American Trypanosomosis

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Learning Objectives

1. To have knowledge about alternate modes of disease transmission apart from the classical vector transmission through inoculation.
2. To know various forms of clinical manifestations depending on the routes of infection.
3. To have an understanding of the importance of microscopic examination in diagnosis and strain identification by molecular techniques.

Introduction

The protozoan parasite *Trypanosoma cruzi*, responsible for causing American trypanosomosis, was discovered by the Brazilian scientist Carlos Chagas in the year 1909. The disease is endemic to large parts of Latin American countries, with the exception of the Caribbean islands. However, in recent decades, it has progressively been diagnosed worldwide highlighting its growing significance in the USA, Europe, Canada, Eastern Mediterranean and Western Pacific countries. Out of all, people mostly from Latin America are more prone to be infected with *T. cruzi*, and it is considered one of the neglected diseases. Chagas disease is mainly communicated to human beings through contact with faeces/urine of infected blood-sucking bugs, viz. kissing bugs or conenose bugs (belonging to subfamily Triatominae). Among these *Triatoma infestans*, *Triatoma dimidiate*, *Rhodnius prolixus* and *Panstrongylus megistus* are considered as being the most important vectors.

History

Approximately, 7–10 million years ago, *T. cruzi* ancestors were probably introduced to South America via bats. Several travellers and physicians documented records of patients with disease symptoms similar to American trypanosomosis during the sixteenth century. Nevertheless, the critical role of triatomine bugs as vectors in transmitting Chagas disease remained unexplored until 1909. Identification of *T. cruzi* and triatome bugs as the transmission vector of Chagas disease came into limelight only at the beginning of the twentieth century. The disease was first described by Carlos Ribeiro Justiniano Chagas in a 2-year-old baby named

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S. C. Parija, A. Chaudhury (eds.), *Textbook of Parasitic Zoonoses*, Microbial Zoonoses,
https://doi.org/10.1007/978-981-16-7204-0_13

Berenice suffering from fever and swollen lymph nodes and with hepatosplenomegaly. Trypanosomes which were identical to those seen in the gut of triatomine bugs were seen in the patient's blood. He named the parasite *Trypanosoma cruzi* in honour of Oswaldo Cruz. As a tribute to his remarkable discovery, the World Chagas Disease Day was established to be celebrated on 14 April in memory of the date of the year 1909 when Carlos Chagas diagnosed the first human case of the disease.

Taxonomy

Trypanosoma cruzi taxonomic classification is based on *An Illustrated Guide to the Protozoa*, 2000, by John J. Lee. The genus *Trypanosoma* belongs to the family Trypanosomatidae, order Kinetoplastida, subphylum Mastigophora and phylum Sarcomastigophora in the subkingdom Protozoa and kingdom Protista.

T. cruzi are stercorarian trypanosomes which undergo posterior station (hindgut) development in vectors and are transmitted via faecal contamination of bite site to infect blood and tissues of vertebrate hosts.

Genomics and Proteomics

T. cruzi consists of mitochondrial genome composed of 30 copies of 20–50 kb maxicircles and thousands of copies of ~1 kb minicircles, which together comprise the kinetoplast DNA or kDNA. The whole genome sequencing was done in 2005. It has revealed that the diploid genome contains a predicted 22,570 proteins encoded by genes, of which 12,570 represent allelic pairs. Over 50% of the genome consists of repeated sequences that comprises retrotransposons and genes for large surface molecules. It has a highly plastic genome, an unusual gene organization and complex mechanisms for gene expression such as polycistronic transcription, RNA editing and trans-splicing.

T. cruzi belongs to heterogeneous population comprising a pool of strains that shift between the domestic and sylvatic cycles involving human beings, vectors and animal reservoirs of the parasite. Extensive study on *T. cruzi* populations from different origins demonstrated the presence of a variant strain with marked characteristics. At present, six distinct genealogies of *T. cruzi* are classified into Tc-I, II, III, IV, V and VI discrete typing units that vary in geographical distribution, host specificity and pathogenicity. Completion of the genome sequence of the *T. cruzi* CL Brener strain 31 possibly opens prospects for the development of novel therapeutic and diagnostic techniques.

A total of 2784 proteins in 1168 protein groups from the annotated *T. cruzi* genome in its life cycle have been identified by peptides mapping. Protein products were identified from 91,000 genes annotated as "hypothetical" in the sequenced genome. The four parasite stages appear to use different energy sources like histidine for stages present in the insect vectors and fatty acids by intracellular amastigotes.

The Parasite Morphology

T. cruzi is characterized by three morphological forms, viz. trypomastigote, epimastigote and amastigote.

Trypomastigote

It is found in the peripheral circulation measuring about 20 μm in length and generally slender and exhibits pleomorphism. They are present as elongate slender dividing forms (with long free flagellum) or stumpy non-dividing infective (metacyclic) forms with no free flagellum. They have a thin, irregularly shaped membrane, with centrally positioned nucleus and a posteriorly situated kinetoplast (Fig. 1). A flagellum arises at the kinetoplast and traverses the entire length of the parasite and extends beyond it. A single mitochondrion is present inside the kinetoplast that drives the flagellum. In stained preparations, trypanosomes generally are seen in a C or U shape. The identification of the parasite is usually

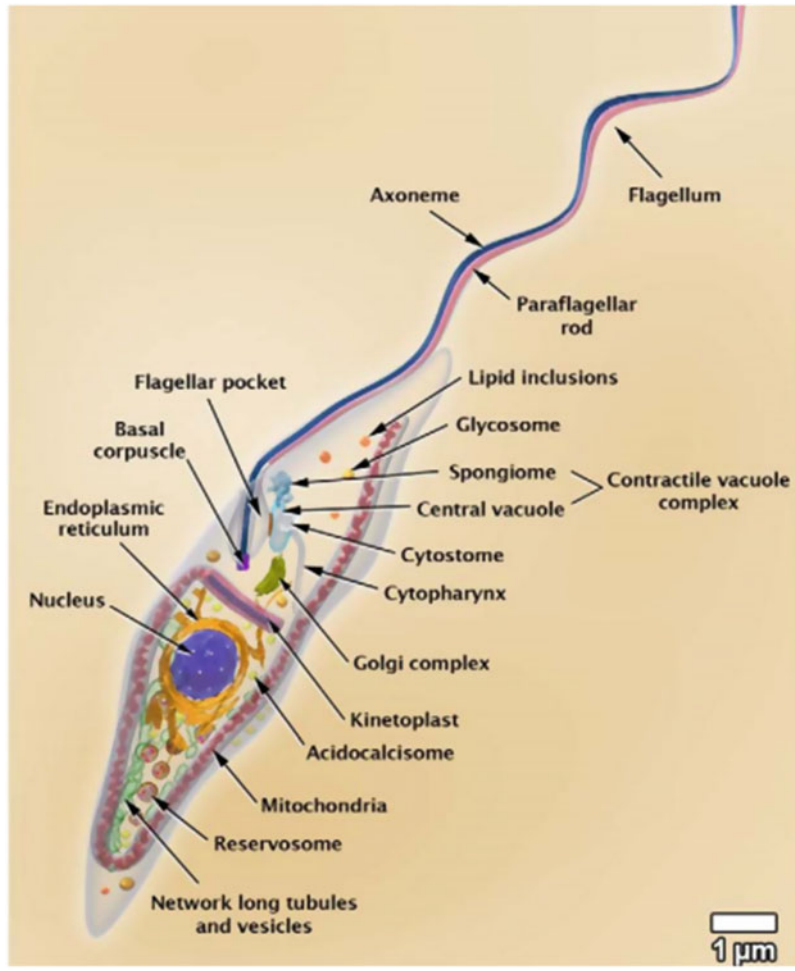

Fig. 1 Schematic representations of *Trypanosoma cruzi* trypomastigote organelles – 3D model (Source Teixeira et al. 2012)

made by its morphological features and needs to be invariably different from *Trypanosoma rangeli*, a non-pathogenic flagellate that infects humans in Central and South America and is transmitted by the same vectors that transmit *T. cruzi.*

Epimastigote

This stage is more or less similar to the trypomastigote stage except that the kinetoplast is located anterior to the nucleus (Fig. 2). Size of the epimastigote measures 10–35 µm in length by 1–3 µm in width.

Amastigote

These are present within the host cells. They are generally round in shape, and the flagellum becomes nearly unapparent (Fig. 3).

Cultivation of Parasite

Specialized systems are available to grow the epimastigotes in axenic culture media. The parasite count is carried out by haemocytometer or automated methods. This helps in assessing the rate of growth or killing potential in drug assays.

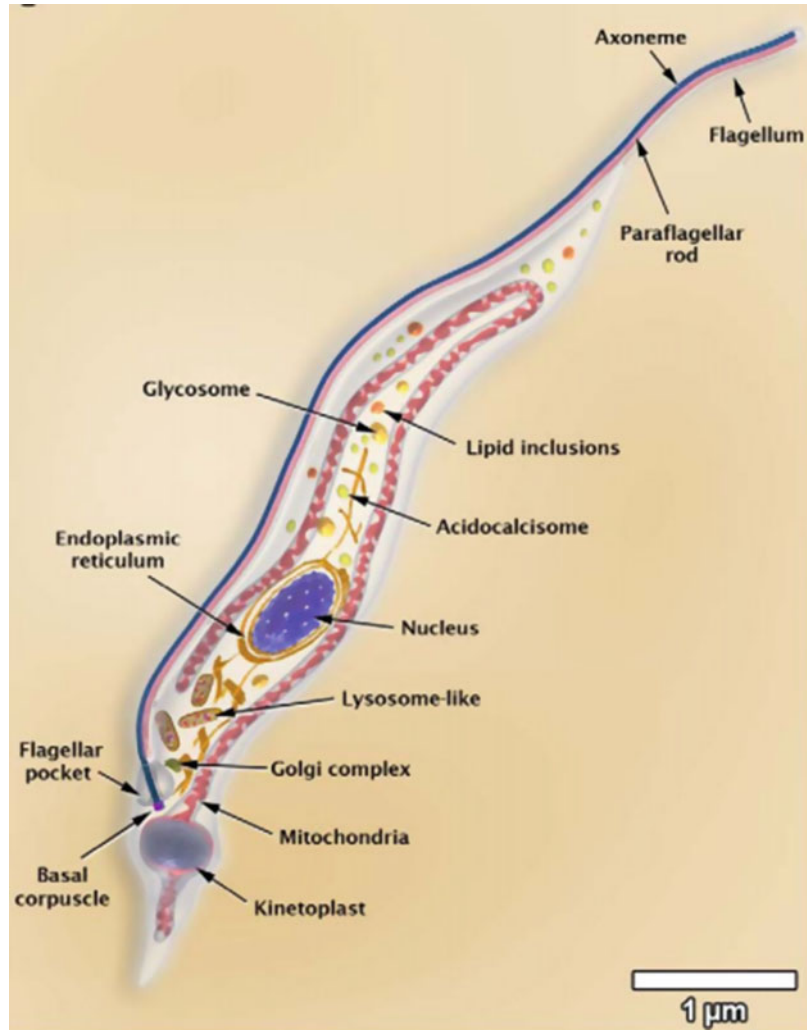

Fig. 2 Schematic representations of *Trypanosoma cruzi* epimastigote organelles – 3D model (Source Teixeira et al. 2012)

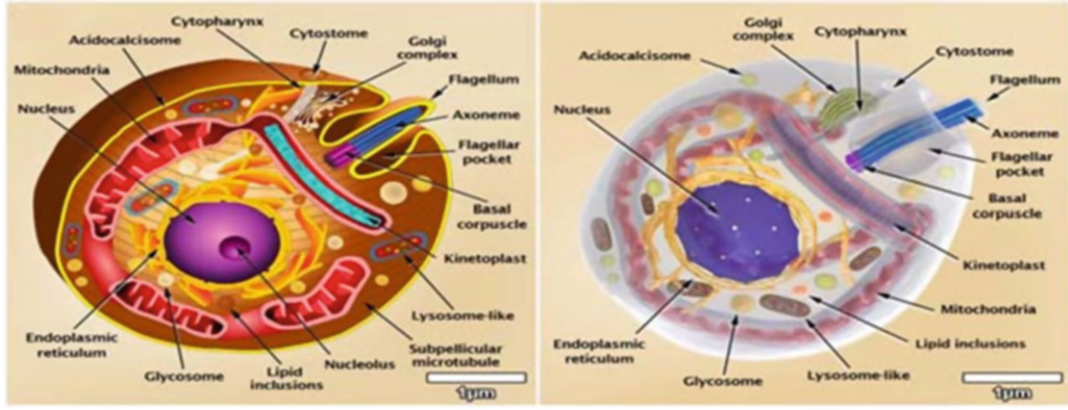

Fig. 3 Schematic representations of *Trypanosoma cruzi* amastigote – 2D and 3D models (Source Teixeira et al. 2012)

Laboratory Animals

Mouse is an excellent model for the study of both acute and chronic *T. cruzi* infections. Thus the murine model is most commonly used to assess the activity of new drugs against *T. cruzi*. Other laboratory animals include rodents, dogs, guinea pigs and primates.

Life Cycle of Trypanosoma cruzi

Hosts

Primary Host

Humans, animals living in close proximity to humans (cats, dogs, wood rats, opossums).

Intermediate Host

Triatomine bugs (*Triatoma infestans*, *Rhodnius prolixus*, *Triatoma dimidiata* and *Panstrongylus megistus*).

Infective Stage

Metacyclic trypomastigotes are the infective stage.

Transmission of Infection

T. cruzi infective form present in reduviid bug faeces enters through the bite wound or scratch wounds but does not invade intact skin. Infective forms are also transmitted to humans by blood transfusion, organ transplantation and contaminated food and drink, through breast milk and congenitally through the placenta (Figs. 4 and 5).

The life cycle of *T. cruzi* includes both vertebrate and invertebrate hosts comprising three well-defined developmental stages (trypomastigotes, epimastigotes and amastigotes). These developmental stages have evolved so that they are conditioned to their individual surroundings which serve multiple purposes which include improved transmission potential, evasion of host immune system and long-term survival. The circulating trypomastigotes in the blood are non-dividing forms which can infect new cells of various tissues in the body. In the cytoplasm of the host cells, trypomastigotes are transformed into aflagellate amastigotes which are the dividing form of *T. cruzi* in mammals.

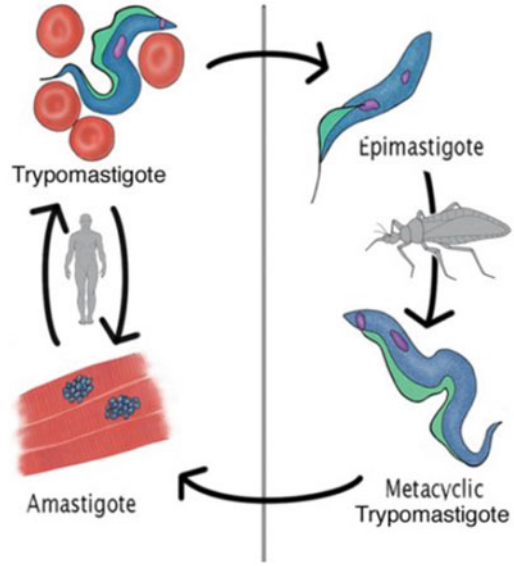

Fig. 4 Developmental stages of *Trypanosoma cruzi* in vertebrate and invertebrate. (Adapted from: Jimenez 2014)

These amastigotes undergo repeated multiplication over a period of 4–5 days and are again transformed into flagellate trypomastigotes and in the process cause death of the infected host cell. The trypomastigotes are released in circulation which can infect new host cells, or they may be taken up by the reduviid bug during their bite. In the gut of the insect, the trypomastigotes metamorphose into rapidly dividing epimastigotes. After a period of a few weeks, these epimastigotes become the metacyclic form, which is the infective stage for mammalian hosts.

Transmission Pathways

In Latin America, *T. cruzi* parasites are mainly transmitted to the host through contact with faeces/urine of infected blood-sucking triton bugs. The primary vectors to humans are the species that inhabit human dwellings, viz. *T. infestans*, *R. prolixus*, *T. dimidiata* and *P. megistus*. The triatomine bugs typically live

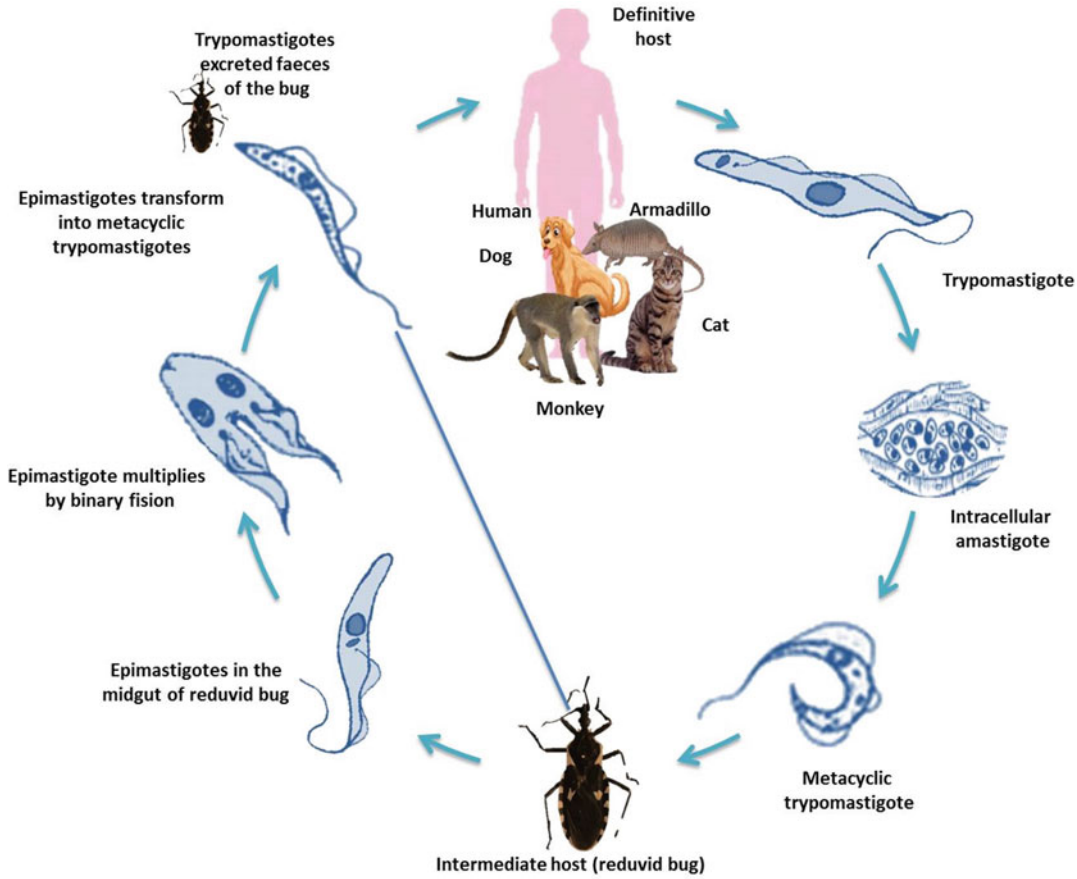

Fig. 5 The life cycle of *trypomastigotes cruzi*

in the walls or roof cracks of homes and peridomiciliary structures. The bugs are nocturnal in nature and are active during nights as and when they feed on mammalian blood. They usually bite on the face/near eyelids and are habituated to defecate or urinate close to the bite site. The parasite enters the body when the individual scratches the bite area causing microabrasions and thus facilitating the entry of contaminated bug excreta.

In addition to classical transmission by the vector, Chagas disease is also transmitted through consumption of food contaminated with *T. cruzi.* Food material may be contaminated with bug faeces, and ingestion of such food is a cause of food-borne transmission, associated with more severe morbidity and high mortality. Other transmission pathways include blood transfusion and organ transplantation or across the placenta during pregnancy. According to recent reports, 22.5% of new infections occurred through congenital transmission. It has also been reported that the infection is capable of being transmitted sexually.

Pathogenesis and Pathology

Pathogenesis of the disease during the early phase is reflective of parasite multiplication and immunological reactions of the host to the parasite. The progression of the infection and parasite replication is controlled by a combination of innate response in the form of NK cells and macrophages and the adaptive response by the proliferation of parasite-specific antibodies. This response is triggered by various pro-inflammatory cytokines like TNF-α and IFN-γ. Chronic phase of the disease is associated with progressive

multiplication of the parasite and concomitant tissue injury and damage along with immunopathological mechanisms. Up to 30% of infected people develop cardiac anomalies and 10% exhibit digestive, neurological or mixed anomalies. As the disease advances, the heart becomes enlarged with cardiac muscle fibres being replaced by scar and fat tissues. Parasites are rarely detected in the heart tissue since they are present at very low levels particularly at later stages of the disease. There may be a massive loss of nerve endings in the heart, colon and oesophagus in the chronic stages of the disease. This condition may contribute to arrhythmias and cardiomyopathy, while in the colon and oesophagus, loss of nervous system control leads to organ dysfunction, blockage of the oesophagus or colon and finally the enlarged organs.

Immunology

The recognition of *T. cruzi* by the immune system depends on both innate and adaptive immune responses of the body. At the outset, the pathogen-associated molecular patterns get recognized by Toll-like receptors of B and T cells which play an important role in bridging humoral and acquired immunities. The innate and adaptive immune responses are characterized by the recruitment of macrophages, dendritic cells, NK cells and B and T lymphocytes along with the cytokines produced by these cells. IFN-γ has an important role as it enhances the production of nitric oxide by macrophages that can destroy the intracellular *T. cruzi*. The key mechanism for systemic protection against *T. cruzi* infection is attained by CD4+ Th1 lymphocyte. It stimulates the production of IL-2 and IFN-γ that in turn trigger the proliferation of cytotoxic CD8+ T lymphocytes. CD8+ T cytotoxic cells produce IFN-γ which in turn activate macrophages, and these activated macrophages along with the perforins produced by CD8+ T cells are instrumental in killing parasite-infected cells. Thus, Th1 response plays a crucial role in *T. cruzi*, while humoral immunity does not play a considerable role. Effective immune evasion mechanisms adopted by the parasite include modulation of the complement system and exerting inhibitory effects on the monocyte-macrophage cells which leads to the chronic phase of the Chagas disease.

Infection in Humans

The initial acute phase lasts for about 2 months after infection. During the long-term chronic phase, the parasites are hidden mainly in the heart and digestive muscles.

The acute stage of the disease is often mild that includes nonspecific manifestations like pyrexia, headache, lymphadenopathy and hepatosplenomegaly. A nodule may appear, and if it is on the eyelid, the condition is known as *Romaña's sign*, and if it is in any part of the body on the skin, it is termed a *chagoma*. Severe acute disease may occur in fewer than 5% infected persons and may turn fatal due to inflammation and fluid accumulation in the heart or brain.

The *indeterminate* chronic Chagas disease is often asymptomatic. However, in 14–45% of people, the disease is manifested in cardiac form with cardiomegaly with cardiac failure and abnormalities in the microvasculature. Further, in 10–21% of people, digestive system involvement is associated with mega-oesophagus or mega-colon. Mega-oesophagus predisposes to odynophagia/dysphagia and acid reflux. Mega-colon may result in constipation or even blockage of intestinal blood supply. About 10% of cases develop neurological manifestations like numbness and altered reflexes or movement.

Symptoms may differ for people infected with *T. cruzi* through other modes of transmission. Persons infected through ingestion of contaminated food and water with faeces of reduviid bug develop severe signs within 3 weeks of consumption. This may include severe nausea and vomiting and dyspnoea, with acute abdominal and chest pain. In infections due to blood transfusion or organ transplantation, the features are similar to those of vector-transmitted disease. Immune-compromised individuals (HIV patients) or those receiving immunosuppressive therapy suffer from severe symptoms associated with inflammation in the brain and surrounding tissue or even brain abscesses.

Infection in Animals

The clinical signs of American trypanosomosis are variable and nonspecific in animals. Dogs acquire infection through faeces of infected reduviid bugs. The bugs often defecate on or near the wounds of the animals and dogs ingest the faeces when licking their wounds. Dogs are also infected by eating infected insects or eating rodents that are infected with *T. cruzi* parasites.

Most infected dogs demonstrate lethargy, decreased appetite and weight loss. In more severe cases, dogs develop signs of heart failure and arrhythmias. Pet owners can observe signs such as fainting, exercise intolerance, vomiting and diarrhoea. Sudden death may occur due to heart failure. Other animals including non-human primates do not typically show any signs of illness.

Epidemiology and Public Health

In the past two decades, Chagas disease has spread to more uninfected areas compared with its evolution since over 9000 years ago. Human activities leading to environmental changes like deforestation are the main culprit for the spread of Chagas disease. Infection caused by *T. cruzi* existed among wild animals but later spread to domestic animals and humans, with relative intensification, since beginning of the twentieth century. *T. cruzi* has been isolated from more than 100 species of wild and domestic mammals. Racoons, wood rats, opossums, non-human primates and dogs are typical mammalian reservoirs. The wide variety of mammalian hosts that *T. cruzi* can infect and the fact that chronically infected animals have persistent parasitaemia result in an enormous sylvatic and domestic reservoir in enzootic regions. This in turn contributes to establishment of the domiciliary cycle of transmission of the parasite in human dwellings.

Chagas disease is a burning public health problem in South America, causing more than 10,000 deaths per year (Fig. 6). Current situation clearly emphasizes that the disease is increasingly becoming a global health concern due to migration of people infected with *T. cruzi* from endemic countries to other parts of the world (Fig. 7 and Table 1). The total estimated number of Chagas patients outside Latin America is more than 4 lakhs with the USA being the most affected country accounting for three-fourths of all cases. Vectors are important in endemic areas, while in non-endemic countries, the main routes of transmission are blood and congenital transmission.

Diagnosis

Detection of *T. cruzi* infections is carried out by conventional parasitological, serological and molecular techniques (Table 2).

Microscopy

The trypomastigotes are most abundant in the peripheral blood during bouts of fever, but may be difficult to detect during the chronic stage of the disease. Fresh specimen of unstained blood or CSF should be examined to observe the motile parasites. Both thick and thin blood films are prepared like malaria parasite and stained by Giemsa or a similar stain. In microscopy, the trypomastigotes are slender and 15–20 μm in length with pointed posterior ends, and they typically appear C or U shaped in appearance. Free flagellum and an undulating membrane may be visible. The kinetoplast is subterminal in position. The sensitivity of blood specimen detection with microscopy ranges from 50% to 95% and is influenced by several factors, ranging from the quality of the microscopic equipment to the expertise of the observer. Amastigotes can be detected in biopsy specimens.

Blood screening for parasite is vital to prevent infection through transfusion and organ transplantation. Conventional microscopy may not detect the infection when the parasitaemia is exceptionally low.

Serodiagnosis

Serological methods are based primarily on the detection of *T. cruzi* circulatory antibodies in the serum. The most commonly used methods are ELISA, indirect haemagglutination, immunoblotting technique and immunochromatographic and

Fig. 6 Endemic zones of Chagas disease (Source: *Wikimedia Commons*)

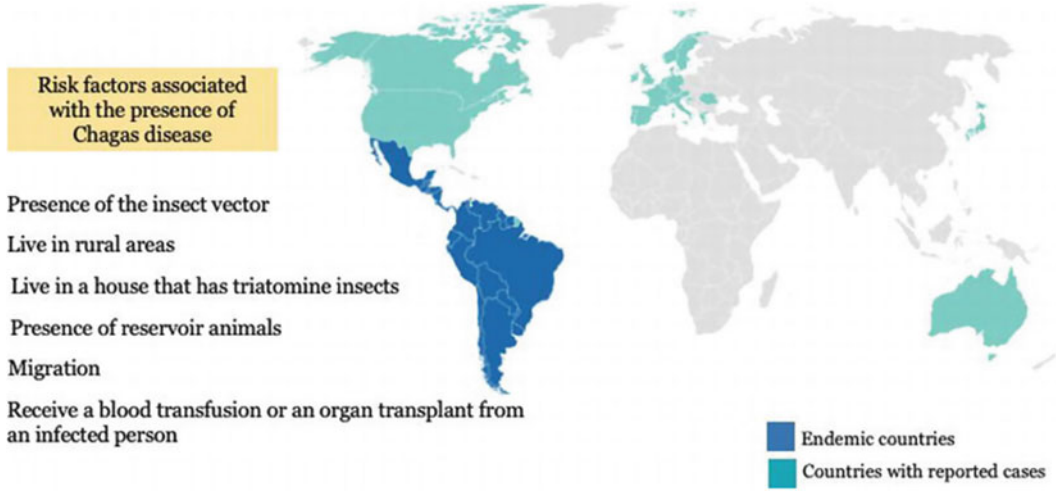

Fig. 7 Distribution of Chagas disease (WHO 2010) (DOI: https://doi.org/10.5772/intechopen.86567)

Table 1 Global distribution of Chagas disease (American trypanosomosis)

Estimated global cases	Distribution	Transmission pathway
Less than 1000 cases	Portugal, Norway, Germany, Austria, Greece	Immigrants
1001–10,000 cases	Australia, Japan, Canada, France, UK, Italy	Immigrants
10,001–100,000 cases	Spain, Costa Rica, Guatemala	Immigrants, blood transfusion
100,001–1,000,000 cases	USA, Bolivia, Peru, Chile, Colombia, Ecuador, Venezuela	Immigrants, blood transfusion, vertical transmission
1,000,001 and above	Bolivia, Peru, Chile, Colombia, Ecuador, Venezuela, Brazil, Argentina, Mexico (endemic countries)	Majority by vector bites (*Triatoma* bugs)

Table 2 Diagnostic methods for American trypanosomosis

Diagnostic approaches	Methods	Targets	Remarks
Parasitological methods (blood screening)	Optical microscopy and microhaematocrit	Aims to visualize the presence of trypomastigotes	Sensitivity varies depending on the stage of infection *Limitations:* Cannot detect chronic phase infections due to low parasitaemia
Immunodiagnostics	Indirect haemagglutination ELISA, IFAT and Western blot	*Trypanosoma cruzi* epimastigote antigens and recombinant proteins (rTc24) are used to target IgG anti-*T. cruzi* antibodies in the blood of infected patients	Best suitable for the diagnosis of the disease even in chronic phase where the parasitaemia is very low *Limitation:* Significant cross-reactivity with *Leishmania* spp.
Molecular assays	Conventional PCR, real-time PCR	Satellite DNA of *Trypanosoma cruzi* and IAC plasmid DNA	High sensitivity and specificity *Limitations:* PCR is not helpful in routine diagnosis

indirect immunofluorescence using crude lysates of the parasite as antigen, recombinant protein or synthetic peptides. These tests despite being highly sensitive and specific show cross-reactivity with *Leishmania* spp. Western blot technique is specific for the detection of *T. cruzi* antibodies using excretion-secretion antigens and/or recombinant proteins. Flow cytometry is useful particularly for differential diagnosis between *T. cruzi* and *Leishmania* infections. The immunochromatographic rapid tests have shown sensitivity and specificity values from 97 to 100% and employ the use of recombinant antigens like H49 and 1F8. The ease of performance and interpretation makes it very useful in field studies.

Molecular Diagnosis

Molecular diagnosis is useful for the accurate detection and characterization of different strains of *T. cruzi*. Several types of PCR including conventional PCR, nested PCR and real-time PCR have been evaluated in the recent past. However, their application is not extensive due to certain limitations. PCR may not be overly sensitive in chronic cases due to exceptionally low level of circulating parasites. Sensitivity of PCR is also influenced by the method of DNA extraction. The low level of parasites in chronic disease results in the PCR-based methods to have sensitivities only of about 45–65%, while specificity remains close to 100%.

Xenodiagnosis

This method is more sensitive than traditional methods. In this, laboratory bred triatomine bugs which are maintained in birds are allowed to feed on the suspected patient. The bug faeces are then examined for the metacyclic forms.

Combination of PCR and xenodiagnosis is much useful to diagnose Chagas disease especially in disease-endemic areas with low parasitaemia.

Treatment

The primary objective of the treatment of Chagas disease is to eliminate *T. cruzi* parasites in the infected host and to prevent the conditions to progress to irreversible lesions associated with the disease. The outcome of treatment by antiparasitic agent often depends on the phase of the disease and the age of the infected individual.

In the acute illness, benznidazole and nifurtimox are highly effective if given soon after infection. Treatment of the chronic phase may not be successful in most of the cases. However, symptomatic treatment of chronic patients is often lifesaving and the sole alternative for this disease. Target-specific treatment for cardiac or digestive or neurological manifestations becomes the need of the hour in critical complicated cases.

Both benznidazole and nifurtimox are contraindicated in pregnant women or in individuals with kidney or liver complications. Nifurtimox is also contraindicated in the backdrop of neurological or psychiatric disorders.

Prevention and Control

Chagas disease is a complex socio-economic and environmental health problem. Till date no vaccine is available for Chagas disease. In endemic areas, vector control has been the most effective method of prevention. Screening of blood and organs for the parasite is mandate to prevent infection through transfusion and organ transplantation. The World Health Organization (2005) recognized Chagas disease as one of the neglected tropical diseases and recommended the following approaches to prevent and control the disease: (1) spraying residual insecticides in and around peri-domiciliary; (2) using bed nets to prevent bite wounds from bugs; (3) maintaining hygiene in food preparation, transportation, storage and consumption; (4) screening of blood, tissue and organ samples before transfusion from donors and recipients; (5) starting antiparasitic treatments in children and women of childbearing age before pregnancy; and (6) screening newborns and other children of infected mothers and providing treatment in early stages.

Case Study

An adult immigrant from El Salvador went to the emergency room of a US hospital with fever and confusion that did not respond to antibiotic treatment. CT scan showed a brain lesion. The patient was HIV-positive, and his last visit to El Salvador was 1 year earlier. A spinal tap showed low glucose and high protein levels in the CSF. In addition, organisms were found in the CSF of size approximately 20 μm. Immunofluorescence assay for antibodies to *T. cruzi* in the CSF was negative but positive in the serum at 1:128.

1. Which other trypanosome is endemic in South and Central Americas, and how it can be differentiated from *T. cruzi*?
2. What are the factors which cause reactivation of chronic Chagas disease?
3. What are the precautions needed to be taken in laboratory while handling the specimen of blood from a patient with suspected Chagas disease?

Research Questions

1. How to develop highly specific and sensitive serological diagnostic tests for *T. cruzi*?
2. How to formulate specific treatment regimen for chronic trypanosomosis?
3. What are the vaccine targets which have been identified for *T. cruzi*?

Further Readings

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