Current Heart Failure Reports (2022) 19:279–289
https://doi.org/10.1007/s11897-022-00568-9

COMORBIDITIES OF HEARTH FAILURE (J. TROMP, SECTION EDITOR)

Chronic Chagas Disease—the Potential Role of Reinfections in Cardiomyopathy Pathogenesis

Christian Olivo Freites[1] · Hendrik Sy[2] · Amal Gharamti[3] · Nelson I. Agudelo Higuita[4] · Carlos Franco-Paredes[5] · José Antonio Suárez[6] · Andrés F. Henao-Martínez[7]

Accepted: 11 July 2022 / Published online: 11 August 2022

Abstract

Purpose of the Review Chagas disease is a neglected anthropozoonosis of global importance with significant cardiovascular-associated mortality. This review focuses on the *Trypanosoma cruzi* reinfections' role in chronic Chagas cardiomyopathy pathogenesis. We discuss and summarize the available data related to pathology, pathogenesis, diagnosis, and treatment of reinfections.

Recent Findings Reinfections influence the genetic and regional diversity of *T. cruzi*, tissue tropism, modulation of the host's immune system response, clinical manifestations, the risk for congenital infections, differences in diagnostics performances, response to antiparasitic therapy, and the natural history of the disease. Animal models suggest that reinfections lead to worse outcomes and increased mortality, while other studies showed an association between reinfections and lower parasitemia levels and subsequent infection protection. In some regions, the human risk of reinfections is 14% at 5 years. Evidence has shown that higher anti-*T. cruzi* antibodies are correlated with an increased rate of cardiomyopathy and death, suggesting that a higher parasite exposure related to reinfections may lead to worse outcomes.

Summary Based on the existing literature, reinfections may play a role in developing and exacerbating chronic Chagas cardiomyopathy and are linked to worse outcomes. Control efforts should be redirected to interventions that address structural poverty for the successful and sustainable prevention of Chagas disease.

Keywords Chagas · *Trypanosoma cruzi* · Reinfection · American Trypanosomiasis

Introduction

American trypanosomiasis—also known as Chagas disease—is a vector-borne anthropozoonosis disease caused by the hemoflagellate parasite *Trypanosoma cruzi* (Trypanosomatidae). The organism infects mononuclear cells and has a predilection for the cardiac, digestive, nervous systems, skin, and soft tissues [1, 2]. The most common form of transmission is vector-borne, accounting for 70% of cases. The blood-sucking bugs belong to the genera of hematophagous Triatominae (Reduviidae family) [3–5]. Other forms of transmission include vertical transmission [6], accounting for approximately 26% of cases. Oral transmission [7], blood products [8], solid organ transplantation [9], and laboratory accidents [10] have also been reported [11]. The World Health Organization (WHO) estimates that around 75 million individuals

This article is part of the Topical Collection on *Comorbidities of Heart Failure*.

✉ Andrés F. Henao-Martínez
andres.henaomartinez@cuanschutz.edu

[1] Division of Infectious Diseases, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA

[2] Internal Medicine Department, Mount Sinai Health System, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[3] Internal Medicine Department, Yale-Waterbury Hospital, Yale School of Medicine, New Haven, CT, USA

[4] University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

[5] Federico Gómez, Hospital Infantil de México, Mexico City, México

[6] Clinical Research Department, Investigador SNI Senacyt Panamá, Instituto Conmemorativo Gorgas de Estudios de La Salud, Panamá City, Republic of Panama

[7] Department of Medicine, University of Colorado Anschutz Medical Campus, 12700 E. 19th Avenue, Mail Stop B168, Aurora, CO, USA

are at risk for infection. Chagas disease is endemic in 21 Latin American countries, where approximately eight to ten million people are infected [11, 12]. Due to population mobility, the disease is not restricted to endemic areas [12]. In the USA, 300,167 individuals live with *Trypanosoma cruzi* infection, of whom 30,000–45,000 have cardiomyopathy; and 63–315 congenital infections occur every year [13]. The disease has two phases. An acute phase can be asymptomatic or present as an acute febrile undifferentiated syndrome. The chronic phase consists of an indeterminate form where patients are infected and asymptomatic (70% of cases) and a determinate form where the patient may present with cardiomyopathy (20–30% of cases) or a gastrointestinal mega-syndrome (megaesophagus or megacolon: 10% of cases) [14–16]. Symptomatic acute disease (~1%) is characterized by fever, lymphadenopathy, hepatosplenomegaly, heart failure, pericardial effusion, myocarditis, pericardial effusion, and heart failure or meningoencephalitis. Chagas disease can reactivate in the setting of immunosuppression and manifest as fulminant heart failure, panniculitis, or central nervous system rim-enhancing lesions and encephalitis [17, 18]. The annual cardiomyopathy risk reaches 2% and 5% among patients with the indeterminate form and acute infection, respectively [19.●●●●]. The parasite inoculum may influence cardiovascular disease severity and outcome [20]. Chagas disease acquired orally has been linked to a higher parasite load and is characterized by a shorter latency period and more severe cardiovascular disease than that associated with vectorial transmission.

The main burden of the disease comes from Chagas cardiomyopathy development. Once established, cardiomyopathy has an annual overall estimated mortality of 8%, of which 6.3% is of cardiovascular origin [21.●●●●]. Cardiovascular mortality mainly comes from heart failure (3.5%), followed by sudden death (2.6%) and strokes (0.4%). Cumulative heart failure mortality could reach 30% at 10 years and 50% at 20 years, assuming exponential growth (Fig. 1). The annual cardiovascular mortality is comparable to untreated AIDS [21.●●●●]. Chagas cardiomyopathy associated heart failure attributable mortality surpasses other common forms of heart failure [22].

Geography influences the proportion of Chagas diseases among cohorts of patients with heart failure. In Latin American registries of heart failure patients, 0.6–20% were Chagas-related [23–25]. These numbers are expected to be substantially lower in non-endemic regions such as the USA or Europe.

People living in endemic areas are at risk of infections. Reinfections could influence the risk for congenital disease [26] or reactivation in the setting of transplantation [17]. It is unclear how repetitive exposure to the parasite through continuous vector exposure in endemic areas contributes to the development of chronic Chagas cardiomyopathy and heart failure.

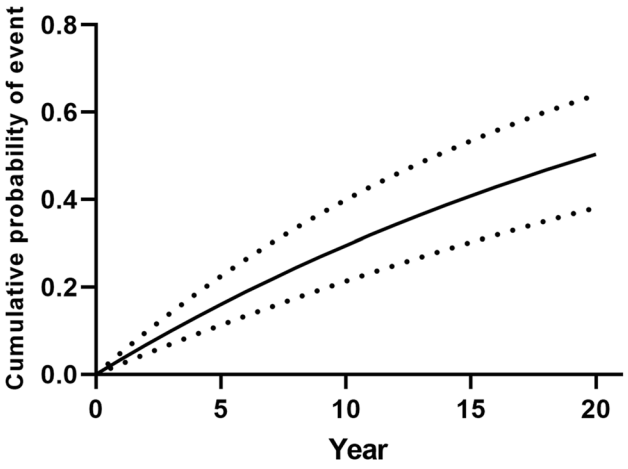

Fig. 1 Cumulative probability of Chagas-related heart failure mortality

This article summarizes the literature findings on Chagas disease reinfection and discusses its implications on Chagas pathogenesis and cardiomyopathy development. We explore the risk factors for reinfection, the pathological manifestations, the outcomes in experimental models, and the relevance of reinfection in diagnosis and treatment.

Trypanosoma cruzi Reinfection Definition

A standard definition for Chagas reinfection does not exist. Reinfections are defined as a new infection after recovery or a new superimposed infection. Theoretically, reinfection occurs with a new detectable blood smear parasitemia in a patient with an already established chronic infection after a recent re-exposure (vector or oral). This needs to be differentiated from reactivation disease in the immunocompromised hosts without recent exposure [27].

Determinant Factors for *Trypanosoma cruzi* Reinfections

The population at risk for reinfection is heterogeneous and determined by factors such as the living conditions, occupation, and intra-domiciliary presence of animals. These and other factors can modulate the risk and rate of *T. cruzi* reinfections:

Endemic Areas

The highest risk for reinfection with *T. cruzi* exists in endemic areas. Chagas disease is endemic in 21 continental Latin American countries, particularly in poor, rural areas of Central and South America, where residents are continuously exposed to competent vectors. In Latin America, the prevalence of *T. cruzi* infection is highest in Bolivia (6.1 cases per 100 inhabitants), Argentina (3.6), Paraguay (2.1), Ecuador (1.4), El Salvador (1.3), and Guatemala (1.2). The Gran Chaco region includes eastern Bolivia, western Paraguay, Northern Argentina, and parts of Brazil. In the hyperendemic region of the Bolivian Chaco, the incidence of *T. cruzi* infection fluctuates between 0.1% and 4% per year [1], and the all-age prevalence in rural areas is > 50% [28]. In the USA, enzootic cycles of *T. cruzi* transmission exist in southern states. However, only a few autochthonous infections have been reported [1].

Tomasini et al. developed models that predict the transmission of *T. cruzi* discrete typing units (DTUs) that were detected in Chaco province, Argentina. The model simulated *T. cruzi* transmission in an environment corresponding to a rural village made of contiguous houses. A fitted model using a hybrid genetic algorithm of epidemiological data of Argentinean rural villages found a 56% rate of mixed infections by different genotypes of *T. cruzi* in humans. The best model predicted 0.032 (0.008–0.042) annual reinfections per individual, with 98.4% occurring in the chronic phase of the disease. Based on these low yearly rates, the risks of reinfection were predicted to be 14% (4–18%) and 60% (21–70%) after 5 and 30 years, respectively, with most individuals being reinfected 1–3 times over their lifetime [29.●●••].

Housing

The vector usually infests the corners and fissures inside poorly constructed homes associated with poor hygiene practices [30]. Mott et al. described a high rate of seroreactivity in residents of unplastered mud-stick houses, mud-brick houses, and plastered mud-stick homes [31]. Houses near henhouses, pigsties, and animal pens increase vectorial transmission [32]. Coura et al. reported that wood, fibers (e.g., piaçava fiber), and palm leaves used as roofing material in rural areas could carry infected wild triatomines to the homes. The uncontrolled deforestation of certain areas in endemic countries with subsequent construction of urbanized areas increases the exposure of individuals to reservoirs and the vector [32, 33].

Socioeconomic Determinants

Certain subsistence activities and customs practiced in rural areas of Latin America provide an opportunity for repetitive *T. cruzi* infections [34]. Certain fruits and juices, such as acai, bacaba, guava, and sugar cane, have been associated with outbreaks of Chagas disease through oral transmission [35]. Due to economic and social crises, there has been an increased number of migrants from endemic areas to Europe and the USA [36]. Also, the geographic distribution widening of the disease increases the population at risk. Climate change enormously influences the rate of vector-borne infections. Tamayo et al. described the effect of increasing temperature on the vector and the parasite. They noticed a rapid appearance, an increase in the infective forms of *T. cruzi*, and a decreased development time in the vector, suggesting an increased probability of infection in higher temperatures [37]. Type of occupation, migrants in transit, and residence of areas affected by climate changes can be crucial socioeconomical risk factors for Chagas infection and reinfection.

Vector

Transmission of *T. cruzi* in the domestic and peridomestic cycles is related to the density and index of intradomiciliary infection of the vector and the vector species [38]. A revision of the impact of Chagas control in Latin America showed a reduction in the morbidity and mortality of Chagas after eliminating domestic vectors and transfusion-related transmission [39]. Sustained vector exposure increased the risk of Chagas cardiomyopathy among women in Bolivia. Among 302 infected women, the risk of having ECG abnormalities consistent with Chagas cardiomyopathy was higher in older women (OR 1.06 [95% CI 1.01–1.12] per year) and those with vector exposure (OR 3.7 [95% CI 1.4–10.2]). Interestingly, vector exposure showed an inverse relationship between maternal parasitemia and mother-to-child transmission. The authors hypothesize that pathogenic and protective effects may result from frequent exposure to infected vectors and repeated superinfection by the parasite [40].

Parasite Strains

The complex domestic, peridomestic, and wild cycles of this parasite include a diverse group of mammalian reservoirs (dogs, cats, raccoons, opossums, armadillos, woodrats, and others) [3, 41]. Due to its genetic diversity, the parasite has undergone multiple nomenclature changes; two major lineages of the parasite, *T. cruzi* I and *T. cruzi* II, were initially defined [42]. There are currently six DTUs, TcI to TcVI, TcI being the most abundant DTU extending from South to North America. *T. cruzi* has one of the largest genomes of the Trypanosomatids [43], with considerable genetic diversity within DTUs. [44] This genetic diversity is associated with different tissue tropisms, trypanocidal agent susceptibilities, and interactions with the host immune system. Triatomines can be infected by multiple *T. cruzi* strains

simultaneously [45]. Data from various endemic areas have shown that mixed infections with more than one *T. cruzi* DTU are more prevalent in humans than in triatomes [46]. It is important to note that the isolation of different *T. cruzi* strains or DTUs in an individual does not establish a reinfection event.

Animal Exposures

Dog ownership has been suggested as a risk factor for *T. cruzi* transmission [47]. Dogs have been shown to increase house infestation with species adapted to human households such as *Triatoma infestans* [48] and non-domiciliated species such as *Triatoma dimidiata* [49]. By contrast, the model of Tomasini et al. based in the Chaco province of Argentina found that one or two dogs only slightly increased the prevalence of *T. cruzi* infection and did not necessarily explain the observed levels of mixed infections[29.●●●●]. However, the findings suggest keeping dogs outside of dormitories to lower transmission to humans [29.●●●●] [47]. Cats are also an important reservoir in the domestic cycle. However, their role is minor compared to dogs [50, 51]. The presence of the peri domiciliary animals, like opossums, may pose a public health threat in areas where the other components of the parasite cycle are present [52]. Opossums have been linked to acute Chagas outbreaks acquired by ingesting contaminated food products. These marsupials are known to harvest trypomastigotes in their anal glands and can contaminate food products.

Pathophysiology of Reinfection

Deposition of contaminated feces with the parasites occurs in the skin or mucosal areas during or immediately after a vector's blood meal [14]. *T. cruzi* will replicate at the inoculation site. The released trypomastigotes would infect multiple cellular lines once they enter the bloodstream. Despite having the capacity to infect any nucleated cell [53], the parasite has a tropism for cardiac muscle and gastrointestinal tract neurons. As soon as a cell is infected, the parasite differentiates into an amastigote form and replicates intracellularly (Fig. 2). Cell rupture ensues, releasing trypomastigotes that will infect other cells. The acute phase can last from 2 weeks to months [54]. The chronic phase can be indeterminate—characterized by a lack of symptoms and no evidence of cardiac injury based on electrocardiographic and echocardiographic evaluation—or determinate, characterized by cardiomyopathy or gastrointestinal pathology [14]. During the acute phase, diffuse myocarditis characterized by vacuolization, cytolysis, and fibrillar degeneration is typically found. The initial inflammatory response is composed of neutrophils

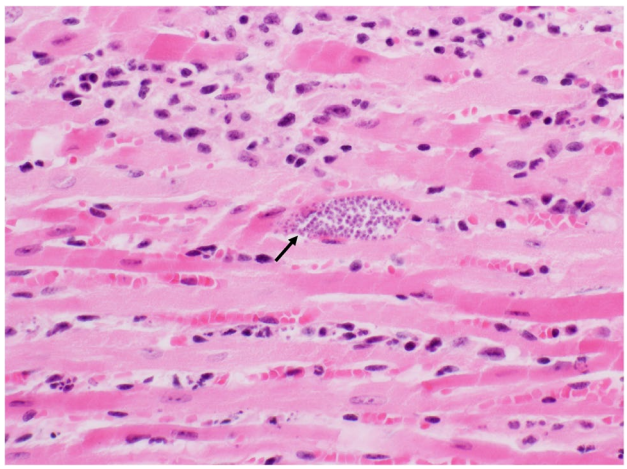

Fig. 2 H&E stain, high power magnification showing parasite amastigotes (arrow), and associated myocarditis

and macrophages and is later replaced with lymphocytes, eosinophils, mast cells, and plasma cells [55]. Polyclonal activation of B cells and subsequent hypergammaglobulinemia can be seen in the acute phase of Chagas disease [56]. Cellular immunity is vital to control the parasite burden in tissues. Cytokines, chemokines, antibody production, and complement activation produce tissue-level changes characterized by interstitial fibrosis and myocardial fiber hypertrophy [57]. Chronic Chagas cardiomyopathy consists of focal areas of low-grade myocarditis with interstitial fibrosis. In these areas, parasites are scarce, and the primary immune cells are T lymphocytes [57, 58]. In the gastrointestinal tract, *T. cruzi* causes myenteric plexus injury due to immune cross-reactivity of the flagellar antigen of *T. cruzi* and myenteric neurons. [59]. Autoimmunity may play a role in the pathogenesis of the disease [60], although controversial.

Heart failure in chronic Chagas cardiomyopathy may develop after repetitive cardiac injury events from subsequent reinfections. These changes can ultimately lead to myocardial abnormalities, including ventricular wall dilation, mitral and tricuspid valve regurgitations, and decreased ejection fraction [61]. Other pivotal mechanisms are the development of left ventricular apical aneurysms and cardiac arrhythmias. Autopsy findings in advanced Chagas heart failure have found evidence of cardiac denervation, specifically intramural neuronal depopulation, ganglionic injury, and reduction of subepicardial neurons [62]. In addition to dysautonomia, cardiac tissue parasite persistence has also been implicated in Chagas-related heart failure. Hypothetically, reinfection could lead to increased myocarditis and risk of advanced heart failure. Gastrointestinal manifestations of the disease—especially megacolon—shared the neural denervation component pathogenesis.

Animal Studies

During reinfection studies, multiple animal models have failed to replicate consistent results of the pathophysiological changes, likely due to the genetic diversity of the parasite, differences in immune host response, and the interaction between the parasite and the host (Table 1). Lauria-Pires and Teixeira studied superinfection in mice with low and high virulence clones. Their results showed that mice infected with low virulence organisms that were subsequently infected with highly virulent parasites did not have statistically significant differences in mortality and histopathological lesions compared with single infected mice [63]. These results suggested immune protection with reinfection. Machado exposed crossbreed dogs to 5 different types of reinfections [64]. A progressive decrease in parasitemia was noted with every reinfection, and the antibody titers during the chronic phases were higher in reinfected dogs than in the single infections. Despite multiple infections, alterations were found exclusively in the cardiac tissue. No significant increase in tissue damage was noted in reinfected dogs independent of the isolates [64].

In contrast, Bustamante noticed higher levels of parasitemia with reinfections with *Tulahuen* strain in albino Swiss mice compared with single infected mice, especially in groups receiving higher parasite loads in the reinfection event [65]. Later in 2007, Bustamante noted differences in reinfections with different parasite strains. *Tulahuen* strain-infected and reinfected mice had higher parasitemia levels and structural cardiac damage than *SGO-Z12* infected mice. That study also showed a significant reduction in beta-adrenergic receptor affinity, concluding that reinfection can produce severe disease, disease progression, and exacerbation of the disease [66]. Andrade studied the effect and differences between single, double, and triple infections (single, mixed, and reinfections) with different strains (Colombian, *21SF*, and *Y* strain). The study proved the coexistence of three different strains within the same mice. Reinfections resulted in the exacerbation of already-existing cardiac lesions in chronically infected mice and demonstrated that the order and strain of the reinfection influenced the outcome [67]. These results are likely explained due to the specific histiotropisms of different strains of the parasite. Restriction fragment length polymorphism (RFLP) studies revealed infection differences in single infected mice with only one strain [68].

The skeletal muscle was the largest reservoir of trypomastigote nests. Reis Machado found decreased parasitemia with reinfection, and higher levels of TNF-alpha, IFN-γ, and moderate to severe inflammatory infiltrate in reinfections with the Colombian strain compared to the Y strain [69]. Perez et al. evaluated the effect of mixed infections versus reinfections. During the study, simultaneous mixed infections did not produce acute morbidity compared to reinfections that impacted the course of the disease [70].

The mode of transmission and inoculum size may also affect the course of the infection. This has been tested by comparing intraperitoneal injections versus oral infections in mice [71]. Lewis et al. study showed that the parasites were "pan-tropic" during acute infections in mice. During the chronic infection, the gastrointestinal tract functioned as a reservoir for *T. cruzi*. In immunosuppression states, the infection was present in multiple organs. Differences in the immune control of *T. cruzi* occur between tissues. The strain differences in various tissues may also be related to tissue-related host responses and not strictly to tropism. They proposed that the cardiac damage accumulates over time according to the frequency of transient episodes of parasitism rather than persistent infection. The severity of fibrosis in the heart depends on host-parasite strain combinations, and different combinations showed heterogeneity of symptoms and disease progression rates [72]. Mortality is also affected by reinfections. Perez and Andrade et al. demonstrated sudden deaths in experimental mice models of reinfections; however, not all the deaths were related to cardiac dysfunction, suggesting that could be multiple mechanisms related to the mortality in reinfections [67, 70]. Meningoencephalitis—a clinical manifestation that can occur as part of acute Chagas disease or reactivation in immunosuppressed hosts—could also contribute to mortality in animal models of reinfection [67, 70].

Parasite Strains

In humans, the variations of tropism can also be seen within one organ, and multiple variations of one DTU can predominate in specific areas of an organ [17]. Apart from the inflammatory changes at tissue levels, the diversity of *T. cruzi* influences the host's humoral immune response. Del Puerto et al. evaluated the effect of specific DTUs infection and their association with clinical forms of Chagas disease for 7 years. No lineages or sublineages were significantly associated with any clinical manifestations in Bolivian chronic Chagas patients [73]. However, this study was performed in a specific geographical area, and variations between strains from other areas were not evaluated. Burgos et al. demonstrated that blood strains in heart transplant patients with mixed infections differ from those in tissues or even within the same organ [17]. From animal studies' findings, it is fair to hypothesize that different *Trypanosoma cruzi* strains may possess various patterns and severity of myocarditis [74, 75], which may correlate with heart failure risk and staging. The lack of clear human validation or an established geographic clinical variation of cardiomyopathy severity and heart failure makes this hypothesis less apparent.

Human Studies

In an experimental model, Dos Santos et al. measured the linkage between the phylogenetic divergence of *T. cruzi* and immunoglobulin G (IgG) levels. *T. cruzi* I was more efficient in

Table 1 Summary of experimental Chagas studies related to reinfections

Study	Animal model	Infection type	Strain	Outcome
Lauria-Pires &Teixeria 1997 [63]	BABLB/c mice	Single infection (h1,h2, m3,m4), reinfection (initially with h1/h2 and later with m3/m4)	Low virulence clones (h1, h2) and high virulence clones (m3, m4)	Low-level parasitemia was noted after being challenged with virulent clones. Mortality ratios and histopathological lesions were similar between low virulent clones single infected mice and reinfected mice with high virulent clones
Machado 2001 [64]	Cross-bred dogs	Single infections (147, SC-1) and reinfections (with 147, SC-1, multiple times in different orders)	147 and SC-1 strains	No deaths were observed during reinfection. Parasitemia levels decreased with the number of reinfections. Simultaneous presence in tissues of both strains was proven in three animals. Antibody titers were higher in reinfected dogs
Bustamante 2002 [65]	Albino Swiss mice	Single infection and reinfections	*Tulahuen* strain	Higher parasitemia level in reinfected mice, especially in groups receiving higher parasite loads in the reinfection event
Andrade 2006 [67]	Swiss Webster mice	Single infection and reinfection	Colombian (Col), Col + 21SF, Col + Y, Col + 21SF + Y, Col + Y + 21SF	Reinfections resulted in the exacerbation of already-existing cardiac lesions in chronically infected mice and demonstrated that the order and strain of the reinfection influenced the outcome. Proved the coexistence of three different strains within the same mice
Bustamante 2007 [66]	Albino Swiss mice	Single infection and reinfection	*Tulahuen* and SGO-Z12	*Tulahuen* strain infected and reinfected mice had higher parasitemia levels and structural cardiac damage than SGO-Z12 infected mice. Also, the study showed a significant reduction in beta-adrenergic receptor affinity with reinfections
Reis Machado 2014 [69]	C57BL/6 mice	Single infection and reinfections	Colombiana and 30	Found decreased parasitemia with reinfection and higher levels of TNF-alpha, IFN-y, and moderate to severe inflammatory infiltrate in reinfections with the Colombian strain compared to the Y strain
Presti 2014 [68]	Albino Swiss mice	Single infection and reinfections	*Lucky* and *Casibla*	Restriction fragment length polymorphism (RFLP) studies revealed infection differences in single infected mice with only one strain. Muscle sections from single infected mice presented mild to moderate inflammatory infiltrates; reinfected mice showed severe inflammatory infiltrates and the presence of amastigote nests in both tissues. Different parasite populations were found in circulation and the tissues within the same mice
Perez 2018 [70]	Swiss mice	Single infection, mixed infection, and reinfection	TcI (C8 clone 1) and TcIV (10R26)	Simultaneous mixed infections did not produce acute morbidity compared to reinfections that impacted the course of the disease. Sudden deaths were seen in the reinfection group

over-expressing all subclasses of specific anti-parasite IgG than other *T. cruzi* strains. Benznidazole treatment also induced IgG alterations. These can differ depending on the stage of the disease when the medication was administered [76]. In humans, Llaguno et al. studied IgG changes in patients with Chagas disease 4 years after treatment with benznidazole. At 48 months, the total levels of IgG were decreased. In patients with cardiomyopathy and exacerbated clinical forms, increased IgG1 and decreased IgG3 were observed. Patients with the cardiac form also presented a decreased IgG3/IgG1ratio [77.•]. Higher anti-*T. cruzi* antibodies correlated with an increased rate of cardiomyopathy and death in a long-term follow-up study suggesting higher parasite exposure can work as a surrogate for reinfections [78.••••].

Reinfections have also been evaluated in specific scenarios like congenital transmission. Burgos et al. assessed the genetic diversity of *T. cruzi* in infected mothers and infants with congenital Chagas. They found no association between a particular genotype and vertical transmission. The strains present in the mothers and the infants (including twin deliveries) were nearly identical [79].

Although there is no specifically human clinical evidence of the role of reinfections in cardiomyopathy development, some animal studies suggest that repeat infections may lead to a higher degree of cardiac injury.

Diagnosis

The diagnosis of Chagas disease is challenging and typically based on microscopic analysis of blood smears or tissue, polymerase chain reaction (PCR), and serologic studies (Fig. 3). The diagnosis of acute Chagas, reactivation, and congenital Chagas relies on PCR and microscopic analysis of blood smears. The diagnosis of chronic Chagas is based on serological tests [80].

Multiple strains can be involved in the pathogenesis of the disease [17]. During reinfections, the difference in the tropism of *T. cruzi* strains can cause the parasite identified in the blood to be different from the one causing tissue disease. Animal models have demonstrated a lower parasitemia with repetitive infections, making the diagnosis more difficult by microscopic analysis and PCR[64]. The differences in humoral response based on the *T. cruzi* strain raised concern about the sensitivity and specificity of serological tests [76, 77.••]. There is a need for studies profiling the Th2 responses based on different strains and the effect of reinfections and mixed infections. Wide use of PCR in endemic areas and rural fields may aid in understanding detectable parasitemia as a marker for Chagas disease progression related to reinfections, intrinsic parasite features, host immune control, or a combination of them. Reinfections events remain very challenging to detect and diagnose. During Chagas cardiomyopathy and heart failure diagnosis, we recommend using the standardized AHA classification of 4 stages: A, B1, B2, C, and D [61]. AHA classification uses electrocardiographic or echocardiographic changes and the presence of heart failure symptoms. Key clinical features include cardiac arrhythmias (commonly left anterior fascicular block (LAFB), incomplete or complete right bundle branch block (RBBB), ST-T changes, or monomorphic PVCs), dilated cardiomyopathy, ventricular aneurysms, and wall motion abnormalities. Symptoms are attributed to pulmonary congestion and hypervolemia. Although unclear, reinfection can present with possible heart failure exacerbation or an acute on chronic heart failure due to additional myocarditis. One of the main prognostic predictors used in clinical practice is the Rassi score. As in other forms of heart failure, NHYA class III/IV, ejection fraction, age, and cardiomegaly are the main mortality prognostic risk factors [21.••••, 61].

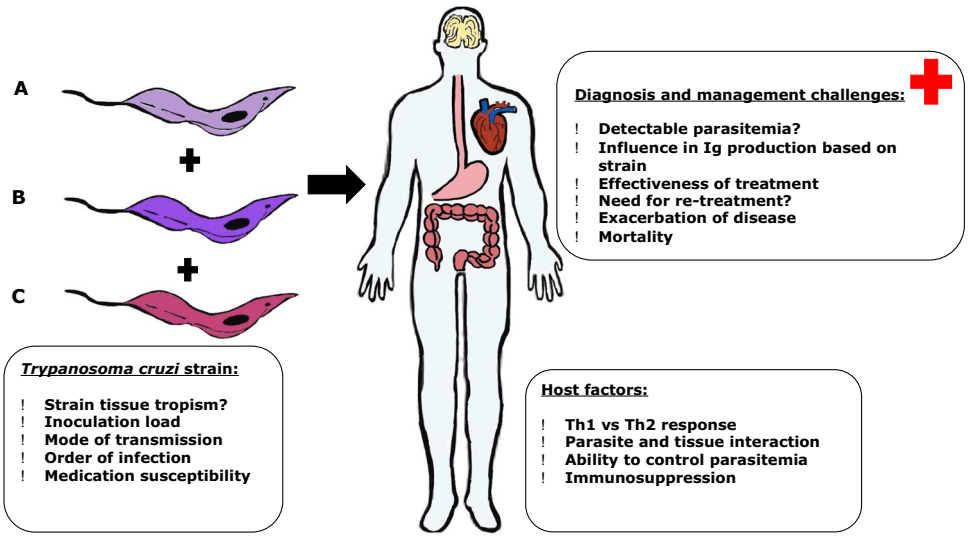

Fig. 3 Factors involved in diagnosis and treatment of Chagas disease during reinfections

Congenital Chagas transmission occurred among 5% of infected mothers [81]. PAHO guidelines recommend screening women of childbearing age or with chronic Chagas disease, including heart failure, and their newborns.

Treatment

The challenge in the treatment of latent infection includes a lack of immune protection after initial infection, decreased efficacy of the treatment during the indeterminate and chronic phases [82], and the human immune system's inability to eradicate the parasite in most cases. Chronic Chagas disease carries a staggering high annual mortality of 8% [21.●●●●], and any efforts to decrease mortality and progression are urgently needed. Currently, two drugs are available to treat Chagas disease, benznidazole and nifurtimox. Benznidazole is approved in the USA for the treatment of Chagas disease [83]. A limitation in the treatment trials of Chagas is that they do not routinely test for the strains that cause the disease in trial participants.

Bustamante and Tarleton demonstrated in an animal model that previous infections, active infections, and previously treated infections do not protect against reinfection with *T. cruzi*. Mice previously treated with benznidazole challenged with cyclophosphamide have an increased reactivation risk [84]. A T-cell-mediated inflammatory response is usually induced after the initial *T. cruzi* replication cycle. However, parasites remain capable of infecting cells initially with minimal immune system stimulation [84]. Teston et al. evaluated the benznidazole susceptibility of natural populations of *T. cruzi* DTUs (I, II, IV) from the states of Amazonas, Parana, and Minas Gerais. The overall cure rate was 62.5%. However, cure rates differ between DTUs and even within the same DTUs from multiple locations (27 to 100%). In the study, 9/23 strains were sensitive, 9/23 were intermediate, and 5/23 were resistant to benznidazole. There was no predominance of resistance patterns between the strains. Differences in cures rates between TcI strains from Amazonas and Parana were noted, highlighting the genetic diversity within DTUs [85]. A natural infection cure without medication administration is a rare phenomenon and usually takes a considerable amount of time [86–88]. The presence of reinfection phenomena may suggest that some infected patients may benefit from repeated treatment courses, along with strict vector control. The BENEFIT trial failed to show any benefit of anti-trypanosomal therapy to reduce cardiovascular events or death at 5 years in patients with already established Chagas cardiomyopathy [89]. In endemic regions with poor vector control, reinfections may account for the observed lack of antiparasitic therapy benefits. However, therapy with benznidazole or nifurtimox is largely ineffective once heart failure is clinically manifested. Also, any reinfection episode may precipitate an exacerbation in a stable heart failure patient on cardiac pharmacotherapy.

Conclusion

The literature reviewed in this article exposes the challenges in studying *T. cruzi* reinfections. The differences in host response between multiple animal models, the lack of a standardized model, the enormous genetic diversity of the parasite, and the subsequent variations in the individual host immune responses make the study of this area extremely complex. These data uncover the need to develop tools to study human reinfections that consider the DTUs and geographic locations of the specific strain. Other essential study variables to consider are host-specific genotypes and immune responses.

The development of new treatment modalities against this multisystemic disease is required to overcome the diverse tissue tropism and possible reservoirs of this disease in the human body. Based on the existing literature, reinfections may play a role in developing chronic Chagas cardiomyopathy and heart failure and may be linked to worse outcomes. This stresses the importance of implementing public health measures to continue strict vector control and improve house conditions of populations at risk.

Declarations

Conflict of Interest The authors declare no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

1. Pérez-Molina JA, Molina I. Chagas disease. Lancet. 2018;391:82–94.
2. Urdaneta-Morales S. Chagasâ€TM disease: an emergent urban zoonosis. The Caracas Valley (Venezuela) as an epidemiological model. Front Public Health. 2014. https://doi.org/10.3389/fpubh.2014.00265.
3. Kjos SA, Marcet PL, Yabsley MJ, Kitron U, Snowden KF, Logan KS, Barnes JC, Dotson EM. Identification of bloodmeal sources and Trypanosoma cruzi infection in triatomine bugs

(Hemiptera: Reduviidae) from residential settings in Texas, the United States. J Med Entomol. 2013;50:1126–39.
4. Mills RM. Chagas disease: epidemiology and barriers to treatment. Am J Med. 2020;133:1262–5.
5. Shikanai-Yasuda MA, Carvalho NB. Oral transmission of Chagas disease. Clin Infect Dis. 2012;54:845–52.
6. Luquetti AO, do Nascimento Tavares SB, da Rocha Siriano L, de Oliveira RA, Campos DE, de Morais CA, de Oliveira EC. Congenital transmission of Trypanosoma cruzi in central Brazil. A study of 1,211 individuals born to infected mothers. Mem Inst Oswaldo Cruz. 2015;110:369–76.
7. Alarcón de Noya B, Díaz-Bello Z, Colmenares C, et al. Large urban outbreak of orally acquired acute Chagas disease at a school in Caracas, Venezuela. J Infect Dis. 2010;201:1308–15.
8. Cancino-Faure B, Fisa R, Riera C, Bula I, Girona-Llobera E, Jimenez-Marco T. Evidence of meaningful levels of Trypanosoma cruziin platelet concentrates from seropositive blood donors. Transfusion. 2015;55:1249–55.
9. Huprikar S, Bosserman E, Patel G, et al. Donor-derived Trypanosoma cruzi infection in solid organ recipients in the United States, 2001–2011. Am J Transplant. 2013;13:2418–25.
10. Herwaldt BL. Laboratory-acquired parasitic infections from accidental exposures. Clin Microbiol Rev. 2001;14:659–88.
11. Manne-Goehler J, Umeh CA, Montgomery SP, Wirtz VJ. Estimating the burden of Chagas disease in the United States. PLoS Negl Trop Dis. 2016;10:e0005033.
12. Chagas disease in Latin America: an epidemiological update based on 2010 estimates [press release]. World Health Organization, February 6, 2015 2015.
13. Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United States. Clin Infect Dis. 2009;49:e52–4.
14. Tanowitz HB, Kirchhoff LV, Simon D, Morris SA, Weiss LM, Wittner M. Chagas' disease. Clin Microbiol Rev. 1992;5:400–19.
15. Benziger CP, do Carmo GAL, Ribeiro ALP. Chagas cardiomyopathy: clinical presentation and management in the Americas. Cardiol Clin. 2017;35:31–47.
16. Bonney KM, Luthringer DJ, Kim SA, Garg NJ, Engman DM. Pathology and pathogenesis of Chagas heart disease. Annu Rev Pathol. 2019;14:421–47.
17. Burgos JM, Diez M, Vigliano C, et al. Molecular identification of Trypanosoma cruzi discrete typing units in end-stage chronic Chagas heart disease and reactivation after heart transplantation. Clin Infect Dis. 2010;51:485–95.
18. Burgos JM, Begher S, Silva HMV, Bisio M, Duffy T, Levin MJ, Macedo AM, Schijman AG. Molecular identification of Trypanosoma cruzi I tropism for central nervous system in Chagas reactivation due to AIDS. Am J Trop Med Hyg. 2008;78:294–7.
19.•• Chadalawada S, Sillau S, Archuleta S, et al. Risk of chronic cardiomyopathy Among patients with the acute phase or indeterminate form of chagas disease: a systematic review and meta-analysis. JAMA Netw Open. 2020;3:e2015072. **This study evaluated the risk of chronic Chagas cardiomyopathy among asymptomatic patients with the indeterminate form. There is a significant annual risk of cardiomyopathy of 2% among asymptomatic patients.**
20. Borges DC, Araújo NM, Cardoso CR, LazoChica JE. Different parasite inocula determine the modulation of the immune response and outcome of experimental Trypanosoma cruzi infection. Immunology. 2013;138:145–56.
21.•• Chadalawada S, Rassi A Jr, Samara O, et al. Mortality risk in chronic Chagas cardiomyopathy: a systematic review and meta-analysis. ESC Heart Fail. 2021;8:5466–81. **This study evaluated the mortality risk among patients with chronic Chagas cardiomyopathy. There is a substantial annual mortality risk of 8%.**
22. Shen L, Ramires F, Martinez F, et al. Contemporary characteristics and outcomes in Chagasic Heart Failure compared with other nonischemic and ischemic cardiomyopathy. Circ Heart Fail. 2017. https://doi.org/10.1161/CIRCHEARTFAILURE.117.004361.
23. Perna ER, Barbagelata A, Grinfeld L, García Ben M, Címbaro Canella JP, Bayol PA, Sosa Liprandi A. Overview of acute decompensated heart failure in Argentina: lessons learned from 5 registries during the last decade. Am Heart J. 2006;151:84–91.
24. González-Zambrano H, Amaya-Tapia G, Franco-Ramos MC, López León-Murguía OJ. Prevalence of Chagas heart disease in dilated cardiomyopathy. Arch Cardiol Mex. 2020;91:50–7.
25. González-Zambrano H, Amaya-Tapia G, Franco-Ramos MC, López León-Murguía OJ (2021) Prevalence of Chagas heart disease in dilated cardiomyopathy. Archivos de cardiologia de Mexico (English ed Internet). https://doi.org/10.24875/acme.m21000188.
26. Torrico F, Vega CA, Suarez E, Tellez T, Brutus L, Rodriguez P, Torrico M-C, Schneider D, Truyens C, Carlier Y. Are maternal re-infections with Trypanosoma cruzi associated with higher morbidity and mortality of congenital Chagas disease? Trop Med Int Health. 2006;11:628–35.
27. Bowman NM, Balasubramanian S, Gilman RH, et al. Deep sequencing to detect diversity of Trypanosoma cruzi infection in patients coinfected with human immunodeficiency virus and Chagas disease. J Infect Dis. 2022;225:243–7.
28. Samuels AM, Clark EH, Galdos-Cardenas G, et al. Epidemiology of and impact of insecticide spraying on Chagas disease in communities in the Bolivian Chaco. PLoS Negl Trop Dis. 2013;7:e2358.
29.•• Tomasini N, Ragone PG, Gourbière S, Aparicio JP, Diosque P. Epidemiological modeling of Trypanosoma cruzi: low stercorarian transmission and failure of host adaptive immunity explain the frequency of mixed infections in humans. PLoS Comput Biol. 2017;13:e1005532. **Study evaluated the anticipated Chagas re-infection rates in a rural community in Argentina.**
30. Rodríguez-Morales AJ, Von A, Franco-Paredes C. Achievements and challenges in controlling Chagas disease. Bol Med Hosp Infant Mex. 2011;68:101–9.
31. Mott KE, de Oliveira TS, Sherlock I, Morrow RH, Hoff R, Muniz TM, Lehman JS, Draper CC. House construction, triatomine distribution, and household distribution of seroreactivity to Trypanosoma Cruzi in a rural community in northeast Brazil *,†. Am J Trop Med Hyg. 1978;27:1116–22.
32. Coura JR. Chagas disease: control, elimination and eradication. Is it possible? Mem Inst Oswaldo Cruz. 2013;108:962–7.
33. Guhl F, Ramírez JD. Poverty, migration, and Chagas disease. Curr Trop Med Rep. 2021;8:52–8.
34. Coura JR, Borges-Pereira J. Chagas disease: 100 years after its discovery. A systemic review Acta Trop. 2010;115:5–13.
35. Coura JR. Transmissão da infecção chagásica por via oral na história natural da doença de Chagas. Rev Soc Bras Med Trop. 2006;39:113–7.
36. Schmunis GA. Epidemiology of Chagas disease in non-endemic countries: the role of international migration. Mem Inst Oswaldo Cruz. 2007;102(Suppl 1):75–85.
37. Tamayo LD, Guhl F, Vallejo GA, Ramírez JD. The effect of temperature increase on the development of Rhodnius prolixus and the course of Trypanosoma cruzi metacyclogenesis. PLoS Negl Trop Dis. 2018;12:e0006735.
38. Brener Z, Andrade ZA, Barral-Netto M,eds.Trypanosoma cruzi e Doenc¸a de Chagas. 2nd ed. Rio de Janeiro: Editora Guanabara Koogan, 2000:344–78.
39. Dias JCP, Silveira AC, Schofield CJ. The impact of Chagas disease control in Latin America: a review. Mem Inst Oswaldo Cruz. 2002;97:603–12.

40. Kaplinski M, Jois M, Galdos-Cardenas G, et al. Sustained domestic vector exposure is associated with increased Chagas cardiomyopathy risk but decreased parasitemia and congenital transmission risk among young women in Bolivia. Clin Infect Dis. 2015;61:918–26.
41. Roellig DM, Ellis AE, Yabsley MJ. Oral transmission of Trypanosoma cruzi with opposing evidence for the theory of carnivory. J Parasitol. 2009;95:360–4.
42. Izeta-Alberdi A, Ibarra-Cerdeña CN, Moo-Llanes DA, Ramsey JM. Geographical, landscape and host associations of Trypanosoma cruzi DTUs and lineages. Parasit Vectors. 2016. https://doi.org/10.1186/s13071-016-1918-2.
43. De Pablos LM, Osuna A. Multigene families in Trypanosoma cruzi and their role in infectivity. Infect Immun. 2012;80:2258–64.
44. Zingales B, Miles MA, Campbell DA, et al. The revised Trypanosoma cruzi subspecific nomenclature: rationale, epidemiological relevance and research applications. Infect Genet Evol. 2012;12:240–53.
45. Ribeiro G Jr, Gurgel-Gonçalves R, Reis RB, Santos CGSD, Amorim A, Andrade SG, Reis MG. Frequent house invasion of Trypanosoma cruzi-infected triatomines in a suburban area of Brazil. PLoS Negl Trop Dis. 2015;9:e0003678.
46. Ortiz S, Villarroel R, Cancino B, Jercic MI, Solari A. Trypanosoma cruzi discrete typing units in patients of Chagas disease and Triatoma infestans after insecticide spraying in Chile. International Journal of Environmental and Agriculture Research. 2017;3:01–8.
47. Cohen JE, Gürtler RE. Modeling household transmission of American trypanosomiasis. Science. 2001;293:694–8.
48. Gürtler RE, Cecere MC, Lauricella MA, Petersen RM, Chuit R, Segura EL, Cohen JE. Incidence of trypanosoma cruzi infection among children following domestic reinfestation after insecticide spraying in rural northwestern Argentina. Am J Trop Med Hyg. 2005;73:95–103.
49. Dumonteil E, Nouvellet P, Rosecrans K, Ramirez-Sierra MJ, Gamboa-León R, Cruz-Chan V, Rosado-Vallado M, Gourbière S. Eco-bio-social determinants for house infestation by non-domiciliated Triatoma dimidiata in the Yucatan Peninsula, Mexico. PLoS Negl Trop Dis. 2013;7:e2466.
50. Mott KE, Muniz TM, Mota EA, Hoff R, Sherlock I, Draper CC, Oliveira TS. Trypanosoma cruzi infection in dogs and cats and household seroreactivity to T. cruzi in a rural community in northeast Brazil *,†. Am J Trop Med Hyg. 1978;27:1123–7.
51. Gürtler RE, Cecere MC, Lauricella MA, Cardinal MV, Kitron U, Cohen JE. Domestic dogs and cats as sources of Trypanosoma cruzi infection in rural northwestern Argentina. Parasitology. 2007;134:69–82.
52. Yeo M, Acosta N, Llewellyn M, et al. Origins of Chagas disease: Didelphis species are natural hosts of Trypanosoma cruzi I and armadillos hosts of Trypanosoma cruzi II, including hybrids. Int J Parasitol. 2005;35:225–33.
53. Lewis MD, Kelly JM. Putting infection dynamics at the heart of Chagas disease. Trends Parasitol. 2016;32:899–911.
54. Mateus J, Pérez-Antón E, Lasso P, et al. Antiparasitic treatment induces an improved CD8+ T cell response in chronic Chagasic patients. J Immunol. 2017;198:3170–80.
55. Andrade ZA. Immunopathology of Chagas disease. Mem Inst Oswaldo Cruz. 1999;94(Suppl 1):71–80.
56. Bermejo DA, AmezcuaVesely MC, Khan M, et al. Trypanosoma cruzi infection induces a massive extrafollicular and follicular splenic B-cell response which is a high source of non-parasite-specific antibodies. Immunology. 2011;132:123–33.
57. Rossi MA, Ramos SG, Bestetti RB. Chagas' heart disease: clinical-pathological correlation. Front Biosci. 2003;8:e94-109.
58. Marcon GEB, de Albuquerque DM, Batista AM, Andrade PD, Almeida EA, Guariento ME, Teixeira MAB, Costa SCB. Trypanosoma cruzi: parasite persistence in tissues in chronic chagasic Brazilian patients. Mem Inst Oswaldo Cruz. 2011;106:85–91.
59. Goldstein AM, Thapar N, Karunaratne TB, De Giorgio R. Clinical aspects of neurointestinal disease: pathophysiology, diagnosis, and treatment. Dev Biol. 2016;417:217–28.
60. Bonney KM, Taylor JM, Daniels MD, Epting CL, Engman DM. Heat-killed Trypanosoma cruzi induces acute cardiac damage and polyantigenic autoimmunity. PLoS One. 2011;6:e14571.
61. Nunes MCP, Beaton A, Acquatella H, et al. Chagas cardiomyopathy: an update of current clinical knowledge and management: a scientific statement from the American Heart Association. Circulation. 2018;138:e169–209.
62. Marin-Neto JA, Cunha-Neto E, Maciel BC, Simões MV. Pathogenesis of chronic Chagas heart disease. Circulation. 2007;115:1109–23.
63. Lauria-Pires L, Teixeira AR. Superinfections with genetically characterized Trypanosoma cruzi clones did not aggravate morbidity and mortality in BALB/c mice. J Parasitol. 1997;83:819–24.
64. Machado EM, Fernandes AJ, Murta SM, Vitor RW, Camilo DJ Jr, Pinheiro SW, Lopes ER, Adad SJ, Romanha AJ, Pinto Dias JC. A study of experimental reinfection by Trypanosoma cruzi in dogs. Am J Trop Med Hyg. 2001;65:958–65.
65. Bustamante JM, Rivarola HW, Fernández AR, Enders JE, Fretes R, Palma JA, Paglini-Oliva PA. Trypanosoma cruzi reinfections in mice determine the severity of cardiac damage. Int J Parasitol. 2002;32:889–96.
66. Bustamante JM, Novarese M, Rivarola HW, Lo Presti MS, Fernández AR, Enders JE, Fretes R, Paglini-Oliva PA. Reinfections and Trypanosoma cruzi strains can determine the prognosis of the chronic chagasic cardiopathy in mice. Parasitol Res. 2007;100:1407–10.
67. Andrade SG, Campos RF, Sobral KSC, Magalhães JB, Pereira Guedes RS, Guerreiro ML. Reinfections with strains of Trypanosoma cruzi, of different biodemes as a factor of aggravation of myocarditis and myositis in mice. Rev Soc Bras Med Trop. 2006;39:1–8.
68. Presti MSL, Lo Presti MS, Esteves BH, et al. Circulating Trypanosoma cruzi populations differ from those found in the tissues of the same host during acute experimental infection. Acta Trop. 2014;133:98–109.
69. Reis Machado J, Silva MV, Borges DC, da Silva CA, Ramirez LE, dos Reis MA, Castellano LR, Rodrigues V, Rodrigues DBR. Immunopathological aspects of experimental Trypanosoma cruzi reinfections. Biomed Res Int. 2014;2014:648715.
70. Perez CJ, Thompson RCA, Keatley SK, Walsh AL, Lymbery AJ. The effect of reinfection and mixed Trypanosoma cruzi infections on disease progression in mice. Acta Trop. 2018;178:107–14.
71. Dias GB, Gruendling AP, Araújo SM, Gomes ML, de Omelas Toledo MJ. Evolution of infection in mice inoculated by the oral route with different developmental forms of Trypanosoma cruzi I and II. Exp Parasitol. 2013;135:511–7.
72. Lewis MD, Francisco AF, Taylor MC, Jayawardhana S, Kelly JM. Host and parasite genetics shape a link between Trypanosoma cruzi infection dynamics and chronic cardiomyopathy. Cell Microbiol. 2016;18:1429–43.
73. del Puerto R, Nishizawa JE, Kikuchi M, et al. Lineage analysis of circulating Trypanosoma cruzi parasites and their association with clinical forms of Chagas disease in Bolivia. PLoS Negl Trop Dis. 2010;4:e687.
74. Rodriguez Angulo HO, Santi-Rocca JS, Fortes AJ, Guerrero N, Girones N, Fresno M, Laboratorio de Activacion del Sistema

immune (2013) Trypanosoma cruzi strains belonging to distinct DTU causes different myocarditis patterns in infected mice. Eur Heart J 34:P3873–P3873.
75. Rodriguez HO, Guerrero NA, Fortes A, Santi-Rocca J, Gironès N, Fresno M. Trypanosoma cruzi strains cause different myocarditis patterns in infected mice. Acta Trop. 2014;139:57–66.
76. dos Santos DM, Talvani A, da Mata Guedes PM, Machado-Coelho GLL, de Lana M, Bahia MT. Trypanosoma cruzi: genetic diversity influences the profile of immunoglobulins during experimental infection. Exp Parasitol. 2009;121:8–14.
77.• Llaguno M, da Silva MV, Helmo FR, et al. IgG subclass analysis in patients with Chagas disease 4 years after benznidazole treatment. Acta Parasitol. 2021;66:1499–509. **The study team demonstrated that after four years of treatment with benznidazole, the pattern of IgG and subsets changes. Also noted an increased IgG1 and decreased IgG3 in patients with cardiomyopathy and exacerbated clinical forms.**
78.•• Nunes MCP, Buss LF, Silva JLP, et al. Incidence and predictors of progression to Chagas cardiomyopathy: long-term follow-up of Trypanosoma cruzi-seropositive individuals. Circulation. 2021;144:1553–66. **This study showed that higher antibodies against *T.cruzi* are associated with increased rates of cardiomyopathy and increased mortality, suggesting that higher parasite exposure changes the morbidity and mortality of this condition.**
79. Burgos JM, Altcheh J, Petrucelli N, Bisio M, Levin MJ, Freilij H, Schijman AG. Molecular diagnosis and treatment monitoring of congenital transmission of Trypanosoma cruzi to twins of a triplet delivery. Diagn Microbiol Infect Dis. 2009;65:58–61.
80. Hochberg NS, Wheelock A, Hamer DH, Marcus R, Nolan MS, Meymandi S, Gilman RH. Chagas disease in the United States: a perspective on diagnostic testing limitations and next steps. Am J Trop Med Hyg. 2021;104:800–4.
81. Howard EJ, Xiong X, Carlier Y, Sosa-Estani S, Buekens P. Frequency of the congenital transmission of Trypanosoma cruzi: a systematic review and meta-analysis. BJOG. 2014;121:22–33.
82. Pecoul B, Batista C, Stobbaerts E, et al. The BENEFIT Trial: where do we go from here? PLoS Negl Trop Dis. 2016;10:e0004343.
83. Morillo CA, Echeverria LE. New treatment regimens for Chagas disease: light at the end of the tunnel? Lancet Infect Dis. 2021;21:1057–8.
84. Bustamante J, Tarleton R. Reaching for the Holy Grail: insights from infection/cure models on the prospects for vaccines for Trypanosoma cruzi infection. Mem Inst Oswaldo Cruz. 2015;110:445–51.
85. Teston APM, Monteiro WM, Reis D, Bossolani GDP, Gomes ML, de Araújo SM, Bahia MT, Barbosa MGV, Toledo MJO. In vivo susceptibility to benznidazole of Trypanosoma cruzi strains from the western Brazilian Amazon. Trop Med Int Health. 2013;18:85–95.
86. Francolino SS, Antunes AF, Talice R, Rosa R, Selanikio J, de Rezende JM, Romanha AJ, Dias JCP. New evidence of spontaneous cure in human Chagas' disease. Rev Soc Bras Med Trop. 2003;36:103–7.
87. Dias JCP, Dias E, Filho OM, Vitelli-Avelar D, Correia D, Lages E, Prata A. Further evidence of spontaneous cure in human Chagas disease. Rev Soc Bras Med Trop. 2008;41:505–6.
88. Bertocchi GL, Vigliano CA, Lococo BG, Petti MA, Viotti RJ. Clinical characteristics and outcome of 107 adult patients with chronic Chagas disease and parasitological cure criteria. Trans R Soc Trop Med Hyg. 2013;107:372–6.
89. Morillo CA, Marin-Neto JA, Avezum A, et al. Randomized trial of benznidazole for chronic Chagas' cardiomyopathy. N Engl J Med. 2015;373:1295–306.