Chagas disease (American trypanosomiasis) threatens the Americas

Accepted 19th September, 2013

ABSTRACT

Trypanosomiases are human and animal diseases caused by protozoans of the class Zoomastigophora. The causative agents share common characteristics like flagella movements and transmission by arthropods; for instance, African Trypanosomiasis (sleeping sickness) is transmitted by the bite of tse-tse fly (Glossina palpalis and Glossina morsitans). However, there are other mechanisms of transmission, such as congenital, in which Trypanosoma equiperdum causes dourine in horses. Trypanosoma cruzi can be found in four morphological stages. The infection by this parasite is known as Chagas disease, which affects heart, esophagus, colon, and other organs. Prevention of Chagas disease is focused on the destruction of vectors and the improvement of dwellings. The aim of this report is to describe the trypanosomiasis that affects humans in America.

Key words: Trypanosomiasis, chagas disease, triatome, myocardiopathy, megaesophagus, America.

INTRODUCTION

Chagas disease or American trypanosomiasis results from the infection with Trypanosoma cruzi, a protozoan first observed by the Brazilian scientist Carlos Justiniano Ribeiro das Chagas, usually known only as Carlos Chagas, in 1909. Since then, this parasite has been found widespread in the American continent.

In Veracruz, Mexico, Triatoma dimidiata was described as a probable vector of human trypanosomiasis (Hoffman, 1928). Triatoma pallidipennis was reported in Oaxaca (Mazzotti, 1936) and also the first two human cases with the disease (Mazzotti, 1940).

In 1965, two types of Chagasic myocarditis were studied in post-mortem cases (Biagi and Arce 1965), while the third case of chagasic myocardiotyph with the first of megaesophagus were published (Salazar-Schettino et al., 1979; Salazar-Schettino et al., 1984). The first case of mega colon was reported in 1986 (Tay et al., 1986). Triatome insects (arthropods) are widely distributed throughout the American continent, from 42nd parallel north (USA) to 49th parallel south (Argentina).

Morbidity and mortality rates of the disorder are variable among the different countries and related to several factors, including: variation of pathogenicity and virulence of T. cruzi strains, lack of proper epidemiological surveys, need of medical attention in rural zones, endemic and non-endemic areas, and lack of information about the condition (Little et al., 1966). In Southern America, for instance, the severity of symptoms and irreversible damage to heart and other organs are frequently the cause of disabilities and death.

EPIDEMIOLOGY

According to the World Health Organization (WHO) in the American Continent, it is estimated that 35 million people are infected and 100 million are exposed to the disease. Chagas disease affects mainly the South, East, Central, and North-west regions of Brazil causing severe heart failure in patients or sudden death in young people (woodcutter’s death) (Doyle et al., 2000). Intestine and esophagus are also damaged by the disease, usually in people living in Minas Gerais, São Paulo and Goias (Pires et al., 2000). In Argentina, the incidence rate...
indicates 10 million persons at risk of getting the disease and approximately 2.5 million already infected (Brigada et al., 2010). In Chile, the number of infected persons was 150,000 while in Peru, it was 80,000 and Venezuela has more than 4 million persons at risk (Maya et al., 2010).

In South America, the most frequent transmitter bugs are: Triatoma infestans, Rhodnius prolixus, Rhodnius pallecens, among others (Pires et al., 2000). The infection is primarily enzootic; hence, wild, domestic and peridomestic animals are the reservoirs such as Dacyops novencinctus (armadillo), Neotoma sp. (wild rat), Peromyscus sp., Rattus norvergicus (rat), Didelphys marsupialis (opossum) and Canis familiaris (dog) (Bautista et al., 1999).

Mexican territory between 0 m and 2,200 m above sea level is considered as a probable endemic area. In this territory, triatomine insects infected by T. cruzi have been found inside dwellings (Bautista et al., 1999). Approximately, 500 case reports of Chagas disease have been demonstrated through parasitology tests and more than 10,000 by serological diagnosis in the States of Oaxaca, Chiapas, Jalisco, Michoacan, Guerrero, Zacatecas, Yucatan, Veracruz, Estado de Mexico, Sonora, Nayarit and Tabasco (Bautista et al., 1999; Ortega et al., 1976).

Vectors of T. cruzi in Mexico are distributed mostly in the Pacific slope (Tay et al., 1986; Palencia and Juliá, 1960; Tay et al., 1967). The most important genera are: Triatoma with 26 species and sub-species, Rhodnius, Dipetalogaster, Paratriatoma, Panstrongylus and Belminus, latter genera including one or three species each (Tay et al., 1979). Several Triatoma sp. share home habits like Triatoma pallidipennis and Triatoma barberi (Bautista et al., 1999; Vidal-Acosta et al., 2000).

Triatomines are known by many names depending on the state, for instance, “Pick” (Maya language), which proved they were known even before the arrival of Spanish conquistadors, Compostela bug, Talaje, nosed and kissing or flying bug (although, most of them do not fly) (Tay et al., 1973). Vectors have different dimensions, but they usually measure 1.5 to 3.5 cm. They mainly show night habits, although, some species bite at full sunlight (Tay et al., 1992).

**LIFE CYCLE AND MORPHOLOGY**

*T. cruzi* can be found in nature in four main morphological stages: trypomastigote, promastigote, epimastigote and amastigote.

Trypomastigote is a flagellate with elongated body measuring 20 to 25 microns in length. It has a large vesicular nucleus and subterminal kinetoplast posterior to the nucleus, which is mainly formed by DNA and mitochondria.

It has an undulating membrane which is joined throughout the cell body; the anterior portion is free to move actively as a whip. Cytoplasm is slightly granular and when the parasite is stained with Giemsa or Wright dyes, it becomes pale blue. The nucleus has carmine color and kinetoplast is purple. This stage is found as metacyclic trypomastigote in blood of mammals and also in the posterior intestine of infected triatomines. This is the infectious form in humans, animals and triatomines (blood trypomastigote) when they take a blood meal from an infected mammal.

The promastigote stage is flagellated and elongated with a size between 20 to 25 microns in length. The kinetoplast is located in the anterior portion, away of the nucleus. The free flagellum emerges from the anterior portion of the body without forming an undulating membrane. This stage is of very brief duration.

Epimastigote is fusiform measuring 20 to 25 microns long. In this stage, the kinetoplast migrates from the anterior portion of the body to be located closer to the nucleus. The flagellum forms a small undulating membrane and the cell multiplies profusely in the intestine of triatomines and also in vitro to give rise to metacyclic trypomastigotes (Figure 1).

Amastigote, also known as leishmanoid or Leishman - Donovan bodies has a rounded shape with measurement between 2 to 2.5 microns. Using an electron microscope, the flagellum is observed inside of a bag. In addition, it has a large nucleus and kinetoplast. This morphologic stage is found within mammalian host cells in which it multiplies profusely.

The life cycle begins when a triatomine bug bites an infected mammal and ingests circulating trypomastigotes, which pass into the midgut and transform into epimastigotes. They multiply by longitudinal binary fission and in few days, there is the presence of metacyclic trypomastigotes (infectious for a new mammalian host).

When an infected triatomine feeds on the face of a patient, usually around the mouth (hence, the name, kissing bug) or in other parts of the body, sometimes, it remains for several hours on the patient (usually asleep). The transmitter ingests several times its own body weight in blood and defecates on the skin or mucosa, depositing together with the feces infective metacyclic trypomastigotes. These triatomines are efficient in the transmission of *T. cruzi* to humans.

Trypomastigotes penetrate the skin through the hole left by the proboscis at the time of the bite, with the legs dragging the feces to the hole (*T. cruzi* is not transmitted by the bite of the triatomine). If the person scratches the bite wound, fingers will be contaminated with the feces, and when awakening, if this person rubs the eyes, infection will occur.

Once in the mammal, the metacyclic trypomastigotes are introduced into the cells of tissue near the site of penetration, where they differentiate to form amastigotes, multiply by binary division, fill the cell, which cannot longer hold more parasites and eventually breaks (Tomlinson et al., 1995). Amastigotes invade the bloodstream, quickly
Figure 1. Triatoma vector of chagas disease.

Table 1. Chagas disease (American trypanosomiasis).

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<thead>
<tr>
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<td>Sarcomastigophora</td>
<td>Mastigophora (flagellar</td>
<td>Zoomastigophora (eukaryote</td>
<td>Trypanosomatidae</td>
<td>Trypanosoma</td>
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transform into blood trypomastigotes, spreading hematogenously throughout the whole body, penetrate once again into cells, become amastigotes, multiply profusely, break the cells and repeat this mechanism. The life cycle is completed when a triatomine, free of infection, bites and ingests blood together with blood trypomastigotes (Table 1).

**MODES OF TRANSMISSION OF AMERICAN TRYPANOSOMIASIS**

People can become infected with *T. cruzi* in several ways:

**Triatomic bug feces**

It is the most relevant form, because this is the natural mechanism of the infection and occurs when a triatomine defecates on the host depositing together with the feces metacyclic trypomastigotes. This stage can penetrate intact skin (demonstrated at least by animal experimentation) through the hole left by the proboscis when biting, scratching, or rubbing the eyes with contaminated fingers, through oral or nasal mucosa and also when eating infected triatomines (in some towns people think they have aphrodisiac properties) (Martinez-Ibarra et al., 2001).

**Transplacental transmission**

During the second half of pregnancy, blood trypomastigotes in the mother’s bloodstream can cross the placental barrier and infect the newborn.

**Blood transfusion**

Similar to malaria infection, transmission through blood transfusion occurs relatively frequent, especially in countries with lack of adequate control of blood donors. *T. cruzi* maintains its viability at 4°C for up to two months.

**Breast milk**

Transmission in milk is very rare; it has be described only in one case by this route.

**Handling of infected animals**

This mechanism occurs specially among hunters who skin animals and handle their infected meat.
Laboratory infections

These usually occur while working with cell cultures or with infected blood from animals that are used to maintain T. cruzi strains.

Other modes of transmission

Even when transmission by oral route has been demonstrated experimentally, inoculating flagellates directly in the oral mucous of mice, or using flies (Musca domestica), these mechanisms are definitely of little importance in T. cruzi transmission to humans (Ortega and Tay, 1972).

PATHOGENESIS AND CLINICAL ASPECTS

The parasite attacks the host in several ways. Nevertheless, destruction of cells of reticuloendothelial system and other tissues, by growth and multiplication of the flagellates are probably the most important modes (Cohen and Gurtler, 2001). Köberle referred to the production of a trypanotoxin in the Chagas myocarditis, and the production of "megas" (mainly megaesophagus and megacolon).

Attempts to report the etiopathogenesis of the lesions caused by T. cruzi are very variable and often contradictory. Meira de Oliveira makes an interpretative synthesis describing two theories: the inflammatory, by evidence of inflammatory component from injuries, and the allergic, in which the lesions are caused by immunoallergy process due to products of parasite disintegration as well as tissues damaged by them.

It is clear that parasites in myocardium block nerve conducts, resulting in dysfunction (bundle branch block), dilatation (cardiopathies) and also myocardial inflammation (acute myocarditis) with cellular infiltration (polymorphonuclears and mononuclears) that subsequently result in mononuclear inflammation with fibrosis (chronic chagasic myocarditis). Parasites invade many tissues and organs such as heart, brain, liver, spleen, lymph nodes and muscle causing lesions and various pathological conditions (Sánchez-Lerm et al, 2007).

Once T. cruzi enters the body, there is an incubation period that usually lasts 4 to 14 days. During this time, the parasites undergo transformations as aforementioned, and penetrate the host cells, initiating the known pathogenic mechanisms, leading to:

Acute phase

In most cases, there are no symptoms or signs of entry of the parasite into the body. Between 5 and 10% of patients develop acute symptomatic stage. Even when it may be present at any age, it is much more common in children.

The acute stage lasts between 7 and 30 days; however, the infection remains latent and eventually results in cardiopathies. During this time, approximately 10% of patients die.

Among the most visible symptoms and signs is the Roña - Mazza sign or ophthalmoganglionar complex, which is painless and characterized by unilateral bipoalpebral edema, conjunctival hyperemia, low conjunctival discharge, dacryocystitis and local adenopathy, compromising the nodes near the site of entrance of the protozoan, and spontaneously disappearing in about 15 days.

Inoculation chagoma

It usually appears on the face, and rarely affects other parts of the body; it is a subcutaneous nodule with regional microadenitis located at the site of penetration of the parasite. It is often confused with other skin conditions (boil and tumors, etc).

Latency stage

This occurs after acute phase; parasites multiply slowly inside the cells, and those that become trypomastigotes in bloodstream are destroyed by antibodies. There is almost complete reduction of symptoms, keeping this situation for long time (15 to 20 years) without spontaneous relief (Cossy et al, 2009).

Chronic phase

During this stage, the infection may remain silent for many years (10 or more) and be symptom free. The patients with chronic Chagas disease, often have irreversible damages in heart and other organs (esophagus and intestine, etc), which are discovered by electrocardiographic or radiological (Dantas and Aprile, 2006).

Cardiomegaly, with cardiac insufficiency predominantly of the right side, alterations of electrocardiogramm (ECG), which indicates incomplete right bundle branch block and left anterior hemi-block, are the more outstanding findings at this stage.

Patients can live many years free of symptoms or with apparent dyspnea, palpitations, precordial pain or cardiac insufficiency. Sudden death can occur in a person without important symptoms shown previously. The prognosis of myocarditis is insidious and there may be sometimes vascular-type strokes caused by thrombi.

DIGESTIVE FORMS

An important number of patients with chronic Chagas disease (rate variable and not well defined in many coun-
tries) develop the so-called “megas” frequently meagesophagus and megacolon. Megas are rarely found in other organs. In meagesophagus condition, there is destruction of autonomous ganglia located in the visceral walls, resulting in esophageal motility disorders, with appearance of dysphagia, epigastric pain and regurgitations (Madrid et al., 2004). The size of a meagesophagus is usually two to three times bigger than the normal tissue (Dantas and Aprile, 2006).

**Visceral complications**

These symptoms are normally severe, especially in preschool children. It is characterized by fever not higher than 38°C, hepatosplenomegaly, generalized polyadenitis, anasarca, diarrhea, bronchial signs, cardiomegaly and meningoencephalitis.

When the heart is affected, there is myocarditis of variable intensity. There have been reported mild cardiomegaly cases without changes in heart rate and severe cases with important cardiomegaly and alterations of ECG due to bundle branch block and cardiac insufficiency. The prognosis of patients is apparently benign and the recovery takes a few months. Meningoencephalitis can also be found in young children with alterations of CSF as a bad prognosis.

**Congenital Chagas disease**

It occurs when blood trypanomastigotes are transmitted from mother to fetus through the placenta, resulting in prematurity, hepatomegaly, splenomegaly, myocardial and CNS complications in the newborn.

**DIAGNOSIS**

To establish the diagnosis of Chagas disease, it is important to consider two main aspects: epidemiological and clinical. At first, the place of living is something to take into account; for instance, it is hard to think about Chagas disease in a patient who had been living all his life on the floor 102 of Empire State building in New York, USA; conversely, if such patient comes from an endemic area, the situation changes completely.

In addition, patients are questioned for possible visits to endemic areas, because many people do not have outward signs or symptoms (Romaña- Mazza sign, inoculation Chagoma, adenopathies and fever, etc) and the clinical manifestations of chronic stage may lasts up to 10 years or more to appear. The knowledge of the transmitters and biting history are important. Any person living in endemic areas and presenting pathological heart conditions such as cardiopathies and myocarditis, etc or meagesophagus and megacolon are suspected to have the disease.

Demonstration of the parasite, which is difficult, is performed through direct parasitological tests. Trypomastigotes can be found in bloodstream, especially during acute stage of the infection. The following methods are used to identify the protozoan.

**Direct test in blood**

To perform this test, a drop of blood by puncturing the finger or earlobe is collected (syringe is used when larger quantities of blood are needed in serology and cell cultures). Blood is placed on a slide and covered with a coverslip to be immediately observed under the microscope.

Trypomastigotes can be seen with actively movement and displacing red blood cells, which facilitates their observation. It is recommended to make thick or thin blood smears and staining to determine morphological and staining characteristics. Giemsa, Wright, Leishman or any other dyes used to stain blood parasites are employed.

**Hemoculture**

Special culture media are used, for instance, the Novy, Nicolle and MacNeal (NNN), Nakamura and others, which are optimal for trypanosomatids growth in vitro. On these, 1 to 5 ml of blood obtained by venipuncture is added. This method gives good results, especially at the final stage of the acute phase when trypomastigotes become low in patient's blood.

**Inoculation of experimental animals**

The experimental white mouse (Mus musculus) is very susceptible to infection with T. cruzi; therefore, when injecting infected blood from a suspected patient into mouse intraperitoneally, trypomastigotes will grow in it easily. Ten days after inoculation, parasites can be detected in blood from infected mice.

**Xenodiagnosis**

This method is employed to detect trypomastigotes using triatomines. However, there are reports indicating that this technique produces adverse reactions in certain patients. To perform it, the use of laboratory-reared triatomine nymphs is preferred (from eggs), free of infection (better to use native species from the region the patient comes from).

Nymphs are put in a little cardboard box covered with nylon tulle and then placed on the patient's forearm; this allows the triatomines to bite and take blood to satiety
Histological sections

In post-mortem cases, it is difficult to find nests of amastigotes in organs, although, this test should be performed routinely in people from endemic areas of Chagas disease during the autopsy.

Immunological tests

They are performed to demonstrate antibodies against *T. cruzi* in the suspected patient. Some of the most common techniques are: complement fixation (CF) of Machado and Gerreiro test, hemagglutination (HA), indirect immunofluorescence (IFA), indirect ELISA and PCR. Some of these are highly sensitive (90 to 95%), such as CF, HA, and IFA Immunoblot. These tests are employed in the chronic stage of the disease, when it is very difficult to detect trypomastigotes.

Other resources to integrate the diagnosis are:

**Imaging test:** In ECG, there could be incomplete or complete right bundle branch block, as well as, ventricular extrasystoles and atrial-ventricular blocking.

**Holter monitor:** This can be useful to define the nature of the arrhythmias as well as prognosis of patients. Prognosis can be good or bad according to the high correlation between the presence of arrhythmias with sudden death.

**Radiology:** This type of study is used to confirm the presence of cardiomegaly, besides being able to find alteration of right cavities without evidence of pulmonary congestion as usually occurs in most of the chagasic cases.

**Echocardiography:** This test is very helpful for those suspected patients with ECG and x-ray examinations unchanged. Akinesia of left ventricular posterior wall and apical aneurysm can then be found with or without the presence of trombi.

For practical purposes, it is important to consider the performance of two or more serological or molecular studies to integrate the diagnosis of infection caused by *T. cruzi*, especially when there is need to confirm a case, and also for the control of blood banks and transfusions services, monitoring of cases under chagasic treatment and in epidemiological research studies.

**TREATMENT AND PREVENTION**

There have been used many drugs against the disease, but when effective, they can cause systemic toxicity and intolerance. Currently, the most employed is nifurtimox (Nx), a powerful trypanocidal drug. Nevertheless, the use of benznidazole (Bz) is also suitable.

The administration of Nx and Bz have a successful effect on trypomastigotes and stop the infection; however, they have null effect on amastigotes found inside the cell. Therefore, in chronic cases when cardiopathies and heart insufficiency are present, adequate medication should be employed and caution must be taken when using immunosuppressive drugs against Chagas disease.

According to Dr. Werner Apt (University of Chile), Chagas disease in humans should be treated at any stage, except in the terminal chronic phase. In the clinical acute phase, the ideal drug is Nx. Trials are currently being performed to obtain a vaccine against *T. cruzi* infection, but this is still in the experimental stage.

Prevention of Chagas disease is focused on the destruction of transmitters and the improvement of dwellings in endemic areas. The use of residual insecticides (lindane and gamexane, etc) in such areas must be constant and repeated. It is also important to have health education programs and train the people to combat and eradicate triatomines from their homes. There should be mandatory screening in blood banks and in hemotherapy centers in order to detect antibodies against *T. cruzi*. In some countries *T. cruzi* universal screening of blood donors is already mandatory such as Mexico (NOM-253-SSA1-2012), Honduras, Brazil and Argentina.

**OTHER TRYPANOSOMIASIS**

*Trypanosoma rangeli*

In some countries of America (Guatemala, El Salvador, Venezuela, Colombia), the infection by *T. rangeli* has been reported by Tejera (1920). This protozoan parasite affects mainly animals and occasionally humans.

The vectors are different species of *Rhodnius*, which transmit the infection through the biting and not by the feces like *T. cruzi* (Vallejo et al., 2007; Montfort et al., 2000). Actually, no disease is produced in the vertebrate host; instead, the main goal is to know how to recognize it in order to avoid confusion with *T. cruzi*. *T. rangeli* is larger, measuring 35 microns and its kinetoplast is small and away from the posterior end portion of the parasite.
African trypanosomiasis

The etiological agents of this disease are Trypanosoma gambiense and Trypanosoma rhodesiense. African trypanosomiasis, also known as "sleeping sickness", only affects African countries where the distribution of the disease is wide. The parasite is transmitted by the bite of tsetse flies (Glossina genus), having as reservoirs antelopes and men, with slow clinical prognosis (several years) or acute (less than one year).

There are mainly two forms of African trypanosomiasis in humans; the first is caused by T. rhodesiense: rapidly progressive and fatal in less than a year. The second: caused by T. gambiense with long course and taking several years to kill the patient and causing central nervous system damage. The acute fatal cases mostly cause hemorrhage of lung parenchyma, bone marrow failure and hyperplasia of reticuloendothelial system.

The disease shifts from the acute phase where there is proliferation of trypanomastigotes in blood and lymph nodes to the sleeping sickness, which is chronic, with involvement of central nervous system (Sanner et al., 2000). The disease starts at the end of the first year or early in the second, resulting in death between two and three years.

African trypanosomiasis is suspected when a patient lives or has visited endemic areas and shows acute infection with irregular fever and palpable lymph nodes generally along the back of the neck (Winterbottom's sign) or suffers chronic disease with sleepiness. The finding of trypanomastigotes in fresh blood, spinal fluid, bone marrow and lymph nodes puncture determines the precise diagnosis (Nishimura et al., 2001).

Regarding the treatment, the use of Pentamidine is successful in early stages of the disease, at a rate of 4 mg/kg/day by intramuscularly injection, for 10 days. Melarprosol and triparasamide are also used.

Acknowledgments

We thank Dr. Enrique Graue Wiechers (UNAM, MEXICO), Dr. Rosalinda Guevara (UNAM, MEXICO) and Dr. Klaus T. Preissner (JLU-Giessen, Germany) for helpful discussions, Q.B. Saúl César Rodríguez Mesinas for excellent technical assistance. This work was supported in part by the Dean’s Office of the Faculty of Chemical Sciences and the Director of Strategic Planning and Evaluation at the Universidad Autonoma Benito Juarez de Oaxaca.

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