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REVIEW ARTICLE

## Chagas disease: Current perspectives on a forgotten disease



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**Abstract** Chagas disease is a parasitic zoonosis caused by *Trypanosoma cruzi*, a protozoan whose transmission to humans is primarily vector-borne. It is estimated that 6–8 million people worldwide are infected and that 65–100 million people are at risk of becoming infected. Its clinical spectrum is very broad. During the acute phase, non-specific manifestations develop that may go unnoticed. During the chronic phase, specific manifestations develop that are diagnosed late and increase the morbidity and mortality of those suffering from it. The drugs available to treat it are partially effective, and the efforts made to develop a vaccine remain insufficient. This article reviews the most significant aspects of Chagas disease, from the discovery of the disease to the development of a vaccine, to help train general practitioners and specialists to provide timely care to those suffering from the disease.

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**PALABRAS CLAVE**

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*Trypanosoma cruzi*

**Enfermedad de Chagas: Perspectivas actuales sobre una enfermedad olvidada**

**Resumen** La enfermedad de Chagas es una zoonosis parasitaria causada por *Trypanosoma cruzi*, un protozoo que se transmite principalmente de manera vectorial al ser humano. Se estima que entre 6-8 millones de personas alrededor del mundo se encuentran infectadas y que entre 65-100 millones están en riesgo de infectarse. Su espectro clínico es muy amplio, pudiendo desarrollar manifestaciones inespecíficas durante la fase aguda que pueden pasar desapercibidas y manifestaciones específicas durante la fase crónica que se diagnostican tardíamente e incrementan la morbimortalidad de quienes la padecen. Los medicamentos disponibles para su tratamiento son parcialmente eficaces y los esfuerzos para crear una vacuna aún continúan siendo insuficientes. En este artículo revisamos los aspectos más relevantes de la enfermedad de Chagas desde su descubrimiento hasta la vacuna, con el objetivo de contribuir en la preparación de médicos generales y especialistas para que proporcionen atención oportuna a quienes la padezcan.

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**Introduction**

The World Health Organization (WHO) recognises American trypanosomiasis, or Chagas disease, as one of the 17 neglected tropical diseases, which have persisted in the poorest, most marginalised societies.<sup>1</sup> It is estimated that close to 6–8 million people worldwide are infected with *Trypanosoma cruzi* and that 65–100 million people are at risk of becoming infected.<sup>2</sup> The majority are in Latin America, where the disease represents a major problem with respect to the morbidity and mortality of the general population, and has become a burden that depletes the region's economic resources and affects the social and occupational environment of those suffering from it.<sup>3</sup> International migration has led to the influx of infected subjects from Latin America to the rest of the world, thus rendering the disease a problem for health systems on a global scale.<sup>4</sup>

**Historical background**

The first signs of Chagas disease date back almost 9000 years. Evidence of *T. cruzi* infection has been found in mummies from northern Chile and southern Peru. In the 18th and 19th centuries, explorers and naturalists such as Charles Darwin provided the first reliable descriptions of its existence and behaviour, without making any association between the parasite, vector, and disease. Only in 1909 did Dr Carlos Justiniano Ribeiro das Chagas make this association, thus giving the disease its eponym. Chagas had been sent to work on a campaign to eradicate malaria while new roads were being constructed in Minas Gerais, Brazil. At the same time, he became interested in studying the presence of insects that abounded in dwellings in precarious areas and fed on blood. When he dissected the insect and studied its gastrointestinal tract, he found protozoa which he identified as belonging to the genus *Schizotrypanum* (now *Trypanosoma*). He called them *T. cruzi* in homage to Dr Oswaldo Cruz, an epidemiologist and teacher. Later on, Dr Salvador Mazza managed to bring the disease to the interest of the

scientific community, redefined the route of transmission, and described the signs and symptoms of the acute phase. Romána, Jörg, Diaz, and Laranja described the signs and symptoms of the chronic phase.<sup>5,6</sup> Between the 1950s and the 1960s, migration from rural to urban areas brought about the "urbanisation of the disease".<sup>7</sup> The first programme to control and prevent the disease (Chagas Disease Control Programme) was created in 1960, while the amount of scientific research conducted was increased in the 1970s. The first and only antitrypanosomal drugs, nifurtimox and benznidazole, were developed in 1972 and 1980, respectively.<sup>6,8</sup>

The first cases in Mexico were reported in 1940 by Dr Luis Mazotti, who described its presence in two patients in Tejomulco, Oaxaca. In 1950, Perrín published the first case of chronic Chagas heart disease. In 1956, the Mexican National Campaign to Eradicate Malaria (CNEP) was launched. In this campaign, dichlorodiphenyltrichloroethane (DDT) was systematically sprayed on millions of dwellings located in malarious areas of Mexico that also corresponded with areas of endemic Chagas disease. Between 1966 and 1967, Biagi and Tay conducted seroepidemiological surveys in different Mexican states, reported the first cases of Chagas heart disease confirmed by parasitology, and used the indirect immunofluorescence (IIF) technique for the first time to diagnose the disease. Between 1972 and 1974, Zavala-Velásquez used nifurtimox for the first time for therapeutic purposes. In 1996, Guzmán-Bracho described the first case of congenital Chagas disease in Sahuayo, Michoacán, Mexico. In 1983, systematic treatment with nifurtimox and benznidazole was started at the cost of purchasing a small batch for the Clinical Unit of the Instituto de Salubridad y Enfermedades Tropicales (ISET).<sup>9,10</sup> Finally, in 2009, the Mexican national programme started to offer medicines to treat cases recorded at the state level.<sup>4</sup>

**Epidemiology**

According to WHO estimates, in the 1990s it was calculated that there were close to 16–18 million people infected, 100

million people at risk of acquiring the disease, and close to 45,000 deaths that could be attributed to the development of complications. In this context, the Pan American Health Organization (PAHO) promoted different initiatives to implement and develop measures to control the transmission of Chagas disease by vector, by transfusion, and from mother to foetus, depending on the epidemiological characteristics of each region.<sup>11</sup> As a result of these activities, a significant reduction in the incidence of the disease has been observed in recent years. It is estimated that at present there are close to 6–8 million people infected, 65–100 million people at risk of acquiring the disease, and close to 12,000 deaths per year that can be attributed to the development of complications.<sup>12</sup> The majority of these people are in Latin American, where the disease is endemic in 21 countries. However, movements from rural areas to urban areas and international migration have resulted in the influx of infected subjects from Latin America to the rest of the world, thus rendering the disease a problem for health systems on a global scale.<sup>2</sup>

According to the Mexican National Center for Diseases Control and Prevention (CENAPRECE), in Mexico, 75 cases of Chagas disease were recorded in 2000 (18 in the acute phase, 50 in the asymptomatic chronic phase and 7 in the symptomatic chronic phase), the incidence of which was 0.07 cases per 100,000 inhabitants and the mortality was 0.02% (21 deaths). By contrast, in 2012, 830 cases were recorded (7 in the acute phase, 823 in the asymptomatic chronic phase and 0 in the symptomatic chronic phase), the incidence of which was 0.70 cases per 100,000 inhabitants and the mortality was 0.03% (30 deaths). Thus, from 2000 to 2012 there was a cumulative total of 5,463 cases and an increase in incidence and mortality.<sup>13</sup> However, two-thirds of Mexico has the conditions required for vector-borne transmission to take place and, according to PAHO estimates, in 2006, there were approximately 1,000,000 people infected and 29,500,000 people at risk of acquiring the disease.<sup>14,15</sup> Therefore, it may be inferred that the disease is underdiagnosed.

## Etiology

- a) **Parasite:** *T. cruzi* is an obligate intracellular protozoan, flagellate, and digenean. It belongs to the order *Kinetoplastida*, family *Trypanosomatidae*, genus *Trypanosoma*, and subgenus *Schizotrypanum*.<sup>8</sup> Traditionally it has been classified into two main groups: *T. cruzi* I (linked to the domestic cycle and causing infections and high rates of morbidity and mortality in humans) and *T. cruzi* II (linked to the wild cycle and causing infections and low rates of morbidity and mortality in humans). The latter is in turn subdivided into 5 subgroups (IIa-e), which account for differences in terms of genetic variability, regional distribution, and potential for developing heart or gastrointestinal disease. The current consensus recommends that *T. cruzi* strains be classified into six main groups: *T. cruzi* I-VI.<sup>16-18</sup>
- b) **Vectors:** Triatomines are called triatomines in Mexico and are also known as barbeiros in Brazil, chipos in Venezuela, chiribicos in Colombia, and vinchucas in Argentina, Bolivia, and Chile. They belong to the

order *Hemiptera*, family *Reduviidae*, subfamily *Triatominae*, and are responsible for spreading *T. cruzi*. They are characterised by being obligate haematophages and having nocturnal habits. There are multiple species, and each of them has different biological behaviour. On the basis of their preferred habitat, they are classified into 3 cycles: domestic, peridomestic, and wild.<sup>8,16</sup>

- **Domestic cycle:** This is related to the structure and material with which rural and/or periurban houses are built (walls made of adobe or wattle and daub, and ceilings made of vegetable matter that forms cracks in walls and holes in ceilings, places where triatomines live and reproduce), in addition to the fact that their close relationship with humans and animals constitutes an abundant, easy-to-access food source. *Triatoma infestans* is the main vector residing in the countries of the Southern Cone (Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay), *Rhodnius prolixus* and *Triatoma dimidiata* are the main vectors residing in the Andean countries and Central America, and *Triatoma barberi* is the main vector residing in Mexico.<sup>8,16</sup>
- **Peridomestic cycle:** A connection is formed between the domestic cycle and the wild cycle involving a wide variety of carriers that freely enter and exit homes. *T. dimidiata* is the main peridomestic vector worldwide.<sup>8,16</sup>
- **Wild cycle:** This is related to a wild habitat and may infect many species of mammal. *Panstrongylus megistus*, *Triatoma brasiliensis*, and *Triatoma pseudomaculata* are the main wild vectors in Brazil, *Rhodnius pallescens* is the main wild vector in Colombia and Panama, and *Triatoma pallidipennis* is the main wild vector in Mexico.<sup>8,16</sup>

In Mexico, 34 species of triatomine belonging to 7 different genera have been identified. The genus *Triatoma* is the most abundant with 27 species.<sup>10</sup>

- c) **Carriers:** Natural carriers consist of squirrels (*Sciurus vulgaris*), armadillos (*Dasypus novemcinctus*), coyotes (*Canis latrans*), raccoons (*Procyon lotor*), sheep (*Ovis aries*), lynx (*Lynx rufus*), mice (*Mus musculus*), opossums (*Didelphis marsupialis*), bats (*Murem caecum*), primates (*Aotus sp.*, *Cebuella pygmaea*, *Cebus albifrons*, *Leontopithecus chrysomelas*, *Leontopithecus rosalia*, *Saguinus fuscicollis*, *Saguinus labiatus*, *Saguinus midas*, *Saguinus ustus*, and *Saimiri sciureus*), and foxes (*Rocyon cinereoargenteus*), in addition to certain domestic animals such as guinea pigs (*Cavia porcellus*), cats (*Felis silvestris catus*), dogs (*Canis familiaris*), and rats (*Rattus rattus*).<sup>8,16,19</sup>

## Life cycle

Its reproductive cycle allows it to exist in 3 different forms, each of which provides the parasite with certain adaptive advantages, any of which is capable of developing into any other. Metacyclic trypomastigotes, the infectious form of *T. cruzi*, have a fusiform shape and measure 10–20 μm in length and 1–3 μm in width. They are transmitted to humans through triatomine faeces; while triatomines feed on blood, they defecate on the host's skin, and *T. cruzi* penetrates

through the opening made by the bite and enters the bloodstream through excoriations of the skin caused by scratching secondary to the stinging that the bite causes or by rubbing on the mucosae (conjunctival or nasal). Once inside the body, they are phagocytosed by macrophages in the subcutaneous cellular tissue at the infection site. In the cytosol of this tissue they differentiate into amastigotes. Amastigotes have an ovoid shape and measure 1.5-5µm in diameter. They are capable of replicating by binary fission and of causing cell lysis to turn back into trypomastigotes in order to free themselves and travel through the blood and lymphatic circulation to penetrate any cell in the body, resulting in tropism for myocardiocytes, rhabdomyocytes, and leiomyocytes. In this stage, they may be ingested by another vector that was not previously infected. Within triatomines, trypomastigotes advance towards the medial segment of the gastrointestinal tract. There, they differentiate into epimastigotes, which are better adapted to survive the environment, and replicate again through binary fission. The epimastigotes migrate towards the distal segment of the gastrointestinal tract, where they anchor themselves to the colon epithelium through their flagella, turn back into metacyclic trypomastigotes to be excreted with faeces during the next ingestion of blood, and infect another human, thus closing the transmission cycle.<sup>1,8</sup>

The parasite's reproductive cycle appears to be organised in successive phases; however, there is no specific sequential pattern of progression in terms of differentiation from one form to the next.<sup>18</sup>

## Transmission

**Vector-borne:** This is the main route of transmission to humans and other domestic, peridomestic, and wild species (90%).<sup>8,16</sup>

**Non-vector-borne:** This includes blood transfusions, organ transplants, the vertical route, the oral route, and accidental transmission due to occupational exposure.

### a) Blood transfusions and organ transplants:

- Transmission by blood transfusion is the second most common route of transmission. It occurs due to transfusion from donors who are infected, asymptomatic, and unaware of the presence of the disease. It has been virtually brought under control in developed countries; however, it remains a problem in developing countries where the lack of resources and protocols does not allow for the enforcement of compulsory screening of 100% of donors at blood banks. The risk of transmission by transfusion of a unit of 500ml of whole blood is 12-20%.<sup>16,20</sup>
- Transmission by transplant of organs from infected donors has been reported in cases of heart, pancreas, kidney, and bone marrow transplant with organs from live donors and cadavers. Cases have been reported in Argentina, Brazil, Chile, Venezuela, and other countries. To date, there are no specific data on the incubation period or risk of transmission due to an infected transplanted organ.<sup>16</sup>

### b) Vertical, transplacental, maternal-foetal or congenital route:

This occurs due to the presence of parasitaemia

during pregnancy. While transmission may occur during any disease phase, the most common is the acute phase. This route of transmission had previously been largely limited to rural areas; however, increasing numbers of cases are occurring in cities where the disease did not exist before. This is due to migration of infected women of child-bearing age from rural areas to cities. Cases of congenital Chagas disease have been reported in Argentina, Bolivia, Brazil, Chile, Colombia, Guatemala, Honduras, Mexico, Paraguay, Uruguay, and Venezuela. The risk of congenital transmission varies depending on the *T. cruzi* strain in question, the parasitaemia of the mother and the existence of placental damage. The average risk due to pregnancy is estimated at 5%. To date, there is no conclusive evidence regarding transmission by breast milk.<sup>12,16</sup>

- c) **Oral route (OR):** This occurs due to the ingestion of faeces from triatomines previously infected with *T. cruzi*, the contamination from utensils used to prepare foods, and the consumption of the blood or meat of wild animals. It should be suspected if two or more confirmed acute cases occur simultaneously with an epidemiological link between them, domestic or peridomestic triatomines are absent, and the patient presents with severe signs and symptoms. Outbreaks have occurred following ingestion of sugar cane juice and water contaminated with triatomine faeces in Brazil, Colombia, Mexico, and Venezuela. The average risk of oral transmission is below 1%.<sup>21</sup>
- d) **Accidental due to occupational exposure:** This occurs due to poor or no implementation of biosafety measures when performing procedures in clinical and experimental laboratories. Conjunctival transmission occurs through aerosols produced after centrifugation of contaminated samples, handling of infected animals, and/or accidental puncture with infected needles. The average risk of transmission due to occupational exposure is below 1%.<sup>8,21</sup>

## Immunology

In the course of the past few decades, knowledge of the mechanisms of immune response and the pathophysiology of Chagas disease has increased. *T. cruzi* is known to interact with multiple host cell receptors, including toll-like receptors (TLRs). This leads to the activation of macrophages, neutrophils, and natural killer (NK) cells. These cells result in an intense inflammatory reaction secondary to upregulation of pro-inflammatory cytokines and cause respiratory burst through NAD(P)H oxidase (nicotinamide adenine dinucleotide phosphate oxidase [NOX2]), inducible nitric oxide synthase (iNOS), and myeloperoxidase (MPO). This releases reactive oxygen species (ROS), such as superoxide (O<sup>2-</sup>), and reactive nitrogen species (RNS), such as nitric oxide (NO), in addition to hypochlorous acid (HOCl).<sup>22,23</sup>

Respiratory burst is the main mechanism of response against parasitic infections. ROS play a significant role in innate immune response against infection with *T. cruzi* as they damage its DNA (they cause oxidative changes in amino acids such as cysteine, phenylalanine, methionine,

tyrosine, and tryptophan). RNS limit residual parasitic replication (they act as parasiticides by mobilising zinc from metalloproteins, inhibiting parasitic respiration, and limiting the availability of precursors for DNA synthesis and repair). HOCl is believed to be a mediator of tissue damage. In addition, subsequent reactions may form intermediate products such as H<sub>2</sub>O<sub>2</sub>, OH, nitrogen dioxide (NO<sub>2</sub>), peroxynitrite (ONOO<sup>-</sup>), dinitrogen trioxide (N<sub>2</sub>O<sub>3</sub>), dinitrosyl iron complexes (DNICs), nitrosothiols (RSNOs), and nitroxyl (HNO).<sup>24</sup> However, when ROS are produced in excess for prolonged periods of time, inefficient ROS elimination and reduced antioxidant concentration result in sustained oxidative stress. This causes cytotoxicity and dysfunction in physiological processes. ROS can rapidly oxidise lipids, proteins, and DNA. Lipid peroxidation causes damage to membrane integrity and proteins, while DNA oxidation results in damage to nucleotides, thereby generating mutations and transcription errors that exceed the capacity of cell repair mechanisms and result in structural and functional deterioration.<sup>25</sup>

Moreover, CD4<sup>+</sup> T lymphocytes play a crucial role in the immune response against infection with *T. cruzi*, since they recruit and activate other cells such as macrophages, mast cells, basophils, eosinophils, neutrophils, B cells, and CD8<sup>+</sup> T cells. "Naive" CD4<sup>+</sup> T lymphocytes differentiate up to subdividing into 4 major lineages in relation to the disease phase: T1, T2, T helper 17, and T regulatory (Treg) lymphocytes. These have different functions, cytokine secretion patterns, and transcription factor expression. The T1 lymphocyte subgroup participates in cell-mediated immune reactions that activate macrophages to eliminate intracellular pathogens and is associated with overexpression of IL-2, IL-12, TNF- $\alpha$ , and IFN- $\delta$ , as well as disease control, although it may often also be associated with disease pathogenesis due to the exuberant inflammatory reaction that it causes. The T2 lymphocyte subgroup participates in humourally mediated immune responses that activate eosinophils, mast cells, and IgE to eliminate parasites and is associated with overexpression of IL-4, IL-5, IL-6, IL-10, and IL-13, as well as disease persistence and severity. In addition, "naive" CD4<sup>+</sup> T lymphocytes may differentiate into T helper 17 or inducible T regulatory (iTreg) lymphocytes depending on the cytokine environment to which they are exposed in order to secrete IL-17 or TGF- $\beta$ , respectively.<sup>19,26,27</sup> CD8<sup>+</sup> T lymphocytes play their role in the immune response against infection with *T. cruzi* by eliminating infected cells through the production of IFN- $\gamma$ , perforin, and granzyme B or through the Fas/Fas ligand pathway.<sup>27,28</sup>

## Pathophysiology

The production of virulence factors by *T. cruzi* during the acute phase strongly inhibits the response of the host's immune system, thereby inducing anergia and clonal deletion of T lymphocytes, along with a strong polyclonal stimulation of B lymphocytes that secrete antibodies with a low affinity towards *T. cruzi* antigens. This promotes persistence of the infection and its progression towards the chronic phase of the disease. Within the chronic phase, the mechanisms behind the transition from the asymptomatic phase to the symptomatic phase have not yet been fully

elucidated. However, it is believed that there are many factors involved, such as differences between *T. cruzi* strains, parasite load, infection time, genetic background, and host immune response.<sup>28,29</sup>

Some theories have attempted to explain the pathophysiological process of the disease, including: theory of parasite persistence (this is based on the fact that the presence and replication of amastigotes in host cells cause mechanical rupture and waste secretion which attract pro-inflammatory cells); unified neurogenic theory (this is based on the fact that significant neuron loss in the sympathetic and parasympathetic nervous systems is unrelated to the presence of *T. cruzi in situ*, and is attributed to production and release of a neurotoxin from a parasite nest hidden in the host's body); and autoimmune theory (this is based on the accelerated cytotoxic interaction that exists between lymphocytes related to the immune response to *T. cruzi* and allogeneic myocardiocytes not infested with parasites). Each one shows unique discrepancies, which may be explained from a clinical point of view by the difficulty of determining pathogenicity after a prolonged period of time has elapsed between infection with *T. cruzi* and development of its complications.<sup>19</sup> However, their analysis lies beyond the scope of this review.

## Risk factors

Risk factors for acquiring Chagas disease include: quality of rural dwelling (type of construction and materials used to finish the floors, walls, and ceilings), lack of awareness of the risk of living with triatomines, and absence of epidemiological control and monitoring programmes that involve the community.<sup>30</sup>

## Clinical manifestations

American trypanosomiasis occurs and progresses in phases. The clinical spectrum of the disease is very broad. During the acute phase, non-specific manifestations may develop. During the chronic phase, specific manifestations may develop.

**Acute phase:** This has an incubation period of 4–14 days, starting from parasite inoculation, and a duration of 2–4 months. It is characterised by the absence of symptoms in 95% of cases. The remaining 5% of cases may have signs and symptoms related to the inoculation site or systemic manifestations. Signs and symptoms related to the inoculation site include: Romana's sign (painless, indurated, pruritic, purplish, bipalpebral, unilateral, periorcular oedema that makes it difficult to open the eyelids, thereby causing limited conjunctival secretion, dacryoadenitis, and preauricular satellite adenopathy); and chagoma (a raised, erythematous, oedematous, indurated, and moderately painful subcutaneous nodule that is approximately 3 cm in diameter). These may be associated with enlarged cervical lymph nodes that are approximately 1–2 cm in diameter, painful on palpation, soft in consistency, with well defined edges, and not adhered to deep planes. Systemic manifestations that may occur include the following: fever, asthenia, adynamia, myalgia, arthralgia, headache, myocarditis, and hepatosplenomegaly. Myocarditis may occur with or without manifestations of cardiac

compromise such as tachycardia, gallop rhythm, PR and/or QT interval prolongation, decrease in QRS voltage, premature ventricular contractions, right bundle branch block, T-wave changes, pericarditis, cardiac tamponade, and heart failure.<sup>2,20,30-32</sup>

- **Chagas disease and immunosuppression:** This has a more prolonged incubation period and is more severe than in immunocompetent patients. It manifests through fever, myalgia, arthralgia, multiple dermatoses, and hepatosplenomegaly. Meningoencephalitis, pericarditis, and/or myocarditis may develop, especially in children and the elderly. Dermatoses may manifest through the presence of chagomas (blisters or bullae with abundant trypomastigotes inside); morbilliform, pruritic, and polymorphous erythematous nodules or rashes. The manifestations of meningoencephalitis depend on the size and location of the lesions. Altered mental status, seizures, headache, hemiparesis, and tremors may occur. In patients infected with human immunodeficiency virus (HIV) who have developed AIDS, acute diffuse meningoencephalitis or multifocal necrotising encephalitis with abscesses tends to occur and may progress to coma and death.<sup>33</sup>
- **Congenital Chagas disease:** This is characterised by the absence of symptoms in 70–80% of cases. The remaining 20–30% of cases may have signs and symptoms such as prematurity, smallness for gestational age, poor general condition, oedema, jaundice, respiratory distress, persistent tachycardia, hepatosplenomegaly, and anaemia. Occasionally sepsis, fever, hydrops fetalis, exanthema, petechiae, lymphadenopathy, meningoencephalitis, cerebral calcifications, eye fundus abnormalities, megaesophagus, interstitial pneumonia, myocarditis, heart failure, megabladder, etc. may occur. It may be classified as: asymptomatic, early-symptom (<30 days of life) or late-symptom (>30 days of life). In addition, the presence of HIV or AIDS worsens the clinical course of these patients as previously mentioned.<sup>20,31,32,34</sup>

**Asymptomatic or indeterminate chronic phase:** This is characterised by the absence of symptoms and the presence of positive serology and/or parasitaemia. It may last months or years, depending on the immune status of the patient and the replication rate of the amastigotes. This form persists in up to 30% of patients with the disease, while the rest may progress to the symptomatic chronic form over a period of 10–30 years.<sup>31,32</sup>

**Symptomatic or determinate chronic phase:** This is characterised by the presence of chronic heart disease (cardiomegaly) and/or gastrointestinal disease (megaesophagus, megastomach, megabladder, megagallbladder, megaduodenum, megajejunum, megaileum, and megacolon) with low, fluctuating parasitaemia levels. Antibody titres may be detected in immunocompetent patients. Neurological findings are rare. However, it is known that there is damage to the autonomic nervous system which results in abnormalities in cardiovascular autonomic function. The sympathetic and parasympathetic nervous systems show a reduction in the number of neurons. This compromises the innervation of cardiac muscle and the smooth muscle of the oesophagus, stomach, colon, bronchi, urethra, and bladder.

In addition, the peripheral nervous system shows a decrease in conduction speed, thereby compromising neuromuscular transmission. The presence of signs and symptoms such as seizures, fever, dizziness, syncope, headache, nausea, vomiting, dyspepsia, and fullness associated to prolongation of gastric emptying has been reported.<sup>30-32</sup>

- **Chagas heart disease:** This occurs in 10–30% of cases and represents the main cause of mortality due to this disease (*sudden cardiac death* [55–63%], progressive heart failure [20–25%], and thromboembolic complications [10–15%]). There are overall and segmental contractility abnormalities, arrhythmias (sinus bradycardia, sinus tachycardia, atrial fibrillation, atrial flutter, ventricular tachycardia or premature ventricular contractions), and conduction disorders secondary to damage to the excitation/conduction system (atrioventricular block, right bundle branch block, left bundle branch block or left anterior fascicular block), valve insufficiency, and heart failure. People with dilated cardiomyopathy may have thromboembolic phenomena leading to pulmonary embolism (PE) and/or cerebrovascular disease (CVD). In addition, there is fibrosis which leads to apical microaneurysms in the left ventricle. It manifests through dyspnoea (42%), precordial pain (42%), palpitations (31%), syncope (27%), and faintness (24%). On physical examination, heart murmurs may occasionally be auscultated.<sup>30-32,35</sup>
- **Chagas oesophageal disease (megaesophagus):** Intramural parasympathetic denervation develops, leading to hypertrophy of the muscle layers and progressive loss of contractile capacity. This results in oesophageal dilatation and elongation. It manifests through odynophagia, progressive dysphagia, regurgitation, pyrosis, retrosternal pain, hiccups, sniffing, and coughing. On physical examination, oropharyngeal hyperaemia secondary to the presence of gastro-oesophageal reflux disease may be observed.<sup>30,31,36</sup>
- **Chagas colon disease (megacolon):** Intramural parasympathetic denervation also develops. This results in motor dysfunction and colonic dilatation, thereby causing absorption and secretion disorders. It manifests through tympanites, abdominal distension, and dyschezia secondary to progressive constipation which may lead to the formation of faecalomas, volvulus, and intestinal obstruction and ischaemia. On physical examination, a faecaloma may be palpated in the left iliac fossa or by means of a digital rectal examination.<sup>30,31,36</sup>

## Diagnosis

If Chagas disease is suspected, epidemiological background and clinical manifestations should be considered so that the relevant studies may be ordered. The diagnosis is made through direct or indirect parasitological methods and molecular methods during the acute phase, and through serological and office-based methods during the chronic phase. Direct parasitological methods confirm the presence of *T. cruzi* or its genetic material in samples and include: examination of fresh samples, blood smear, thick film, micro-Strout test, and Strout's concentration method. Indirect parasitological methods include: xenodiagnosis and

blood culture. Molecular methods such as polymerase chain reaction (PCR) may be useful. Serological methods demonstrate the presence of specific anti-*T. cruzi* antibodies in samples and include: enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence (IIF), indirect haemagglutination (IHA), and Western blot (WB).<sup>37</sup>

**Acute phase:** In examinations in which the presence of the parasite is confirmed, confirmation by means of other methods is not required. In examinations in which the presence of the parasite is confirmed through its genetic material, the following should be considered: patients under the age of 9 months and immunocompromised patients require 2 positive results in different samples, while patients over the age of 9 months require 1 positive result and detection of antibodies for the diagnosis to be confirmed.<sup>12</sup>

#### Direct parasitological methods:

- **Examination of fresh samples:** This is based on obtaining a drop of blood from the patient to be observed through a light microscope in search of mobile trypomastigotes. It can be performed in laboratories with minimal resources.<sup>21,37</sup>
- **Blood smear:** This is based on obtaining a drop of blood from the patient to be stained with Giemsa, Romanowsky, or Wright's stain and observed through a light microscope in search of trypomastigotes. It allows the morphology of *T. cruzi* to be identified.<sup>21,37</sup>
- **Thick film:** This is based on obtaining, concentrating, and defibrinating a drop of blood from the patient to be stained with Giemsa, Romanowsky, or Wright's stain and observed through a light microscope in search of trypomastigotes. This is the method of choice to be used in areas where there is also malaria.<sup>21,37</sup>
- **Micro-Strout test:** This is based on obtaining and centrifuging a sample of blood from the patient for the leucocyte fraction to be observed through a light microscope in search of trypomastigotes. It can be performed in laboratories with minimal resources and is especially useful for early detection of congenital Chagas disease.<sup>21,37</sup>
- **Strout concentration method:** This is based on obtaining a sample of blood from the patient, centrifuging it to remove the erythrocyte fraction, concentrating the parasites in the sediment by discarding the supernatant, and observing it through a light microscope in search of trypomastigotes. It can be performed in laboratories with minimal resources.<sup>21,37</sup>

#### Indirect parasitological methods:

- **Xenodiagnosis:** This is based on feeding uninfected triatomines with blood from a patient probably infected with *T. cruzi* and examining the intestine and/or its excretions through a light microscope in search of epimastigotes and/or trypomastigotes. The procedure may be performed naturally or artificially, and the haemolymph and salivary glands of the triatomines used must always be reviewed to detect the presence of *Trypanosoma rangeli*, which is morphologically similar to *T. cruzi* and is often a reason for false positive results.<sup>21</sup>
- **Blood culture:** This is based on obtaining a sample of blood from the patient and seeding it in Tobie's medium to

be observed weekly through a field inverted microscope until it is isolated. Once isolated, it is cultured in liquid media, such as liver infusion tryptose (LIT) or brain heart infusion (BHI), for its maintenance. It is used to increase the concentration of *T. cruzi* to obtain an antigen that can be used for molecular and serological diagnosis. It is especially useful for early detection of congenital transmission.<sup>21</sup>

#### Molecular methods

- **Polymerase chain reaction (PCR):** This is based on obtaining a sample of blood from the patient to be subjected to multiple cycles of denaturation, hybridisation, and amplification of DNA segments from *T. cruzi* (qualitative PCR), or to measure the circulating parasite load (quantitative PCR). It is especially useful for early detection of congenital transmission, transmission by organ transplant, accidental transmission due to occupational exposure, and transmission in cases of immunocompromise.<sup>21,37</sup>

**Chronic phase:** At least 2 serological methods with different principles should be used. If the result is positive in both, the diagnosis is confirmed, whereas if the results are incongruous, a third test should be used to confirm or reject them.<sup>21</sup>

#### Serological methods

**Enzyme-linked immunosorbent assay:** This is based on obtaining a sample of blood from the patient and placing it on polystyrene plates containing *T. cruzi* antigens. If the serum contains anti-*T. cruzi* antibodies, a colourimetric reaction occurs that is detected by adding a second antibody with a specific substrate, which may be observed through a spectrophotometer. It has a sensitivity of approximately 94–100% and a specificity of approximately 96–100%. Its results are considered to be positive when titres are greater than or equal to double the value of the cut-off point for optical absorbance or density, which is different for each specific kit.<sup>3,21,37,38</sup>

**Indirect immunofluorescence:** This is based on obtaining a sample of blood from the patient and placing it on glass plates with wells containing *T. cruzi* antigens (epimastigotes). If the serum contains anti-*T. cruzi* antibodies, a reaction occurs that is detected by adding a second antibody labelled with fluorescein, which may be observed through a fluorescence microscope. It has a sensitivity and a specificity of approximately 98%. Its results are considered to be positive when titres are greater than or equal to 1:32 and may have cross-reactivity with *Leishmania* or *T. rangeli*.<sup>3,21,37,38</sup>

**Indirect haemagglutination:** This is based on obtaining a sample of blood from the patient and sensitising the surface of the erythrocytes with *T. cruzi* antigens that interact with anti-*T. cruzi* antibodies. This causes a reaction that generates agglutination and which may be observed. It has a sensitivity of approximately 88–99% and a specificity of approximately 96–100%. Its results are considered to be positive depending on the cut-off point for each specific kit.<sup>3,21,37,38</sup>

- **Western blot:** This is based on detecting anti-*T. cruzi* antibodies that have been previously separated through electrophoresis and subsequently transferred to a membrane on which an enzyme reaction that detects their presence is performed. It has a sensitivity and a specificity of approximately 100%. To date, there is no commercial kit available for use in Chagas disease.<sup>3,21,37,38</sup>

To diagnose congenital Chagas disease, serological methods are ordered to detect infection in the mother, while parasitological (examination of fresh samples) and molecular (PCR) methods are ordered to detect infection in the child. Umbilical cord blood or peripheral blood is obtained during the first 2 months of life. If the results are positive or there is a desire to make the diagnosis after the first 2 months, ELISA and IIF should be requested at 9–12 months, after the level of maternal antibodies transferred has disappeared.<sup>31</sup>

To diagnose Chagas meningoencephalitis in patients with AIDS, the following are ordered: a head CT scan (single or multiple hypodense areas with perilesional oedema and a mass effect with displacement of the midline), brain biopsy (widespread multifocal, necrohaemorrhagic encephalitis with obliterative angiitis and amastigotes in glial cells, macrophages, and endothelial cells), and lumbar puncture.<sup>33</sup>

To diagnose Chagas heart disease, the following are requested: chest X-rays (cardiomegaly with or without effusion), electrocardiogram (arrhythmias and blocks), echocardiogram (microaneurysms, fibrosis, decrease in contractility, and abnormal ejection fraction), magnetic resonance imaging (MRI) (structural abnormalities), and scintigraphy.<sup>31,32</sup>

To diagnose Chagas oesophageal disease, chest and abdominal X-rays (gastroduodenal series), manometry, and panendoscopy are ordered, as deemed necessary. To diagnose Chagas colon disease, the following are ordered: plain abdominal X-rays, barium enema, and colonoscopy.<sup>31,32</sup>

## Treatment

Currently, there are two anti-trypanocidal drugs available that are used to treat infection with *T. cruzi*: benznidazole and nifurtimox. Both have very high trypanocidal activity during the acute phase of the disease: up to 100% in neonates and up to 80–90% in children and adults identified and treated in a timely manner. However, they have very low trypanocidal activity during the chronic phase of the disease.<sup>4,39</sup>

(a) **Benznidazole:** This is a nitroimidazole-derived trypanocide that is absorbed rapidly in the gastrointestinal tract. **Mechanism of action:** It acts through covalent modification of macromolecules by nitro-reduction of intermediates. **Pack size:** 100 mg tablets. **Dose:** Neonates are administered 5 mg/kg/day divided into 2 doses. Children are administered 10 mg/kg/day divided into 2 doses. Adults are administered 5–7 mg/kg/day divided into 2 doses. **Duration:** 60 days in neonates, children, and adults. **Adverse reactions:** The most common adverse effects are dermatological effects (29–50%).

Skin rashes due to photosensitisation occur during the first 2 weeks of treatment. Peripheral neuropathy is dose-dependent, appears late in the course of treatment, and is an indication for suspending treatment (0–30%). Bone marrow suppression occurs very rarely (<1%). Other reported adverse effects include anorexia and weight loss (5–40%), paraesthesia (0–30%), nausea and vomiting (0–5%), and leukopenia and/or thrombocytopenia (<1%).<sup>40,41</sup>

Prior to administering treatment with benznidazole, the following laboratory tests should be ordered: complete blood count, blood chemistry, serum electrolytes, liver function tests, and general urinalysis. The complete blood count should be repeated every 2–3 weeks throughout and at the end of treatment.<sup>30</sup>

(b) **Nifurtimox:** This is a nitrofurantoin compound that is appropriately absorbed by the gastrointestinal tract. **Mechanism of action:** It acts through reduction of the nitro group to form unstable radicals such as nitroanion, which is highly reactive and produces highly toxic reduced O<sub>2</sub> metabolites such as H<sub>2</sub>O<sub>2</sub> and O<sup>-</sup>. It inhibits pyruvic acid synthesis and interrupts *T. cruzi* carbohydrate metabolism. **Pack size:** 30, 120, and 250 mg tablets. **Dose:** Neonates are administered 10 mg/kg/day divided into 3 doses. Children are administered 15–20 mg/kg/day divided into 4 doses. Adults are administered 8–10 mg/kg/day divided into 4 doses. **Duration:** 60 days in neonates and 90 days in children and adults. **Adverse reactions:** The most common adverse effects are gastrointestinal effects (70%), anorexia and weight loss (50–70%), abdominal pain (12–40%), and nausea and/or vomiting (15–26%). Neurological abnormalities may occur. Irritability, disorientation, insomnia, and trembling are among the most common, and paraesthesia, polyneuropathy, and distal neuritis are among the least common. Peripheral neuropathy (2–5%) is dose-dependent, appears late in the course of treatment, and is an indication for suspending treatment. Other reported adverse effects include headache (13–70%), vertigo (12–33%), myalgia (13–30%), and leukopenia (<1%).<sup>30,32,41</sup>

Prior to administering treatment with nifurtimox, the following laboratory tests should be ordered: complete blood count, blood chemistry, serum electrolytes, liver function tests, and general urinalysis. The complete blood count should be repeated 4–6 weeks after the start and at the end of treatment.<sup>30</sup>

**Indications:** Acute, congenital, reactive, or chronic infection in children under the age of 18, and adults aged 19–50 years with no evidence of advanced cardiomyopathy.<sup>3</sup>

**Contraindications:** Both drugs are contraindicated during pregnancy and in patients with liver and/or kidney failure.<sup>30</sup>

Currently, the WHO and clinical practice guidelines (CPGs) recommend the use of benznidazole as a first-line treatment for Chagas disease, given that there is greater clinical evidence of its efficacy, it is better tolerated by patients, and it has fewer adverse effects than nifurtimox.<sup>3</sup>



Treatment of Chagas heart disease tends to be resistant to routinely used treatments. Anticoagulants are administered as prophylaxis for PE and CVD, while anti-arrhythmic agents are administered for premature ventricular contractions. Pacemakers are implanted for third-degree atrioventricular block, while for congestive heart failure, ventricular assist devices are implanted, vasodilators,  $\beta$ -blockers, and diuretics are administered, or heart transplants are performed, as required. Such treatment is provided in addition to implementation of hygiene and dietary measures.<sup>20,31</sup>

Treatment of Chagas oesophageal disease consists of measures that facilitate oesophageal emptying: lower oesophageal sphincter relaxants, pneumatic dilatation, botulinum toxin, and traditional or laparoscopic cardiomyotomy. Such treatment is provided in addition to implementation of hygiene and dietary measures.<sup>30,31</sup>

Treatment of Chagas colon disease consists of measures that facilitate colon emptying: laxatives, cleansing enemas, and colectomy of the affected area, as required. Such treatment is provided in addition to implementation of hygiene and dietary measures.<sup>30,31</sup>

**Follow-up:** For follow-up of treatment, weekly medical supervision is recommended. For monitoring of treatment response, it is recommended that the decrease in anti-*T. cruzi* antibody titres be documented using IIF, and that titres equal to or greater than 1:16 be considered to be positive. Recovery is considered to consist of persistent negative parasitology and serology (two negative tests with a lapse of twelve months from the end of treatment between each one).<sup>21,30</sup>

## Prevention

Over the past decade, the number of studies aimed at formulating both prophylactic and therapeutic vaccines for infection with *T. cruzi* has increased. Various antigens have been used as immunogens to observe the development of the disease. Recombinant DNA and protein vaccines (ASP-1, ASP-2, CCL4/MIP-1  $\beta$  chemokine, Cruzipain, trans-sialidase catalytic domain, ANYNFTLV epitope, TSSA CD8<sup>+</sup> epitope, GP83, KMP11-H70, LYT1, MASP, PFR2, PFR2-H70, PFR3, PFR3-H70, PFR3m, rTcSP2 and rTcSP2-CHP, Tc13, Tc24, Tc52, TcG1, TcG2, TcG4, TcSP, TcSSP4, TcVac2, TcVac4, TS, and TSA-1), as well as vaccines containing live attenuated parasites (*T. cruzi* and *T. rangeli*), have been used and demonstrated to have various levels of protection with respect to parasitaemia, clinical manifestations, severity of cardiac damage, and survival in the preclinical phase. However, currently, there is no vaccine in the clinical phase, and although the future is promising, the development of an effective vaccine against *T. cruzi* has encountered many difficulties, since Chagas disease is almost exclusive to poor and marginalised societies and therefore lacks commercial incentive for the pharmaceutical industry. Prophylactic vaccines could be administered in areas characterised as being highly endemic, thus preventing infection, while therapeutic vaccines could be administered to patients who are seropositive for *T. cruzi* and in the asymptomatic phase, thus preventing the development of Chagas heart disease. In addition, it is estimated that close to 10,000 deaths per year

would be prevented and up to 600,000 disability-adjusted life years (DALYs) would be saved.<sup>40,42-44</sup>

At present, the main strategies to combat the disease involve preventive strategies such as home improvement, systematic spraying of insecticides, and health education, as well as control of blood samples at blood banks and monitoring of all pregnant women.<sup>39</sup>

## Conclusion

Chagas disease is recognised as one of the 17 neglected tropical diseases that affect millions of people worldwide. It is considered to be the most significant zoonosis in Latin America and is the fourth leading cause of disability (after respiratory infections, gastrointestinal infections, and HIV/AIDS).<sup>45</sup> Although its exact prevalence and distribution is unknown in Mexico, it remains a serious problem for Mexican health services.

The extensive study that has been done of subjects, ranging from the epidemiology of the disease to its diagnosis, has expanded knowledge of the disease and how to combat it. In the past century, multiple vector control and prevention programmes have been carried out throughout areas that are considered to be highly endemic. These have decreased its incidence dramatically. However, the objective of these measures is not to eradicate the disease. A large part of the population remains unaware of the disease, those who are aware of it ignore its presence, and those who suffer from it are neither diagnosed nor treated in a timely manner. In addition, the medicines available to treat Chagas disease are partially effective, and the efforts made to develop a vaccine for populations at risk of acquiring the disease remain insufficient.

Only 1% of the new drugs registered are aimed at controlling neglected tropical diseases, whereas close to 90% of investment in research and development in the pharmaceutical industry is aimed at creating drugs designed for the 10% of the population with the greatest revenue.<sup>46</sup> Therefore, it is essential to combat poverty, spread knowledge on methods of prevention, train general practitioners and specialist physicians to provide timely care to those suffering from it, and increase resources intended to treat it to bring patients suffering from a silent, devastating disease out of oblivion.

## Ethical disclosure

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this study.

**Confidentiality of data.** The authors declare that no patient data appear in this article.

**Right to privacy and informed consent.** The authors declare that no patient data appear in this article.

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## Conflict of interest

The authors declare that they have no conflicts of interest.

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