Lombardy region, northern Italy) (279, 280). Bergamo province is home to the largest Bolivian community in Italy, which consists of over 10,000 individuals and represents approximately 40% of all Bolivians in the country (64). Serological screening for *T. cruzi* in pregnant women has also been implemented at

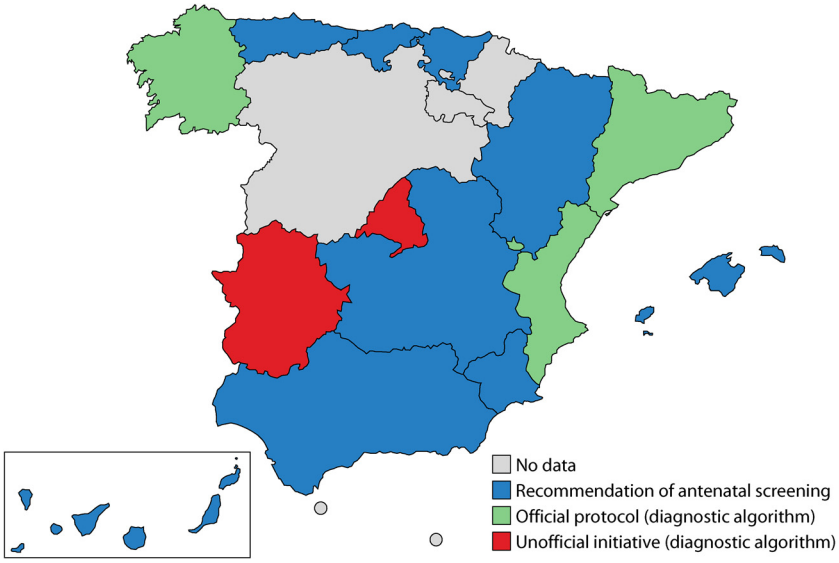

FIG 4 Spanish policies to control congenital Chagas disease (cCD). Regions in blue recommend antenatal screening, but their guides do not include a specific diagnostic algorithm. Aragon follows the recommendations of the Spanish Society of Gynecology and Obstetrics (278). Regions in green have an official guide that includes a diagnostic algorithm for cCD. Regions in red have an unofficial initiative that includes a diagnostic algorithm for cCD. All diagnostic algorithms are detailed in Fig. 5. In the case of Extremadura, antenatal screening for CD is also officially recommended, but the technical document refers to a previously published algorithm (274). No data were found for regions in gray. In the case of Castile and Leon, antenatal screening for CD is recommended in the area of Salamanca (276).

an institutional level in Negrar (region of Veneto), Rome (region of Lazio), and Bologna (Emilia-Romagna region) (181, 281).

In Switzerland, 2,000 to 4,000 people were estimated to have CD in 2021 (282). Although migration from Latin America is growing (283, 284), there is still no national health policy to control cCD in this country (178). Switzerland is a federal republic composed of 26 cantons, only 2 of which, Geneva and Vaud, have implemented cCD prevention programs; these are managed locally in two university hospitals in the cities of Geneva and Lausanne (63, 217, 282, 285). In some other European countries such as Portugal and Germany, local initiatives promoted and applied in individual hospitals are progressively being implemented to detect infected women and screen their newborns (217, 223). Studies on the need for cCD screening strategies have also been reported (e.g., by Carlier [286] in Belgium, Lescure et al. [287] and Brutus et al. [288] in France, Guggenbühl Noller et al. [289] in Germany, Bart et al. [290] in the Netherlands, and Fernandez Turienzo et al. [291] and González Sanz et al. [292] in the United Kingdom).

SPECIAL REMARKS RELATED TO CONGENITAL INFECTION

The primary mechanisms of *T. cruzi* transmission to humans are vectors, blood transfusion, and the mother-to-child route (293). In countries where the disease is endemic, efforts to control CD have focused mainly on the two first pathways, leaving vertical transmission to become an issue of growing concern (294, 295). Indeed, in the absence of a vector, congenital infection represents the main route of transmission, both in countries of endemicity with a certified interruption of vector transmission and in areas where the disease is not endemic (294). Legislation concerning blood transfusion and solid-organ transplantation follows a fairly similar pattern in most countries. Briefly, at-risk blood and organ donors and organ recipients need to be serologically tested. Positive serology in potential blood donors is exclusive, whereas organ transplants from *T. cruzi*-infected patients can generally be carried out after assessing the risk-benefit balance and with proper monitoring; the exceptions are organs from patients in the acute phase of CD and heart and intestinal transplants (43, 225). In contrast, health policies for cCD control differ substantially from one country to another and even

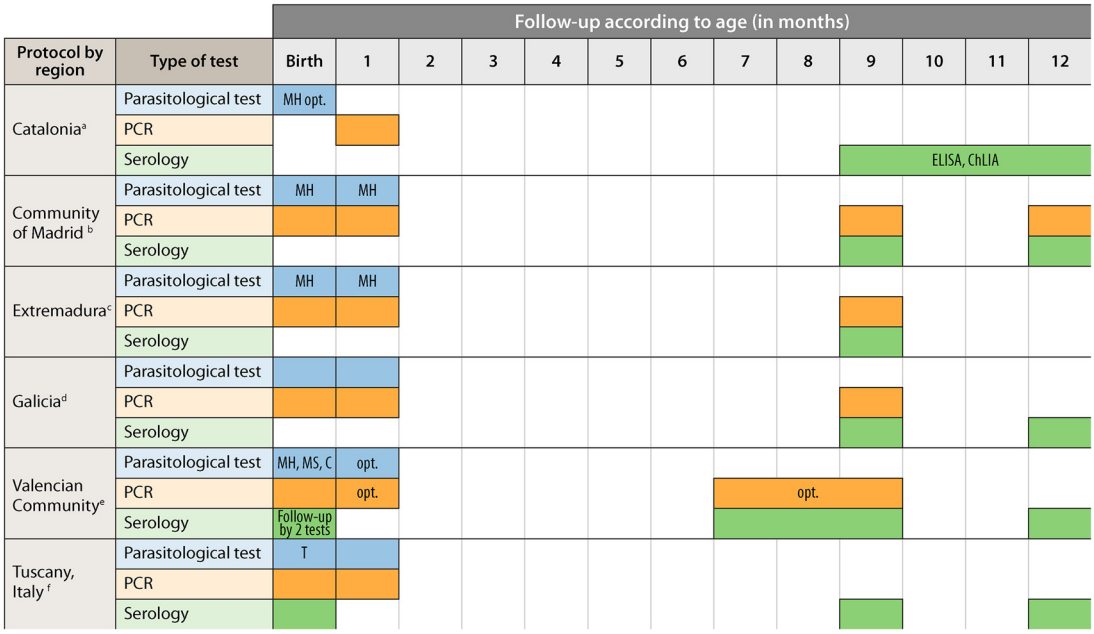

FIG 5 Timeline of the methodologies used in the algorithms for the diagnosis of congenital Chagas disease implemented in Spain and the Italian region of Tuscany. Blue, parasitological tests; green, serological tests; orange, molecular diagnosis. Opt., optional; MH, microhematocrit; MS, microstrout; C, culture; T, thin and/or thick blood smears; ELISA, enzyme-linked immunosorbent assay; ChLIA, chemiluminescence immunoassay. [a]For Catalonia (266), microhematocrit is optional and recommended only for centers with experience in the technique. In the case of a doubtful PCR result, repeat testing should be performed. In the case of a positive serological result, repeat testing with another technique based on a different principle should be performed. If the second test is negative or with a value close to the cutoff, repeat testing with the same technique 2 months afterward should be performed. [b]For the Community of Madrid (267), both parasitological testing and PCR must be carried out at birth and 1 month afterward. A positive PCR result at birth in a sample of umbilical cord blood must be confirmed by another PCR in the peripheral blood of the newborn. Repeat serology should be performed at 12 months only in cases of a positive result at 9 months in order to compare IgG titers. It is not clear which is the serological technique (or techniques) of choice. [c]For Extremadura (268), both parasitological testing and PCR must be carried out at birth and 1 month afterward. It is not clear which is the serological technique (or techniques) of choice. [d]For Galicia (269), both parasitological testing and PCR must be carried out at birth and 1 month afterward. Repeat serology at 12 months should be performed only in the case of a positive result at 9 months in order to compare IgG titers. It is not clear which is the serological technique (or techniques) of choice. [e]For the Valencian Community (272), both parasitological testing and PCR must be carried out at birth and 1 month afterward. Umbilical cord blood can be used for parasitological and molecular testing. Serological testing at birth is performed by IgG and IgM determination. Repeat serology at 12 months should be performed only in the case of a positive result at 7 to 9 months with a reduction of IgG titers compared to those at birth. An increase of IgG titers at 7 to 9 months compared to those at birth means congenital infection. Recommended serological techniques are an ELISA, an indirect immunofluorescence (IIF) assay, and an indirect hemagglutination assay (IHA). Serological confirmation requires coincident positive results by two tests. [f]For Tuscany, Italy (273), both parasitological testing and PCR must be carried out at birth, and parasitological testing and/or PCR must be carried out 1 month after birth. Repeat serology should be performed at 12 months only in the case of a positive result at 9 months with a reduction of IgG titers compared to those at birth. An increase of IgG titers at 9 months compared to those at birth means congenital infection. It is not clear which is the serological technique (or techniques) of choice.

among regions within the same state. Thus, this review is focused mainly on strategies for cCD management on both a global and a regional scale.

In this context, programs to control cCD in countries of endemicity and countries where the disease is not endemic coincide in basing diagnosis mainly on parasitological and serological techniques and to a far lesser degree on molecular tests. However, certain diagnostic aspects require comment, especially regarding serological testing. After passive transmission during pregnancy, maternal IgG antibodies can remain in the bloodstream of the newborn for 8 to 10 months (depending on the technique used for detection) before disappearing (7). Thus, although a negative serological result at birth rules out infection, it is highly improbable if the mother is infected. Likewise, a positive result does not confirm cCD and requires an additional test some months after birth to verify a progressive decline in maternal IgG over time and the possible appearance of antibodies produced by the neonate, which implies a double expenditure of both time and money (7). This explains why only three of the algorithms found in protocols of countries where the disease is endemic

(Costa Rica, Peru, and Venezuela) and two in countries where the disease is not endemic (Valencian Community in Spain and Tuscany in Italy) include a serological analysis at birth, whereas the rest concentrate on testing in neonates aged 6 to 12 months (Fig. 2 and 5). However, the loss to follow-up after the postdelivery discharge of mothers and their newborns and before the definitive serological results are obtained has an important impact on cCD diagnosis and is a reason to avoid a delay in serology (294). Basile et al. (257) reported 140 cases of newborns (17.5%) who did not complete the follow-up in Catalonia, mainly due to the departure of the family to another location. Consequently, all the protocols of both countries of endemicity and countries where the disease is not endemic recommend analyzing a blood sample from the newborn by parasitological techniques at birth, and some also stipulate retesting 1 to 3 months later for greater sensitivity (Fig. 2 and 5). The main drawback of parasitological tests is that they need to be carried out by skilled and trained personnel to obtain accurate results (87).

Molecular tools, a highly sensitive alternative or complement to current methods, could help to improve the early diagnosis of cCD and facilitate the rapid establishment of treatment (161, 296). Although the efficacy of molecular methods for cCD diagnosis has been widely proven (96, 297, 298), they are the "great forgotten" in countries where the disease is endemic. The use of PCR assays is advocated only in Chile, Mexico, Panama, Paraguay, Peru, and Venezuela. In resource-limited regions of endemicity, the implementation of molecular methods for routine screening is difficult to sustain (51, 161), being economically and logistically prohibitive (296). In contrast, in countries where the disease is not endemic, all the diagnostic algorithms recommend giving a PCR test at least once around the time of birth (Fig. 5). Given that low levels of *T. cruzi* DNA of maternal origin can be detected in the neonate at birth (7, 294), the protocols in countries of nonendemicity that include molecular diagnosis require the first PCR at birth to be confirmed 1 or 2 months later or delay the first PCR to 1 month after birth. The most serious omission in cCD control in areas where the disease is not endemic is the lack of health policies at a national level.

Another aspect to consider is that in terms of methodology, new serological and molecular tools have emerged in recent years, but they have not been incorporated into the cCD diagnostic algorithms, which have remained almost unchanged for 2 decades (123, 229, 299). This includes the new-generation serological tests, which, due to their high sensitivity, allow the long-term detection of maternal IgG antibodies in the bloodstream of the neonate and, thus, delay the final diagnosis (7) (see "Serological Diagnosis," above). Indeed, in a recent study, our group reported that the CMIA Architect Chagas assay (currently replaced by Alinity s Chagas; Abbott Diagnostics) showed positive results in noninfected infants aged 10 to 13 months (229). As mentioned above in "General recommendations for congenital Chagas disease screening," in 2019, the WHO recommended delaying serological testing in neonates 8 to 10 months of age (5), but according to the results of the new-generation tests, this may be insufficient (229). Another relatively new molecular approach is LAMP (see "Molecular Diagnosis," above), a ready-to-use, easily handled methodology with good potential for cCD diagnosis (96). Additionally, its low cost compared to the cost of more sophisticated techniques such as PCR may help to extend the use of molecular methods in settings of endemicity with economic constraints. Nevertheless, its effectiveness should be confirmed with further research.

Various studies have carried out economic evaluations of CD screening of pregnant Latin American women and their children and share the conclusion that the detection and treatment of cCD cases at an early stage are a cost-effective strategy (254, 300–304). In 2005, Billot et al. (300) indicated that a proper program to diagnose and control cCD in Bolivia would cost $123 per infected infant, which corresponds to $1.2 per birth in the country. In the case of the United States, targeted screening and treatment would result in lifetime savings of around $1,300 per birth and up to more than $600 million per birth-year cohort (254, 303, 304). In Spain, Sicuri et al. (301) and Imaz-Iglesia et al. (302) also demonstrated that congenital transmission control programs are more economical than the nonscreening option. However, in addition to the various health policies for cCD control, alternative diagnostic algorithms have been proposed by numerous studies in the scientific literature (7, 51,

53, 155, 288, 294, 298), thus expanding the range of available strategies, which hinders the establishment of a common front against the disease.

The implementation of systematic screening for cCD is of the utmost importance in managing the disease. Moreover, studies are necessary to evaluate the impact of control programs (on prevalence and congenital transmission rates, etc.) and detect possible weaknesses and shortcomings of the current protocols that can be resolved in future strategies. The screening of family members of newly diagnosed patients is an additional useful intervention strategy (305). The impact of treating infected women of childbearing age on the incidence of cCD should also be assessed. However, such evaluation studies are still infrequent in the few territories that have established a surveillance system for cCD (181, 257, 306–308). Thus, more efforts are needed to promote an exchange of experiences and information worldwide that could help to create a common efficient strategy for cCD management and control.

CONCLUSIONS

Currently, even though cCD represents the main form of the disease in many countries, most legislation on *T. cruzi* infection concerns transmission through vectors, blood transfusion, and solid-organ transplants rather than the mother-to-child route. To our knowledge, this is the first review to analyze the existing policies and initiatives for cCD prevention and control on a global level. The wide diversity of methodologies and recommended times of testing stipulated in the available protocols highlights the need for a consensus. Currently, each health authority applies its own criteria, leading to a different approach in every region. The lack of a common diagnostic strategy hampers the management of the disease worldwide. National health policies are inexistent in countries where the disease is not endemic, and official protocols at a regional level are scarce. Countries of endemicity have more procedures in place to control cCD, but economic constraints in resource-limited areas restrict the implementation of molecular methods. Furthermore, protocols in both countries of endemicity and countries where the disease is not endemic have not been adapted to the new methodologies developed in recent years.

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We declare no conflict of interests.

ADDENDUM IN PROOF

The Ministry of Health of Argentina updated in 2022 the diagnostic algorithm for congenital Chagas disease (234), which now includes the use of the PCR (if available) from birth up to 10 months of age (preferably as close to the date of birth as possible). This recent update does not appear in Fig. 2 because it came to light after the acceptance of the manuscript.

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