Review of Chagas Disease and Treatment Obstacles to Eradication

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Abstract

The World Health Organization and the Center for Disease Control reports Chagas disease, or American trypanosomiasis, as a major neglected tropical disease prevalent in 21 endemic Latin American countries. The agent of the disease is a single-celled protozoan parasite, *Trypanosoma cruzi*, and common modes of transmission include infected feces of a triatomine bug, crossing the placental barrier, and blood products. The two phases of infection are acute and chronic, and the three most affected body systems are cardiovascular, digestive, and nervous. Although research has led to several advances in the knowledge of CD, there are gaps in treatment and epidemiological research, leaving millions of people susceptible to infection.

Keywords: Chagas disease, Latin America, acute and chronic phases, kissing bugs, combination treatments

Introduction

Since the original identification and description of Chagas Disease (CD) by Carlos Chagas in 1909, scientists have achieved substantial progress in understanding this anthropozoonosis (disease transmitted from animals to humans) as evidenced in the 21 endemic countries by the decrease in number of deaths per year from more than 45,000 in 1985 to 12,000 in 2010 to 7,000 in 2016 (Pérez-Molina et al., 2018). However, even with a broader knowledge of CD, there are nearly 6 to 8 million infected individuals worldwide and between 60 and 80 million susceptible people living in endemic regions. Along with the advances in the last century, new problems and gaps in research are becoming evident, especially during the previous few decades. For example, kissing bugs (Order: Hemiptera, Family: Reduviidae, Subfamily: Triatominae) are the primary form of transmission and, since vector control protocols have been devised, other methods of spreading CD are taking the lead such as congenital transmission, usage of blood products, and food contamination. Additionally, advocacy campaigns identify and communicate the socioeconomic implications of CD yet clinical resources such as diagnostic tests and treatment are still deficient within many Spanish endemic populations.

Physiologically, there are two phases of *Trypanosoma cruzi* (a flagellated protozoan parasite) infection in the human body – acute and chronic. If diagnosis is prompt and treatment is successful, patients progress past the acute phase and either enter the asymptomatic indeterminate or symptomatic form of the chronic phase. Some literature classifies the acute, chronic indeterminate, and chronic symptomatic stages as three separate phases as opposed to two (Telleria and Tibayrenc, 2017). Of the individuals who develop clinical complications during the chronic phase, the three primarily affected body systems are the cardiovascular, digestive, and nervous system, respectively. Scientists are unable to identify the reason

previously infected patients remain in the indeterminate stage indefinitely while others suffer severe illness.

This review article explores recent etiologic studies, the life cycle of *T. cruzi*, effects of CD on humans, current medicinal practices such as diagnosis and treatment, and future challenges requiring further research. A comprehensive exploration into previous and current research regarding forms of treatment is the specific focus.

History

Carlos Chagas was participating in an anti-malaria campaign in 1908 located in Lassance, Minas Gerais, Brazil when a railroad engineer introduced him to kissing bugs containing the agent of CD – *T. cruzi* (Frederick et al., 2018). Before this experience, Chagas had already studied *T. minasense* in monkeys. However, by the age of 33, Chagas completed and published his research which incorporated the causative agent, vector, and human case studies of CD. All three discoveries were made within five months at Manguinhos Institute under his professor and mentor Oswaldo Cruz who founded this institute (Telleria and Tibayrenc, 2017). Chagas was a Brazilian clinician and researcher who contributed greatly to the scientific community's knowledge of CD and was elected in 1929 as a member of the Committee of Hygiene of the Society of Nations, the forerunner of the World Health Organization, before his death in 1934 at the age of 56 (Moncayo, 2010). Salvador Mazza, who characterized the three clinical stages of CD, and Cecilio Romanha, who identified the classic ocular edema symptom, were two other scientists notable for their contribution to the discovery of CD (Telleria and Tibayrenc, 2017).

Case number 1 of CD was found in a girl named Berenice, Carlos Chagas' first patient. Her symptoms as described by Chagas in his 1909 article included edema, fever, and anemia. Lana et al. located Bernice at the age of 78 and restudied her case (Telleria and Tibayrenc, 2017).

She was asymptomatic and, after finding circulating *T. cruzi* in her blood, was found to have the indeterminant chronic form like the 75% of patients diagnosed with CD.

According to Telleria and Tibayrenc, interesting facts, myths, and theories have arisen throughout the study of CD. For example, *T. cruzi* was initially but incorrectly associated to the goiter, which is now known to be a result of iodine deficiency not a pathogen (2017). Many current scientists believe Charles Darwin was affected by and died from Chagas Disease after contracting the protozoan during his time on the Galapagos Islands (2017). Lastly, DNA from the CL Brener strain of *T. cruzi* was the first to be sequenced, which was published in 2005. This strain has a 50% repetitive genome, which is 57% similar to the *Trypanosoma brucei* genome and 44% similar to the *Leishmania major* genome (2017).

Epidemiology

Latin American countries with the highest prevalence rate per 100 inhabitants of CD include Bolivia (6.1), Argentina (3.6), Paraguay (2.1), and Ecuador (1.4) ("Chagas disease in Latin America"). However, countries with the greatest number of CD infected people consist of Argentina (1.5 million), Brazil (1.2 million), Mexico (0.88 million), and Bolivia (0.61 million) (see Appendix A for further epidemiological information). Chagas disease is termed a neglected tropical disease, indicating a direct correlation between poverty and infection. Poorer populations are most affected because triatomine bugs reside within cracks and holes of houses, beneath bark, and in chicken coops, rodent burrows, and dog cages. With that said, the incidence rate of infection due to vector-borne transmission is much larger in Brazil (0.084) and Bolivia (0.081), while that of congenital transmission is largest in Paraguay (0.34), Belize (0.33), and Ecuador (0.32). Per year, there are roughly 41,000 cases of vectorial transmission and 14,000 cases of congenital transmission. Coupled with the fact 6-8 million individuals are currently

infected with CD, this data suggests control methods have been overall successful but not absolute.

In the United States, there have been 7 autochthonous vector-borne, 7 blood transfusion, and 6 organ donor related reported cases (Bern et al., 2011). Autochthonous implies CD was spread from one infected individual to another via a vector in the same geographical area. This occurrence is significant because it suggests the locations where suitable CD vectors are surviving their living conditions is expanding beyond solely Latin American countries. Nevertheless, these numbers are low due to the fact the United States has implemented vector control and blood screening protocols and is home to only 11 triatomine vector species, in comparison to over 100 species in South America (Bern et al., 2011). Chagas disease infections in dogs and livestock have been reported in the southern US, most notably in the state of Texas, where 351 canine cases were described between 2013 and 2014 (Curtis-Robles et al., 2015). Roughly 300,000 immigrants infected with CD live in the United States (Longo et al., 2015). Of the populations living in Andean countries, 25 million people are in danger of contracting the disease (Cura et al., 2017).

Life Cycle of *Trypanosoma cruzi*

The life cycle of *T. cruzi* has three stages – trypomastigotes, epimastigotes, and amastigotes (Carrea et al., 2016). Trypomastigotes are the largest, followed by epimastigotes and amastigotes. Unique structural features of *T. cruzi* are their kinetoplast (conglomerate of mitochondrial DNA situated near the nucleus), undulating membrane (associated with the flagellum to enhance locomotion), glycosome (specialized peroxisomes that perform carbon dioxide fixation, purine salvage, and pyrimidine synthesis, in addition to normal peroxisomal

functions), and acidocalcisome (elemental storage and osmoregulatory organelle) (Telleria and Tibayrenc, 2017). Amastigotes lack both a flagellum and undulating membrane.

The insect vector is the triatomine bug, or "kissing bug" (Order: Hemiptera, Family: Reduviidae). The nickname is derived from the fact triatomine bugs are attracted to the human mouth and eyes region. Kissing bugs' propensity to bite near the mouth and eyes is not random because they smell the higher concentration of carbon dioxide. Three common genera include *Triatoma, Panstrongylus, and Rhodnius.* Living throughout the entire region between southern United States and southern Argentina, these insects exist in both dry and wet climates.

The cycle begins when a triatomine vector, the kissing bug, ingests trypomastigotes from the blood of a mammalian host. A triatomine bug bite wound is visible and distinguishable from a typical insect bite such as from a mosquito because they are bigger, averaging a length of 1 inch. Inside the insect, epimastigotes form as trypomastigotes differentiate. Epimastigotes replicate by binary fission in the small intestine (midgut) and then differentiate into metacyclic trypomastigotes in the large intestine (hindgut). *T. cruzi* are referred to as stercoraria because of their posterior station development in triatomine bugs. The mode of parasite transmission differs from salivaria species such as *Trypanosoma brucei* in tsetse flies, which have an anterior station development. Next, the vector releases metacyclic trypomastigotes in their feces near the bite site on a mammalian host, which breaches the integument barrier through a cut, mucous membrane or conjunctiva, and results in infection. In other words, the moment of human infection is not during the vector's blood meal, rather when the mammalian host mechanically rubs the parasite into the open bite wound.

Intracellular and extracellular stages occur when *T. cruzi* resides within the mammalian host - amastigotes occur intracellularly and trypomastigotes occur in the bloodstream but not

inside blood cells. Inside human cells, the metacyclic trypomastigotes transform into amastigotes and replicate by binary fission. Intracellular amastigotes are able to transform into trypomastigotes when the cell ruptures. From this point, trypomastigotes may infect brain cells and muscle cells such as the esophagus, colon, and heart due to the fact this life cycle stage circulates in the bloodstream. The cycle completes as the secondary host, a vector, bites the primary host, an infected mammal and contracts the protozoan pathogen, becoming infected (see Appendix B for *T. cruzi* life cycle diagram).

While asexual reproduction occurs in triatomine bugs and sexual reproduction in humans, reservoir hosts may contract the parasite, display no signs of infection, yet transmit metacyclic trypomastigotes to humans. In the western United States, woodrats, cayotes, racoons and skunks are common reservoir hosts, while in the eastern United States, racoons, armadillos, skunks, and opossums are common reservoir hosts (Bern et al., 2011). Dogs are especially a concern for disease prevention because they are domestic and have large populations.

Prevention

Organ transplants, blood transfusions, laboratory accidents, and oral (foodborne), placental or congenital transmission are mechanisms of transferring CD other than via vector-borne transmission (Pérez-Molina et al., 2018). Vector control has been the primary mechanism of Chagas Disease prevention. This type of control includes the use of insecticides in infested homes and buildings. Vector control implementation also encourages the use of bed nets, insect repellent, and appropriate clothing. National control programs in developed countries have reduced the prevalence of CD through the enactment of blood screenings. However, the financial burden in several Latin American countries is too immense to enforce preventative measure beyond blood screenings. The yearly global expenditure on CD is $627 million, of

which the US spends $118 million (Meymand et al., 2017). Regarding chronically diseased individuals, antiparasitic treatments are the most promising route of research because they have the potential to be a turning point in the trajectory of the disease, which means developing a newer and more effective drug or vaccine is essential.

Medical Indications

Initially, due to the small size of the protozoan, an acute Chagas infection is asymptomatic. When they do occur, symptoms of the acute phase include fever, malaise, swollen lymph nodes, and inflammation at the infection site. The Romaña sign, named after Cecilio Romanha, is the unilateral swelling of the eyelid and a telltale sign of CD. Infants who congenitally contract the disease often show signs of hypotonia – reduced muscle tone –, fever, anemia, and hepatosplenomegaly – enlargement of both spleen and liver (Rassi et al., 2010). Life-threatening symptoms such as myocarditis and meningoencephalitis of the acute phase occur in less than 1% of cases. The incubation period post inoculation is 1-2 weeks and clinical manifestations subside within 4-8 weeks. Once infected with CD, 100% of untreated and 20% of treated people possess the disease indefinitely (Longo et al., 2015). Therefore, following this phase, patients advance to the chronic phase. The majority of these patients remain in the asymptomatic determinant stage, while 30-40% of untreated and 6-8% of properly treated people become afflicted years or decades later with symptoms of the chronic phase. Cardiomyopathy and megaviscera are two frequent problems associated with the organs affected by the symptomatic chronic phase. The heart and visceral organs represent 14-45% and 10-21% of the chronic symptomatic instances, respectively (Pérez-Molina et al., 2018).

Unexpected death is the foremost concern for patients with amastigotes replicating in their heart cells, which disrupts the conduction system and myocardium. Atrioventricular

blocks, tachycardia, bradycardia, sinus node dysfunction, and left ventricular aneurysms are usual causes of congestive heart failure for people with CD, contributing to poor prognoses. Chronic Chagas cardiomyopathy (CCC) has a 60% death rate due to arrhythmia and, of the children who die from heart disease in Latin American countries, 70% are caused by CCC (Carvalho et al., 2017). Furthermore, there is a stronger correlation between male patients and myocardial fibrosis in comparison to female patients (Assunção et al., 2016).

Gastrointestinal complications as a result of CD are widespread in the southernmost part of South America as opposed to the rest of Latin America, although it is present there as well. Megaesophagus and megacolon put patients at risk for esophageal cancer and persistent constipation, respectively. Specifically, the sigmoid colon and rectum become enlarged in the severe forms of megacolon, leading to abdominal distention, regurgitation, and bowel obstruction. About 5-20% of patients who experience myocardiopathy also have harmful gastrointestinal expressions. Furthermore, neurological involvement exists in up to 10% of cases and presents as neuropathy or polyneuropathy. Roughly 2.7 people suffering from CD induced cardiomyopathy undergo an ischemic stroke each year (Carvalho et al., 2017).

Overall, during symptomatic chronic CD, cardiac, gastrointestinal, and neurological complications are common. Amastigotes may be found in the myocardium, causing congestive heart failure, or in alimentary canal organs such as the esophagus, small intestine, and colon, which causes megaviscera. Less commonly, the brain and spinal cord are infected with amastigotes, resulting in meningoencephalitis or an ischemic stroke. Lastly, immunosuppressed patients are at risk for an exacerbated infection level and thus more likely to undergo symptoms such as meningoencephalitis and lesion formation in the central nervous system.

Pathogenesis

Chagas disease pathogenesis remains a subject requiring ample research due to genetic variability of the *T. cruzi* parasite and of the primary and secondary hosts. A pressing question for doctors and scientists studying CD is why chronic phase symptoms normally come about decades after persisting in a latency stage.

An inflammatory response and rapid antibody production are accompanied by acute phase infection (Pérez-Molina et al., 2018). Amastigotes inhabit tissues such as smooth, cardiac, and skeletal muscle, the brain, red blood cells, macrophages, and gonads. For example, the tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) of the innate immune response, which are proinflammatory cytokines, become activated. IFN- γ is essential for controlling parasite infections, but it also has a tendency to be overproduced, leading to the synthesis of reactive oxygen species and nitric oxide synthases.

The chronic phase of CD is identified by levels of inflammation. Specific *T. cruzi* strains produce varying levels of disease in people because certain strains are more virulent than others. In total, more than 6000 *T. cruzi* strains have been identified, of which strains TcI through TcV are the most common in relationship to CD (Brenière, Waleckx, & Barnabé, 2016). As stated previously, pathogenesis in the chronic phase is still an enigma waiting to be explained. However, in addition to the cytokines produced during the acute phase via $CD4^+$ T lymphocytes, cytotoxic processes are employed during the symptomatic chronic phase via $CD8^+$ T lymphocytes (Teixeira et al., 2011). Briefly, $CD4^+$ T cells, or T helper cells, are involved with innate immunity and are activated by major histocompatibility complex II (MHC II) molecules on antigen-presenting cells (APCs) (Janssen et al., 2003). Their main functions are cytokine secretion and activation of B cells and macrophages. $CD8^+$ T cells, or killer T cells, are involved with adaptive immunity and are activated by MHC I molecules on APCs. Their main functions

are secretion of cytotoxic granules and defense against intracellular parasites. Scientists believe overactivation of $CH8^+$ T cells is the reason for severe tissue damage such as megaviscera and cardiomyopathy during the symptomatic chronic phase (Pérez-Molina et al., 2018). In contrast, the indeterminate chronic phase may be referred to as a host-parasite equilibrium (Andrade et al., 1997).

Diagnosis

Diagnosis of CD varies with the infection phase of the parasite and, occasionally, with the mechanism by which the disease was acquired. Ideal diagnosis is early and confirmed by multiple methods. Wright-Giemsa stained blood, which stains human erythrocytes pink and human leukocytes purple, is sufficient enough for an acute phase diagnosis (Longo et al., 2015). Additionally, trypomastigotes may be seen by examining freshly coagulated blood under the microscope.

The method of choice for diagnosing a congenital Chagas infection is called microhematocrit, which ascertains the ratio of volume of red blood cells to the volume of total blood, allowing for relative quantification. Furthermore, xenodiagnosis introduces the blood from a patient being tested for the disease into a laboratory-bred triatomine insect and analyzes the insect's excrement over a period of two months to determine if the original blood sample contains the parasite. Xenodiagnoses is a suitable diagnostic method for infants who congenitally obtained chronic CD. If an infant has a negative test result for the presence of trypomastigotes and his/her mother was infected at the time of birth, doctors recommend that the infant be tested for anti-*T. cruzi* IgG at nine months. At this stage of development, babies no longer have maternal antibodies. Therefore, retesting the infant is important in ensuring he/she has developed a sufficient number of appropriate antibodies. Polymerase chain reaction (PCR) is

another technique used to diagnosis infants who are at risk of being infected due to congenital transmission. The process of PCR requires three steps – denaturing, annealing, and extending. Denaturing is the separation of DNA at a high temperature (94°C), annealing is the attachment of DNA primers to the resulting single-stranded DNA at a low temperature (54°C) and extending is the synthesis of a new strand of DNA at a medium temperature (72 °C). PCR is preferred over microscopic visualization because it is more sensitive and detects even the slightest existence of a parasite's DNA in human blood.

Those with a chronic phase infection must undergo two of the following diagnostic procedures for a confirmatory diagnosis: serological test of IgG, PCR, enzyme-linked immunosorbent assay (ELIZA), indirect immunofluorescence, or hemagglutinin assay. The necessity for extensive clinical tests is because the parasite is too small during the chronic phase to be detected by microscopy (Edimar et al., 2017). An electrocardiogram (ECG) test typically accompanies the former assays in diagnosing symptomatic chronic CD. Positive results from an ECG include sinus tachycardia and low QRS voltage.

Prognosis

As stated before, the incubation period after vector-borne transmission is 1- 2 weeks. The incubation period is 1- 6 weeks following blood transmission, and 2 days to 3 weeks following oral transmission (2017). There are 5 classifications for patients with CCC. Type A is positive for *T. cruzi.* Type B1 is positive for heart disease and negative for ventricular dysfunction. Type B2 is positive for heart disease and ventricular dysfunction but does not indicate heart failure. Type C is positive for heart disease, ventricular dysfunction, and heart failure. Lastly and most critically, type D patients have extremely advanced and unmanageable heart failure.

Since CCC is the worst manifestation of CD, the mortality prognosis for these patients is 12% after 1 year, 35% after 5 years, and 60% after 10 years. Yet, patients diagnosed with type D have less than a 10% survival rate after a year. Accurate predictors of mortality are left ventricular systolic function, cardiomegaly or ventricular tachycardia (Rassi et al., 2010).

Treatment

The two anti-parasitic medications for acute CD are benznidazole and nifurtimox. However, only the former is approved by the U.S. Food and Drug Administration (FDA) because the latter is more toxic and must be taken in larger doses and for a longer period of time (Menezes et al., 2012).

Benznidazole (Bnz) is the first-line treatment due to its less harsh side effects, which are a result of an enzyme catalyzed reduction of the nitro functional group producing metabolites (Molina et al., 2015). Rashes are frequent side-effects, but dermatitis, fever and/or lymphadenopathy – disease of the lymph nodes – are less common but significant enough that termination of the medication is suggested if they occur. The rarest side effect is bone marrow suppression, which warrants immediate termination and usually hospitalization. General and less serious complications include headache, anorexia, and insomnia. Infected children and adults should take 5-10 mg/kg per day for 60 days (Rassi et al., 2010). Of the CD patients who were administered Bnz during a case study in Spain, 22% terminated the course of treatment within 30 days due to severe side effects and 30% ended treatment before the end of the study (Pérez-Ayala et al., 2011). Women have a higher hypersensitivity to Bnz, an unfortunate fact since females are at a greater risk of infection.

Nifurtimox interferes with pyruvic acid synthesis and carbohydrate metabolism. Approximately 70% of patients using this medication experience gastrointestinal side effects. Other unwanted neurological effects may include irritability, tremors, or insomnia. Rare but more serious physiological side effects involve the peripheral nervous system. These may include pressure on peripheral nerves, polyneuropathy, or neuritis. Infected children should take 15 mg/kg and adults 8-10 mg/kg daily for 60-90 days.

Overall, 80-90% of patients are cured from the acute phase by treatment with benznidazole or nifurtimox at the earliest onset. Recent studies suggest that either of these drugs may successfully treat children with chronic CD due to early exposure. For example, these drugs cure 8% of adults and 62% of children at the asymptomatic indeterminant phase (Menezes et al., 2012). The efficacy of both drugs is higher in children than adolescents and adults. The benefit of taking either anti-trypanosomal treatment for people over the age of 5 has not been demonstrated.

The major courses of treatment for patients with the chronic form is slowing the progression of the disease and managing symptoms. For instance, a symptomatic chronic patient experiencing atrioventricular blocks may need a pacemaker. Ventricular arrhythmias as result of CCC are often treated by doctors with the amiodarone drug, which blocks abnormal cardiac electrical signals, reducing the chances of an irregular heartbeat (Stein et al., 2018). For individuals with refractory heart failure, cardiac transplantation is the ideal course of treatment. Fundoplication is a surgical procedure that wraps the upper portion of the stomach around the lower portion of the esophagus and prevents gastro-esophageal reflux. Nitrates and nifedipine are two drugs that some medical professionals administer to gastrointestinal CD patients to help lower esophageal-sphincter relaxation and assist in directing food and liquids past the esophagus.

A fiber rich diet, intake of ample liquids, and consumption of laxatives are appropriate methods of treating colon motility dysfunction – constipation, diarrhea and/or abdominal cramping. Fecal impaction may occur in later stages of colon dysfunction and may require surgery to manually dislodge contents.

Socioeconomic Challenges of the Disease

Triatomine bugs are prevalent in rural and impoverished areas. Of the neglected tropical diseases, CD is one of the costliest (Nunes et al., 2013). This makes management of the disease difficult for undeveloped and developing countries and low-income households. In 2013, the global expenditure was $7.19 billion per year, which was spent on research, medical treatments, and diagnostic techniques (2013). While this quantity has halved since 2013, CD is still responsible for huge economic hindrances. The United States and Canada are responsible for providing 10% of the yearly funds needed to fight against CD.

Future Research Areas and Opportunities

Three pressing and unanswered questions about Chagas Disease include (1) the pathophysiology of chronic Chagasic cardiomyopathy, (2) role of autoimmunity in pathogenesis, and (3) the relationship between the severity of a CD infection and the virulence of the *T. cruzi* strain. Researching these topics could ultimately lead to the invention of a suitable vaccine or, at the very least, a better understanding of the diversity of the protozoan and the effects it induces in the human body.

Standardized animal models that better represent the effectiveness of new drugs need to be developed. Training healthcare professionals to treat CD patients and relocating them to endemic areas of Latin America has been a challenge but is imperative, especially because long-term care is required for all previously infected patients. Developing sustainable preventative

regulations, devising a plan to make diagnostic equipment and treatment available since CD is a poverty-related infection, creating more advantageous and affordable medications, and conducting epidemiology studies are also sought-after achievements.

Chagas Disease Overview

In short, Chagas disease remains an important area of research due to the number of affected lives each year in Latin America. Since its discovery, the number of annual deaths has significantly decreased, but a lot of work still needs to be done in order to develop a vaccine or a more acceptable medication. The acute phase is better understood in terms of symptoms, physiology, and predictability compared to the chronic phase. Advanced diagnostic techniques have been devised for CD. Diagnosing an infected patient is able to be done fairly quickly and accurately. However, the problem rests in the fact numerous patients do not have access to medical care and, when they do, are limited. Early detection of the disease is crucial for even a remotely positive prognosis. The two anti-parasite medications, benznidazole and nifurtimox, have an increased efficacy when distributed at the earliest onset. As time passes, adult generations are growing older and, since current CD drugs have unknown effects in the elderly, the hope of success in the abundant clinical trials being conducted is high.

Current Treatment Research

Since the two forms of Chagas Disease treatment – benznidazole (Bnz) and nifurtimox (Nfz)– have severe side effects and limited efficacy, research into new forms of medication is imperative. Previously tested medications include ketaconazole, posaconazole, ravuconazole, E5700, amiodarone, K777, aryl-imidamine, and allopurinol (Menezes et al., 2011). While these experiments had promising results, they did not yield a cure. In other words, they lead to symptom alleviation, reduced parasite load, and improved organ function, but did not provide a

cure on the organismal level. Research into the development of novel pharmaceutical medications is time-consuming and expensive, hence the numerous emerging low dose Bnz combination therapy experiments being conducted. In addtition, various vaccine options are also being explored, but success is limited due to the antigenic variation ability of *T. cruzi*.

Drug Combination Therapies

Thiazole and furan compounds. In an experiment testing the effects of drug combinations, three compounds, when individually mixed with Bnz, showed various results. Thiazole compound **2**, furan compound **3**, and thiophene compound **4** were additive, synergistic, and antagonistic against *T. cruzi* epimastigotes, respectively (Aguilera et al., 2018). Compound **4** was removed from research because the *in vitro* studies disproved its effectiveness for treatment of CD in combination with Bnz.

The results of the *in vivo* phase of this experiment, which involved intraperitoneally injecting 5,000-10,000 blood trypomastigotes in male mice, were positive. The experimental groups were orally treated with either compound **2** alone, compound **2** and Bnz, compound **3** and Bnz, or Bnz alone. The dosage schedule of compound **2** alone (50 mg/kg b.w/day), Bnz alone (5, 10, 25, 50, and 100 mg/kg b.w/day) and combination of compound **2** (50 mg/kg b.w/day) and Bnz (5 mg/kg b.w/day) was three cycles of five consecutive day administrations with two day intervals, while the dosage schedule of compound **3** (50 mg/kg b.w/day**)** and Bnz (10 mg/kg b.w/day) was 15 consecutive day administrations (2018). The compound **2** and Bnz experimental group overcame the second parasitemia infection peak at day 37, maintained 100 percent survival at the end of the 60-day trial, and decreased in the number of anti-*T. cruzi* antibodies. This is significant because the survival rates of compound **2** alone and the control group after 60 days were 83 percent. Lastly, the compound **3** and Bnz experimental group

shifted the first maximum parasitemia infection peak from day 21 to day 30, abolishing the second peak, maintained 100 percent survival, and had lower anti-*T. cruzi* antibody levels. This is significant because the survival rate of the control group after 60 days was 85 percent. In comparison to **2** plus Bnz, **3** plus Bnz demonstrated the best outcome against *T. cruzi* due to its synergistic effect, lowering the first and completely eliminating the second parasitemia peaks. In the future, these chemical compounds may prove to potentiate the efficacy of Bnz and lessen its side effects through use of smaller dosages. Pharmacological research, modifying the treatment administration schedules, is currently being conducted.

Vitamins. Cyanocobalamin or vitamin B_{12} (Vit B_{12}) has antiparasitic properties when combined with Bnz and used to treat *T. cruzi* infected mice. Initially, *in vitro* studies using Vit B_{12} on a culture of *T. cruzi* epimastigotes resulted in an indirect relationship between concentration of Vit B_{12} and parasitemia levels (Ciccarelli et al., 2012). However, the decreased growth rate lasted for only a short period of time subsequent to treatment, indicating a reversal of the antiparasitic activity of Vit B_{12}.

In vivo antiparasitic activity was tested despite *in vitro* method results because the short timeframe of efficacy may have been attributed to culture instability. Mice were intraperitoneally infected with 5,000 blood trypomastigotes and treated five days later. Groups 1-5 were administered Vit B_{12} alone (1.5 mg/kg b.w/day), Vit B_{12} (1.5 mg/kg b.w/day) plus Vit C (1.5 mg/kg b.w/day), Bnz (0.75 mg/kg b.w/day) alone, a combination of the three compounds, and a vehicle control, respectively (Ciccarelli et al., 2012). The dosage schedule for all groups was two cycles of 5-day administrations with a two-day interval. The parasitemia levels on day 13 for groups 1-5 were 2.23×10^6, 1.26×10^6, $1.43\ 10^6$, $1.33\ 10^6$, and $4.18\ 10^6$ parasites/ml, respectively. Since group 5 was the control, these results indicated that all experimental groups

experienced a lower parasitemia level due to the treatment, but Bnz alone and Bnz combined with both vitamins had the lowest infection levels. Moreover, no group had a 100 percent survival rate. The control had zero percent survival, Bnz alone and Bnz plus both vitamins had 70 and 80 percent survival, respectively. Therefore, considering both parasitemia levels and percent survival, group 4 treated with Bnz plus Vit B_{12} and Vit C displayed the most optimal results.

During a recent study conducted in the same laboratory as the previous combination assay with Vit B_{12} and Vit C, a team first focused on Vit C's antioxidant and pro-oxidant activities and then tested its ability to potentiate antiparasitic mechanisms of Bnz (Puente et al., 2018). When present at physiological levels, Vit C acts as an antioxidant, neutralizing free radicals. When present at high levels due to pharmaceutical intervention, Vit C is catalyzed by metallic ions and acts as a pro-oxidant, generating reactive oxygen species (ROS). The authors hypothesized that Vit C acts as a pro-oxidant inside the parasite where the concentration is higher due to a smaller cell size but as an antioxidant inside the human body where the concentration is lower due to a larger cell size.

Three life cycle forms of *T. cruzi* were tested during the *in vitro* stage – epimastigotes, trypomastigotes, and amastigotes (Puente et al., 2018). The results of Vit C alone versus Bnz alone were similar between the three parasitic forms. Trypomastigotes, the infective stage of *T. cruzi*, was used in the *in vivo* phase. Mice were intraperitoneally injected with 5,000 trypomastigotes and treated with either Vit C (1.5 mg/kg b.w/day), Bnz (0.75 mg/kg b.w/day), a mixture of both, or a vehicle control seven days later.

The results indicated that Vit C serves as a pro-oxidant when given alone and as an antioxidant when given in combination with Bnz. However, it does not play both roles of pro-

oxidant against *T. cruzi* and antioxidant for the host because similar lysis percentages where determined for the combination and Bnz alone experimental groups. The minimal concentration of Vit C alone needed to employ cytotoxic effects is 2,500 uM. Conclusively, this study suggests that the presence of Bnz reduces the effects of Vit C to merely antioxidant by eliminating its pro-oxidant mode of action.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are harmful substances present in individuals infected with CD. Bnz and Nfx, when metabolized, also produce ROS and RNS, amplifying the toxicity experienced by the patient and leading to multi-organ inflammation, a common symptom of CD. Another study, published by a different laboratory but in the same month as the previous article, also attempted to reduce the oxidative environment, by performing a combination experiment utilizing Bnz (Providello et al., 2018). Ascorbic acid (AA) or vitamin C (Vit C), an antioxidant, was the medication used in combination with Bnz. Male murine models were used and intraperitoneally injected with 10,000 blood *T. cruzi* trypomastigotes. Five groups were formed and included (1) control without infection nor treatment (2) infected control without treatment, (3) infected and treated with Bnz (10 mg/kg b.w/day), (4) infected and treated with AA (7 mg/kg b.w/day), and (5) infected and treated with AA and Bnz (AA+Bnz10; at7:10 mg/kg b.w/day). Drugs were administered for 15 consecutive days starting two days subsequent to infection.

In vivo research determined decreased parasitemia levels in the AA+Bnz10 group compared to the AA and Bnz alone experimental groups (Providello et al., 2018). Additionally, the mice who were administered both AA and Bnz had lower intracellular ROS, diminished cardiac parasitemia and inflammation, and eliminated hepatic inflammation at the end of the 17-day protocol. The findings of this drug association study suggest AA may be another potential

chemical compound that, when combined with Bnz, protects against *T. cruzi* infections by minimizing intracellular oxidative damage and reducing inflammation symptoms. During clinical trials, Bnz plus AA improves cardiac arrhythmia symptoms of chronic CD patients, but additional research is needed.

Pentoxifylline. The chronic form of Chagas disease is predominantly attributed to the disease's morbidity and mortality rates because it causes Chagas cardiomyopathy. Symptoms associated include abnormal heartbeats, altered electrocardiogram (ECG) results, and myocardium inflammation, as well as high and constant levels of parasitemia. Worsening fibrosis gives rise to congestive heart failure and, in several cases, unexpected death.

Nitric oxide (NO) and tumor necrosis factor (TNF) are important components of both acute and chronic phases of CD. In moderate concentrations, the former possesses trypanocidal functions and the latter binds to G protein-couple serpentine receptors, inducing the upregulation of inducible NO synthase (iNOS), which catalyzes the formation of NO (Silva et al., 2003). However, persistent high concentrations of NO have been attributed with the development of CCC. Infliximab is an anti-TNF drug and, when given to acute CD patients, slowed and reduced cardiac tissue damage but did not interfere with parasitemia controls. Bnz is used to lower parasite loads. For patients who do not receive early treatment, CCC develops, meaning Bnz is unable to protect against deteriorating health due to the establishment of immunological imbalances. In short, administered Bnz and naturally synthesized NO and TNF work together to protect the body against a *T. cruzi* infection by initiating an appropriate inflammatory response and attacking the protozoan. Yet, when Bnz is not administered early, NO and TNF are not sufficient enough to treat the parasite infection and their effects actually become harmful due to

prolonged inflammation. Pentoxifylline (PTX) is an immunoregulator that, when combined with Bnz, has shown to alleviate and reduce these symptoms of CCC (Silva et al., 2003).

The Colombian strain of *T. cruzi* was used in a chronic murine model and 100 blood trypomastigotes were intraperitoneally injected. Eighty percent of the infected mice survived with treatment until 120 days post infection, surpassing the acute phase. The first presence of blood trypomastigotes indicates the beginning of the acute phase and a drop-in blood trypomastigotes after a high elevation indicates the beginning of the chronic phase. From parasitemia assays, day 14 and 90 were deemed the onset of the acute and chronic phases, respectively (Vilar-Pereira et al., 2016). During the chronic phase, from 120 to 150 days post infection, mice were orally treated with either (1) Bnz alone (25 mg/kg b.w/day), (2) PTX alone (20 mg/kg b.w/day), or (3) Bnz plus PTX. On day 150, all infected mice that received treatment survived while only 80 percent of the vehicle control group survived.

Results of this *in vivo* study supported the initial hypothesis which stated suboptimal levels of Bnz in combination with PTX would not only lessen CCC symptoms of fibrosis and inflammation, but that the effects would persist past termination of treatment. Notable outcomes of the Bnz plus PTX group included minimized atrial flutter – rapid atrial chamber pumping – and ameliorated second-degree atrioventricular blockages (Silva et al., 2003). Additionally, reversal of the ECG QT wave abnormality, which is described as a frequent cause of death in CCC patients, and rapidly decreased NO and TNF production was evidenced just in the combination therapy group. These finding do not suggest the therapies completely eliminated levels of parasitemia and restored health, rather Bnz plus PTX acted as an adjuvant by normalizing immune system responses and suppressing elevated inflammation. Clinical trials are the next step in research based on the fact the infected murine model experienced an

improved prognosis after treatment with 25 percent of the normal dose of Bnz in combination with the already clinically proven benign drug PTX (Vilar-Pereira et al., 2016).

Vaccinations

80 kDa prolyl oligopeptidase. A recent experiment isolated a *T. cruzi* 80 kDa prolyl oligopeptidase (Tc80) gene from strain RA and cloned it into the pcDNA3.1 plasmid. Tc80 is the virulent protozoan gene responsible for breaking down extracellular matrix components and establishing easy access into the host's tissues and cells (Bivona et al., 2018). The delivery system was *Salmonella enterica* bacteria, the restriction sites were *Hind*III and *XhoI*, and a murine model was utilized. Group I intramuscularly received rTc80 (10 ug) with oligodeoxynucleotides CpG 1826 (10 ug) and group II orally received weakened *Salmonella* with a Tc80 plasmid ($1x10^9$ CFU/mouse). Group III was administered a combination of the previous two and group IV mice, the control, were intramuscularly given phosphate-buffered saline (PBS) and CpG-ODN (10 ug) and orally given weakened *Salmonella* with a blank plasmid. Four doses were administered with three 10-minute intervals.

Functions unique to the immunized mice included counterbalanced parasitemia levels, Tc80 enzyme inhibition, and trypomastigotes cell lysis. Mice treated with weaken forms of the *Salmonella* Tc80-gene carrying plasmids, groups I and II, possessed the highest percent survival, representing the significance of cell-mediated immunity with active antigens as opposed to humoral-mediated immunity with passive antibodies (Bivona et al., 2018). While antibiotic responses are needed during onset of acute CD, cytotoxic responses are essential in order to prevent the long term, lethal consequences accompanying chronic CD. Therefore, the authors' hypothesis stating Tc80 is an effective gene target for the development of a novel vaccine fighting CD infections was supported.

Recombinant enolase. Another study isolated recombinant enolase (rTcENO) and its gene (pBKTcENO) from *T. cruzi* strain H8 (Arce-Fonseca et al., 2018). In glycolysis and gluconeogenesis, intracellular enolase uses either magnesium or manganese to convert 2-phosphoglycerate into phosphoenolpyruvate and water. On the cell surface of pathogens, enolase is also a receptor, hence its utilization as a potential vaccine target. Protein and DNA vaccines were tested in two experiments. During the first, mice were intraperitoneally immunized with rTcENO (10 ug) or the PBS control. During the second, mice were intramuscularly injected with pBKTcENO (100 ug) or the DNA vector control.

Notable results included the presence of antibodies against epimastigotes after both types of immunization. However, 75% of the rTcENO-immunized mice lived compared to the PBS control while none of the DNA vector-immunized mice lived compared to the DNA vector control (Arce-Fonseca et al., 2018). Moreover, IL-2 and IFN-gamma production increased only after rTcENO vaccine deliverance. IL-2 and IFN-gamma are released in response to Th1 and IL-4, IL-5, and IL-10 are released in response to Th2. Th1 and Th2 cell responses play a role in immunoglobulin synthesis, a process necessary in the removal of intracellular and extracellular parasites, respectively. Together, these findings suggest rTcENO vaccine efficacy and pBKTcENO vaccine inefficacy against acute *T. cruzi* infections, laying the foundation for further research.

Amastigote surface protein-2. The most recent publication exploring vaccinations for *T. cruzi* infections is by Ribeiro et al., which studied the effects of adenovirus expressing amastigote surface protein-2 (AdASP-2) injected into the tibialis anterior muscle on mice livers (2019). The groups used during this study included group 1 (50 μL vehicle control), group 2 (150 *T. cruzi* blood trypomastigotes of Y strain and 50 μL vehicle control), group 3 (50 μL of

AdASP-2), and group 4 (150 *T. cruzi* blood trypomastigotes of Y strain and 50 μL AdASP-2). All mice were subcutaneously infected and intramuscularly immunized (2019).

Data collected during this experiment was the gene expression of nine substances associated with the immune system and located in the liver – COX-2, TNF, TNF-α, iNOS, cytochrome c, caspase-3, TLR4, IL-6, and IL-10 (Ribeiro et al., 2019). After histological, genetic, and statistical analysis of acute CD infections, TNF-α, iNOS, TLR4, and IL-10 were shown to be vital inflammatory cytokines that control infection levels. On the other hand, the anti-inflammatory apoptotic pathway via cytochrome c and caspase 3 was not initiated as a result of the AdASP-2 vaccine (2019).

In summary, AdASP-2 removed trypomastigotes after a sublethal exposure and triggered tissue regeneration. In regard to Chagas disease eradication efforts, vaccine AdASP-2 shows promising results.

Unknown Impact on Latin American Countries

Telleria and Tibayrenc, in their book titled *American Trypanosomiasis: Chagas Disease: One Hundred Years of Research,* discuss two reasons for Chagas Disease being regarded as silent (2017). Firstly, the heart and digestive symptoms of the chronic stage often manifest long periods of time after the initial infection, leaving patients unaware of their illness for several years and even decades. Secondly, the social implications associated with CD foster literal silence in both endemic and nonendemic populations. For instance, poverty, economic instability, poor living conditions, and political distress are factors resulting in and caused by Chagas disease. Uneducated locals residing in mud huts of endemic regions that lack strong political governments and influence are most at risk (2017).

The financial burden of CD and the economic crisis most Latin American countries endure also contributes to a lack in prospective cohort studies as well as retrospective case studies. A prospective study follows a patient or group of patients from before the onset of infection over a long period of time while a retrospective study traces a symptomatic patient or group of patients backwards to examine factors leading to infection. Since the former type of experiment is more controlled and contains less opportunity for bias, the results yield accurate epidemiological rates and risk factors in comparison to the latter type of experiment.

A cohort study by Pérez-Ayala et al. was conducted in Madrid, Spain from 2003 to 2009 (2011). Throughout the 7-year study, 1146 and 337 Latin American immigrants were screened by serology and PCR, respectively. Positive results were seen in 31% of the former and 61% of the latter. After further investigation, 97% were from Bolivia, 68% were female, and the majority were between the ages of 30 and 40 (2011). A 2009 case study was conducted on a 47-year-old man in the United States. He initially presented to the American hospital with complaints of laterally radiating abdominal pain and constipation that was intermittent for 15 years but became severe 5 weeks' prior. The patient was born in Central America, immigrated to the US twenty-five years before this hospital visit, and frequently travelled to his birth country. Subsequent to extensive clinical investigations and tests, he was diagnosed with Chagas disease due to a chronic *T. cruzi* infection (Isselbacher et al., 2010).

Chagas disease research such as the cohort study in Spain and the case study in the US not only reinforce the expansion of the disease and its challenging diagnosis but suggest an unknown severity in Latin America. The examined participants from Spain and the patient from the US all immigrated from Latin America, where their infection was acquired and where there is a scarce supply of similar studies being conducted.

The lack of both prospective and retrospective studies combined with the fact lower socioeconomical classes are most affected suggests that an accurate CD prevalence rate is unknown and likely higher than reported.

Conclusion

Over the span of 110 years, incredible progress has been made in regard to minimalizing Chagas disease and its negative impact on Latin American communities. This success was initiated in 1909 by Carlos Chagas who identified the agent (*T. cruzi*), vector (kissing bugs), and clinical manifestation (cardiac, visceral, and nervous) of CD within a 5-month timeframe. Other notable achievements were the discovery of the *T. cruzi* life cycle, determining the metacyclic trypomastigote infective stage and the intracellular amastigote diagnostic stage, prevention techniques such as vector control and screening of blood donation products, pharmaceutical treatments of benznidazole and nifurtimox, and acute and chronic phases of infection.

Significant research into drug combination therapy as well as vaccinations has been conducted. In low dose combinations with Bnz, thiazole and furan compounds delayed the onset of acute parasitemia spikes and lowered the concentration of anti-*T. cruzi* antibodies, Vit C had a reduced mode of action to merely antioxidant, ameliorating the negative impacts of ROS, and PTX lowered the presence of fibrosis and inflammation in CCC patients. The vaccine efforts of rTc80 indicated lower parasitemia levels and trypomastigote cell lysis, while rTcENO showed the production of *T. cruzi* antibodies, and AdASP-2 suggested tissue regeneration and removal of trypomastigotes.

Considering the high volume of promising research yet deficiency of funding for further explorations, CD has not been eradicated. Additional factors contributing to the current prevalence of CD consist of the *T. cruzi* antigenic variation ability, existence of an unknown

number of individuals with an asymptomatic indeterminant phase of infection, expansion of suitable living environments for kissing bugs into the southern US, and limited quantity of prospective and retrospective studies available for review.

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Appendix A

	Prevalence rate of *T. cruzi* infection per 100 inhabitants	**Number of people with a *T. cruzi* infection**	**Number of new annual cases due to vector transmission**	**Number of people with Chagasic cardiopathy**	**Prevalence rate of *T. cruzi* infection per 100 US immigrants**	**Prevalence rate of *T. cruzi* infection per 100 European immigrants**
Argentina	3.6	1,500,000*	1100	380,000*	--	2.2
Belize	0.33	1,000	10	200	--	--
Bolivia	6.1*	610,000	8100	120,000	25.79*	18.1*
Brazil	0.03	1,200,000	46	230,000	--	0.6
Ecuador	1.4	200,000	2,000	40,000	--	0.4
El Salvador	1.3	90,000	970	18,000	3.66	5.6
Guatemala	1.2	170,00	1,300	21,000	2.88	--
Honduras		73,000	930	15,000	2.94	3.7
Mexico	0.8	880,000	6,100*	70,000	--	1.5
Paraguay	2.1	180,000	300	33,000	--	5.5

Table 1. Approximated epidemics and demographics of Chagas disease in 10 Latin American countries (“Chagas disease in Latin America”).

Appendix B

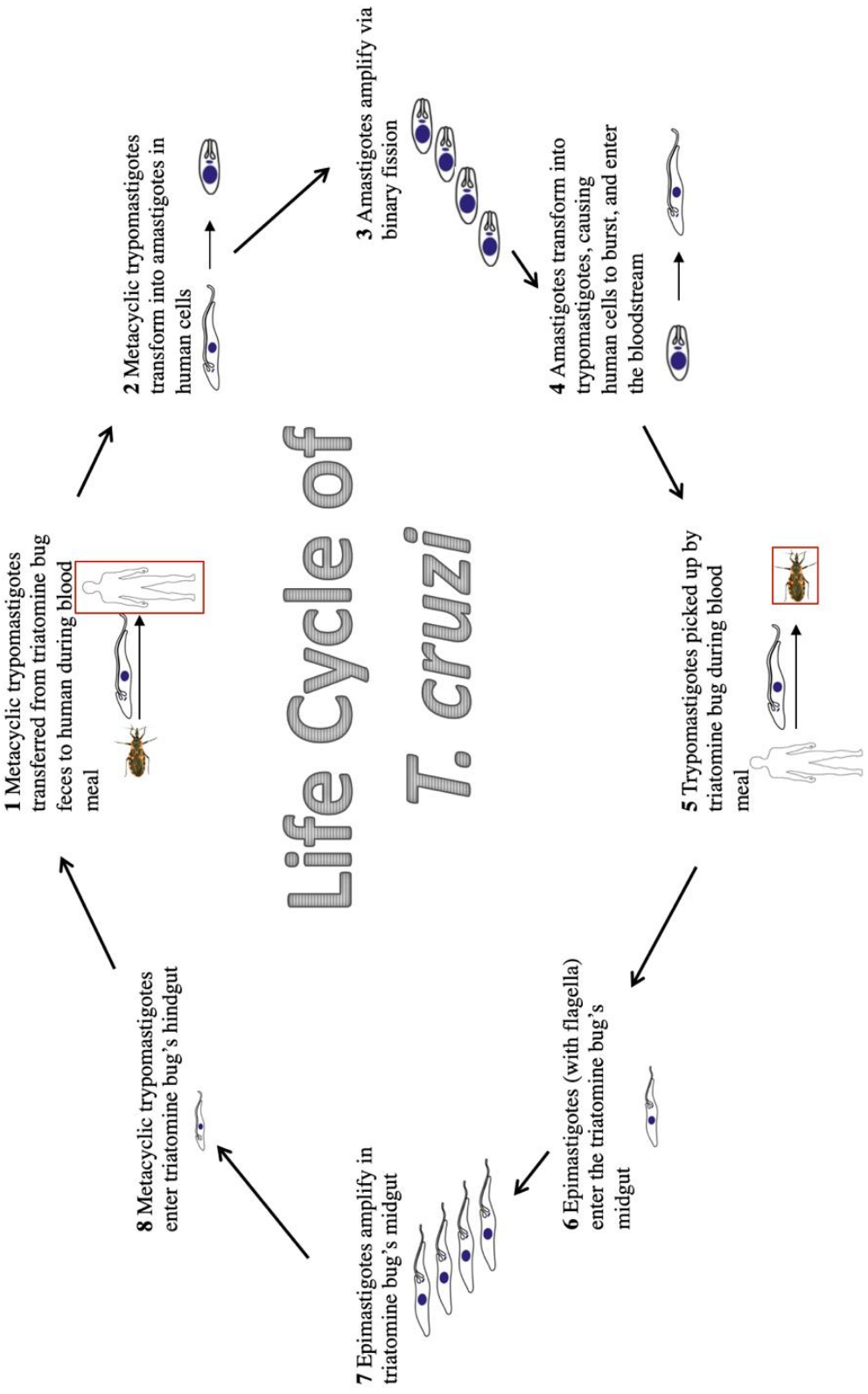

Figure 1. The life cycle of *T. cruzi,* showing various stages and transmission points.