Chapter 4

The Mouse Model as a Tool for Histological, Immunological and Parasitological Studies of *Trypanosoma cruzi* Infection

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Additional information is available at the end of the chapter

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Abstract

The global expansion of Chagas disease is due to the constant migration of individuals from endemic countries with incidence of vector and nonvector transmission of *Trypanosoma cruzi*. The disease is present in its various stages: chronological characteristic signs and symptoms of the infection and its mechanism of immune system and cell and tissue damage. The first stage, which lasts 90 days approximately, is diagnosed by direct methods (blood smears stained with Giemsa, fresh and xenodiagnosis). The indeterminate-chronic stage is asymptomatic, but the growth and intracellular binary multiplication of the trypomastigotes continue promoting cell lysis and allowing parasites to infect other cells, with preferential tropism to organs producing mega syndromes such as cardiomyopathy, myocarditis, meningoencephalitis, megaesophagus and megacolon. Inadvertently, this process is repeated for several years leading to Chagas disease. The mouse inoculation allows checking the parasitemia in vivo and the development of the disease in short time (signs, behavior and tropism), histopathological alterations and detection of antibodies in serum. These parameters may vary when using different strains of *T. cruzi* from different geographical areas; Triatoma species due to their genetic variability are influenced by the environment, nutrition, reservoirs and habitat. The murine model ECA CD-1 has the ability to replicate human findings of Chagas disease.

Keywords: Trypomastigote, Chagas disease, murine model, amastigote, CD-1 strain

1. Introduction

In 1909, the Brazilian doctor and researcher Carlos Ribeiro Justiniano das Chagas discovered the etiological agent of the later called Chagas disease in the triatomine insect (family *Reduviidae*), a flagellate protozoan of the genus *Trypanosoma* and subgenus *Schizotrypanum* and designated the specie adding "cruzi" in honor of his teacher and mentor Oswaldo Cruz, hence the name *Trypanosoma (Schizotrypanum) cruzi*. Later, in 1926, another doctor of Argentine origin, Salvador Mazza described the magnitude of the endemy in Argentina, Bolivia and Paraguay, identifying the hemoflagellate parasite in blood samples, demonstrating in this way, the existence of the trypanosomatida infection, which was given the name of American Trypanosomiasis, since the vectors of *Trypanosoma cruzi* had been found only in America [1].

Chagas disease is a chronic debilitating affectation which impairs the health and the quality of life of infected people all around the world. The estimated number of infected people in the world arose from 30 million in 1990 to 6–8 million in 2010. In the past 20 years, the annual incidence decreased from 700,000 to 28,000 and the burden of Chagas disease decreased between 1990 and 2006 from 2.8 million disability-adjusted life to less than half a million [2]. Chagas disease is in close relation to the socioeconomic status of the population migration between Latin America and the rest of the world, and it currently represents one of the most important public health concerns [3]. The initiatives of the Americas have allowed achieving significant reductions in the number of acute cases and the presence of domiciliary Triatominae vectors in all endemic areas.

Trypanosoma cruzi belongs to the order of the kinetoplastid diseases, a group of parasites that has one or two flagella from a monophyletic group that diverged early from the branch common to all eukaryotic organisms. The morphological feature that distinguishes them is a prominent and paraflagellar structure known as kinetoplast, which corresponds to a condensation of DNA (DNAk), located on the inside of a single mitochondrion, which is branched across the cell. Within the family *Trypanosomatidae*, the *Trypanosoma* genus is most important because it includes a number of human diseases vectors such as *T. cruzi*, *T. brucei gambiense* and *T. brucei rhodesiense*, causal agents of Chagas and Sleeping sickness disease, respectively. Depending on the behavior of the parasite within the vector, the trypanosome genus has been divided into two groups. The first one called stercoraria, includes the trypanosomes that develop in the digestive tract of the vector, with the release of the infective forms in the stool (*T. cruzi* and *T. lewisi*). The second group called Salivaria includes trypanosomes that are initially developed in the digestive tube then passing through the epithelium and reaching the salivary glands, from where the infective forms are inoculated mechanically by bite or sting of the vector (*T. brucei*, *T. congolense* and *T. rangeli*) [4].

1.1. Life cycle

T. cruzi displays a digenetic life cycle alternating its multicellular life between the vertebrate host and its invertebrate vector. The cycle starts in the invertebrate arthropod when the

insect sucks the blood of an animal carrying trypomastigotes in its blood, which gets to the stomach and are transformed into esferomastigotes and the replicative form epimastigotes. Subsequently, parasites migrate to the intestine where they multiply and eventually are transformed into the infective forms metacyclic trypomastigote, staying in the rectal ampulla until they are excreted with feces and urine. In this point, the life cycle continues in humans where the highly infective metacyclic forms aim to penetrate the skin or mucous membranes; although unable to pass through intact skin, they enter the body through skin or mucous membrane abrasions infecting macrophages, fibroblasts, smooth muscle and striated cells, Schwann cells, glial cells and neurons, excepting eosinophil and neutrophil cells. Once parasites have penetrated the cell, proliferation occurs and the trypomastigotes are released in the interior of a parasitophorous vacuole giving rise to amastigotes forms. The life cycle restarts with the insect feeding from an infected animal (**Figure 1**).

1.2. Routes of transmission

We can distinguish three cycles of vector transmission in *T. cruzi*. The primitive or wild cycle is zoonotic in nature. The protozoan parasite circulates between the insect vectors and the wild reservoirs (mammals of small and medium size). More than a hundred wild reservoirs of *T. cruzi* among marsupials, xenarthrans, bats, carnivores, lagomorphs, rodents and nonhuman primates have been described [6].

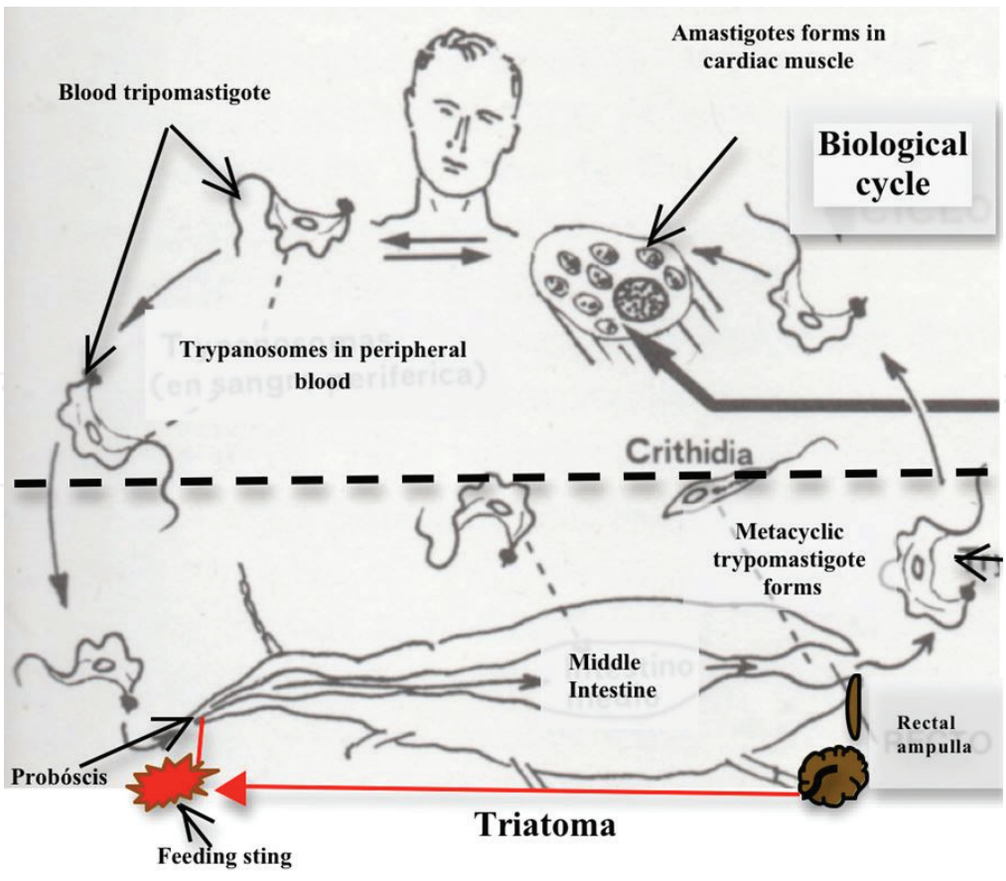

Figure 1. Digenetic biological cycle of *Trypanosoma cruzi*. Adapted by Federico-Mayer Rodolfo† and modified by de Diego-Cabrera José Antonio. Faculty of Medicine. Autonomous University of Madrid [5]. Spain, 1984 [5].

The domestic cycle comprises the infection of humans and the consequent Chagas disease. The domestic cycle is defined by factors in the anthroponotic foci, making people one of the last natural reservoirs of *T. cruzi*. Finally, the peridomestic cycle, comprising peridomestic mammals (rodents, marsupials, cats and dogs), which are in close contact with humans and their residences that have been built invading the habitat of wild triatomas that are attracted by the food and the lights of the houses.

Depending on the eco-epidemiological conditions of the place, both circles can overlap becoming an intradomiciliary cycle, especially when mankind invades the natural habitat of these vectors and builds houses fearing the entrance of reduvids (**Figure 2**).

On the other hand, the infection transmission by blood transfusion has become a serious complication in nonendemic countries, due to the migration of infected individuals from endemic regions [7]. This route is considered the second most important route of transmission in endemic areas [8]. *T. cruzi* resists processes of cryopreservation and thawing and can survive up to 18 days in total blood stored at 4°C. The vertical transmission is also known as a natal or congenital transmission, including prenatal, perinatal and postnatal care. This mechanism of transmission has a variable incidence between 0.1% and 18% according to geographical region [9], and has been regarded as the third in order of importance, next to vector-borne and transfusion transmission. An infected mother can transmit the parasite circulating in her blood during the second half of gestation. Among infected newborns, only 10–30% present symptoms [10].

The infection is not detected until adulthood in the course of the latent or indeterminate phase [11]. Spontaneous abortions have been reported, premature birth, intrauterine growth retardation, stillbirths and various clinical forms that can go from low birth weight, hepatomegaly,

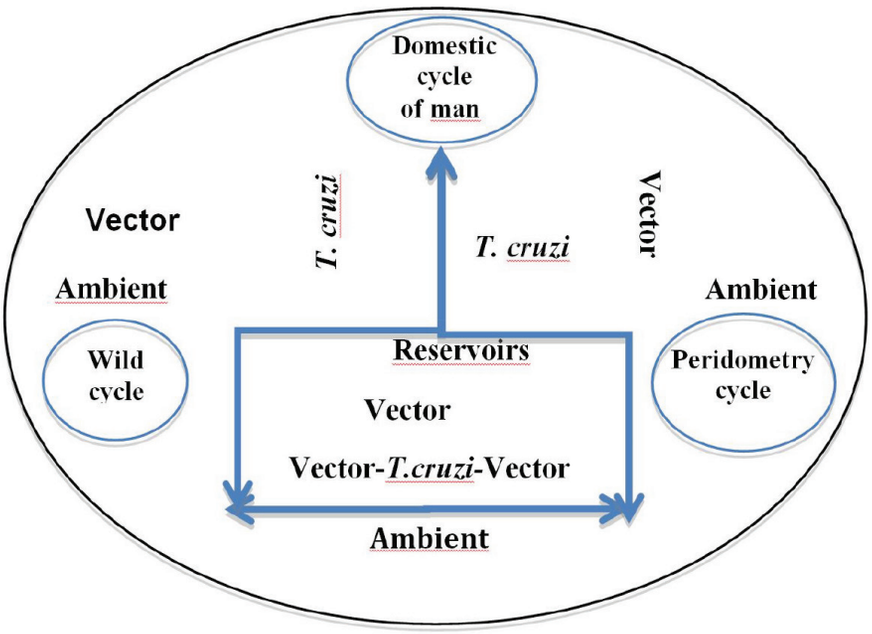

Figure 2. Exchanges between wild, peridomestic and domestic cycles of *T. cruzi* transmission. Adapted from Coura and Pinto Dias [6].

splenomegaly, acute respiratory symptoms, anemia, digestive disorders, Cardiac and Central Nervous System (CNS). The donation of organs has increased the number of infected people in urban areas. It has been informed about the transmission of infection to seronegative heart, bone marrow, liver, pancreas and kidney transplant recipients with variable transmission rates that reach 35% [12]. Patients infected with *T. cruzi* that should receive a transplant also represent a particular challenge, with risk of reactivation of the disease because of the immunosuppression after transplantation. The raw meat from infected rats and rabbit can be induced by the consumption of foods contaminated with triatomines or its feces, or by the ingestion of raw meat from infected mammalian hosts [13]. It must be confirmed by the detection of the parasites in a direct microscopic examination of a blood sample or other biological fluids of the patient. Many cases of numerous outbreaks of acute Chagas Disease are attributed to oral transmission; it has been detected in the Amazon region, due to the consumption of drinks or food contaminated with feces of infected triatomines [14]. It is also important to take into account the laboratory accidents that arise when research animals are handled mainly by postgraduate students, even though they occur in a smaller proportion [15].

1.3. Pathology and mechanism of injury

The disease presents three phases: the acute, chronic asymptomatic (intermediate or dormant) and the chronic symptomatic. The incubation period in the acute phase is 4–10 days and of shorter duration when the route of transmission is blood transfusion. This stage is generally asymptomatic, or it can occur with systemic manifestations that are common to other diseases such as fever, edema, lymphadenopathy, hepatomegaly and splenomegaly. It is accompanied by anorexia, fatigue, myalgia, headache and, occasionally, arthralgia. In some cases, there are signs of inoculation or entrance door, chagomas, lesions that are more frequent in the face and limbs of a forunculoid aspect, pink or violet and indurated borders. A typical sign in children is the bipalpebral edema (sign of Roman-Mazza). In this phase, the trypomastigotes are easily detected in the blood due to the high parasitemia.

In case the acute phase is overcome, there will be an extended period of chronic disease without clinical symptoms that lasts from 5 to 10 years, characterized by low parasitemia and by the presence of anti-*T. cruzi* IgG antibodies. About 30% of seropositive individuals reach the chronic phase, and in a span of 10–30 years, clinical manifestations such as heart disease and digestive megasyndromes show up, which may occur separately or coexist in the same patient. The chronic phase progresses slowly with a predominance of tissue damage. Digestive disorders consist in dilatation of viscera (mainly colon and esophagus, and in two-thirds of cases, the progressive myocarditis leads to the development of chronic Chagas disease heart (CChC). The relative prevalence of the various clinical manifestations varies according to geographic regions. In Argentina, as well as in Venezuela and Central America, the main clinical manifestation is the cardiomyopathy. On the contrary, in Chile and in the central region of Brazil, the mega syndromes are more frequent. This heterogeneous incidence of manifestations in different endemic areas could obey both biological and genetic differences of the circulating parasites [16] and host-related factors (age, gender, ethnicity, exposure to infections, family history of Chagas disease heart) [17], in addition to the introduction by migration of infected people from different countries around the world (**Figure 3**).

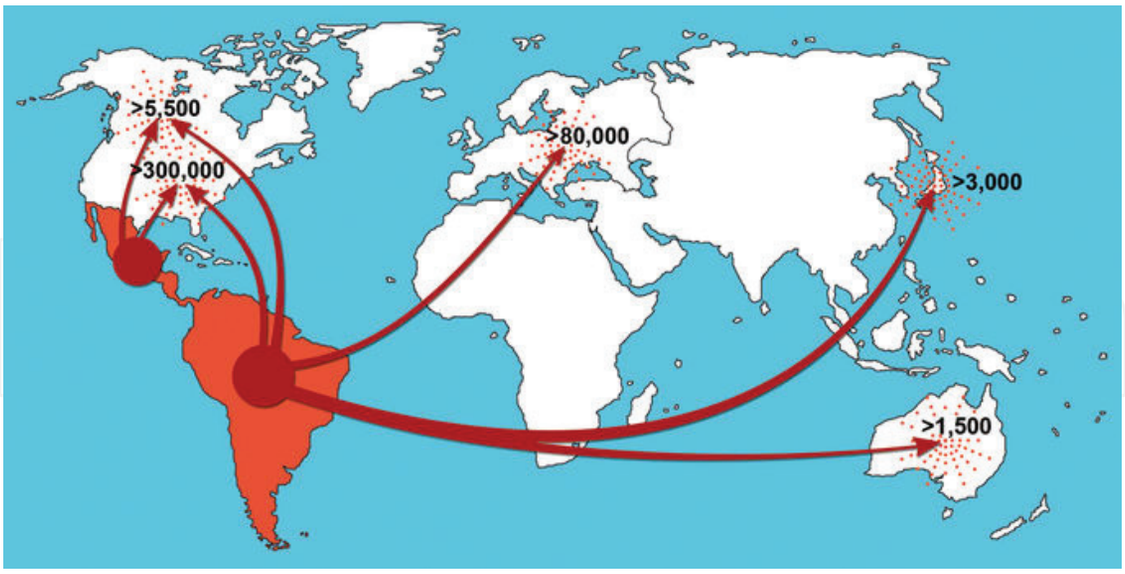

Figure 3. The estimated number of cases of Chagas disease in nonendemic countries, driven by constant migration. (ISGlobal, 2015).

In this last Chagas disease's phase, histological lesions are disseminated in the heart muscle, intestines and nervous system, inflammatory infiltrates composed mainly of CD8+ cells, in addition to nests (pseudocysts) full of parasites in their form of amastigotes [18].

Regarding the treatment, there are currently only two drugs available, benznidazole and nifurtimox. The therapeutic success is closely related to the stage of infection at the time of starting the treatment. Patients in acute phase (regardless of the route of infection), neonates and children, have better therapeutic prognosis [12, 15]. On the other hand, the success of such drugs is discussed in individuals with chronic infection and so far, there is no established therapeutic regimen [19]. The adverse effects are much more important among the adult patients; cases of photosensitivity and skin rashes, nausea, anorexia, weight loss and abdominal pain have been reported.

Recently, a group of biomedical and clinical scientists members of the network NHEPACHA (New Tools for the diagnosis and evaluation of the patient with Chagas disease), based on clinical and immunological evidence, have suggested new paradigms regarding the medicines for the Chagas disease in order to provide better treatment for patients in chronic phase [20].

On the other hand, the study of biochemical and biological characteristics of the hemoflagellate parasite has enabled the identification of new targets for chemotherapeutic agents; an example would be the drug trials with inhibitors of the biosynthesis of Ergosterol, Posaconazole and Ravuconazole, respectively, in patients with chronic Chagas disease.

1.4. Diagnosis of infection

Parasitological methods for detection of the acute phase have great sensitivity (direct methods) [21].

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In the same way, these methods are used for the diagnosis of congenital infection in newborns and in children under the age of 6 months. The lack of maturation of the immune system and the presence of maternal IgG antibodies make, in the latter, the use of the serology for the infection diagnosis impossible [22]. The protozoan *Trypanosoma cruzi* is a powerful antigen and a few months after the initial inoculation, there is a humoral immune response that is effective is controlling the increase of parasitemia, which is mediated by antibodies and enzymes of the complement system. There are antibodies to various antigens of *T. cruzi* (surface, somatic and excretion), which belong to different classes (IgG, IgA, IgM) and subclasses [23–25]. The serologic test for the selection of blood donors must conform to the Official Mexican Standard for Epidemiological Surveillance, Prevention and Control of Vector-borne Diseases [26–28], citing that it must analyze the serum of each donor with two conventional immunological tests; if one of them is reactive, a third one will be carried on in such a way so as to qualify the donor as either positive or negative, with two reactive or two negative tests. In all cases, the laboratory diagnosis should be accompanied with epidemiological and clinical history of the patient, as the current and past source, the type of housing where it is found, the trips that could have been made to endemic areas and the history of blood transfusions and the infected mother.

1.5. Origin of the discrete units (UDTs) typing of *Trypanosoma cruzi*

The evolutionary history of *T. cruzi* infection is closely related to its vertebrate host [29]. The mammal fauna of South America in the cretaceous period mainly consisted of marsupials and

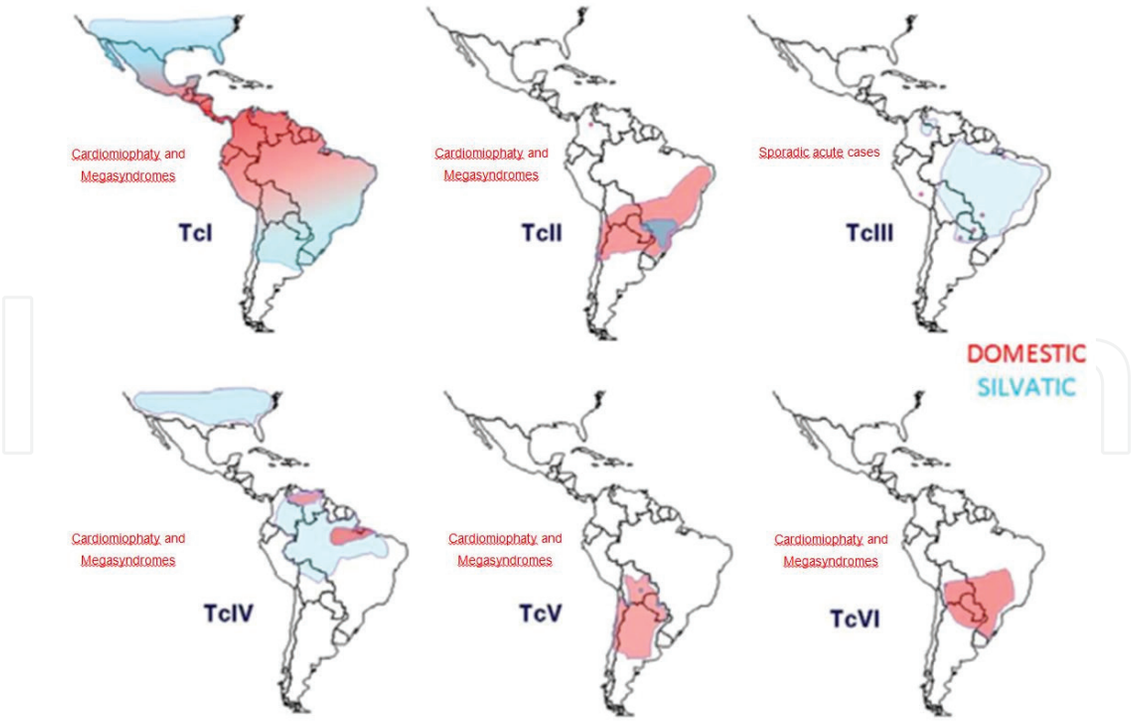

Figure 4. Geographic distribution of *Trypanosoma cruzi*, subpopulations disease phases with the corresponding primary clinical manifestations.

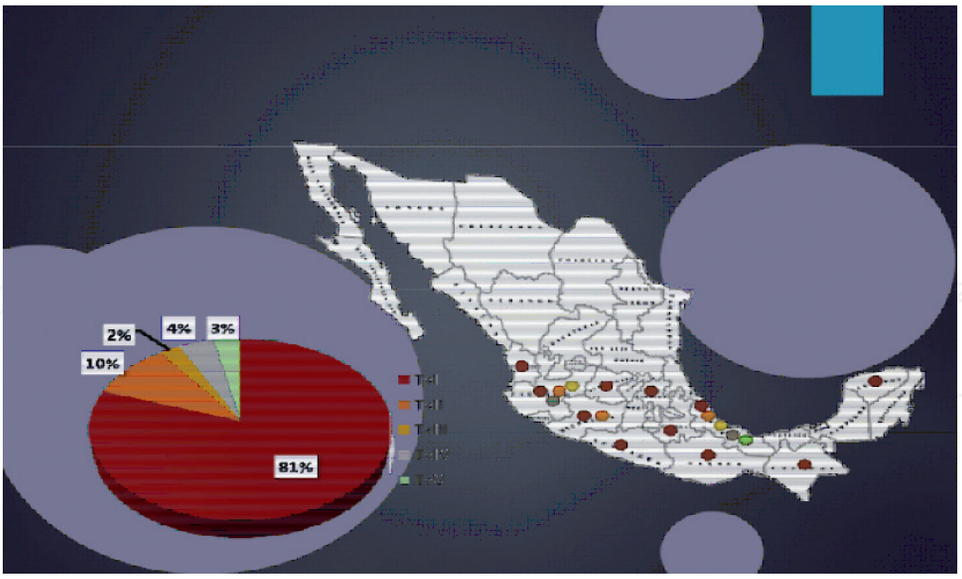

Figure 5. Distribution of *Trypanosoma cruzi* strains in México [35, 36].

placentals, the ancestral of the order Xenarthra (armadillos, anteaters and sloths), which were the natural reservoirs of the parasite at the time. The various ecotopes in whom were these two groups of hosts would have made possible the evolution by clonal propagation of two groups of parasites, which gave rise to the ancestral UDTs, TcI and TcII. It has been suggested that 1Cwi, evolved in association with the marsupial mammals of the genus *Didelphis* (weasels) and *T. cruzi* II, did in relation to the terrestrial mammals, such as armadillos [30]. *Trypanosoma cruzi* is a species composed of heterogeneous populations that circulate in nature between human beings, arthropod vectors, domestic animals, and wild reservoirs [31]. Currently, it is generally accepted that *T. cruzi* is a paradigmatic pattern of clonal evolution with low rate of gene recombination. A constant pattern of *T. cruzi*, behavior cannot be expected since different strains (subpopulations) circulate in nature. Extensive polymorphism promotes variation in infecting capacity, behavior in different hosts (virulence, histotropism, curves of parasitemia), adaptation to different vectors, immune response, stimulation, susceptibility to different chemical compounds, capacity of replication and differentiation, among others. Subpopulations are currently identified in the laboratory by biochemical, immunological and molecular biology assays [32, 33] (**Figure 4**).

Studies in murine experimental models have shown that both the parasite and host genotypes are crucial for tissue distribution and pathophysiology of infection by *T. cruzi* [34]. It has been previously reported that 81% of the Mexican strains of *T. cruzi* belong to lineage TcI and have different capabilities of infection, virulence and processing capacity in vitro, when compared to the other lineages [35, 36] (**Figure 5**).

2. Importance of the murine model in research American trypanosomiasis

2.1. The murine model in biomedical research

Animal models are very useful for studying human diseases because there are hundreds of pathogens that affect both humans and animals. The use of experimental animals in biomedical

research represents a key element for development of new prevention approaches and treatment of transmissible and nontransmissible diseases. Suffice it to recall the rabies vaccines, smallpox, tetanus, diphtheria, whooping cough and polio, the development of several antibiotics, insulin, and the knowledge of the genetic bases of inheritance [37]. No doubt that mice are the most commonly used animal for in vivo assays among experimental animal models in biology and medicine. The use of mice allows the study of mammal's reactions against aggressions like poisoning or infection (viral, bacterial, or parasitic), the study of immune responses and disorders and many others in several different fields like oncology, teratology and embryology [38] (**Figure 6**).

Herein, we present a comparative cross-sectional study involving four *Trypanosoma cruzi* strains obtained from three different species of triatomas captured in endemic areas of the states of Jalisco (*T. longipennis*), Morelos (*T. palidipennis*), Nayarit(*T. longipennis*) and Queretaro (T. Mexicana)

2.2. Collection sites

Several communities from different States of México were included in the present study: San Pablo, Tolimán in Querétaro State, Milpillas of Talpa de Allende in Jalisco State, Sant Catarina in Morelos State and Jala in Nayarit State (**Figure 7**).

2.3. Triatoma collection and maintenance

Cages covered with adhesive tape were used, with the glue facing outward. A live Wistar rat was placed inside the cage. Cages were placed at late night in strategic areas under the loose stones of poultry and farm animal fences, fallen leaves and wooden logs. Cages were collected the next day, early in the morning (**Figure 8**).

Triatomas glued to the surface of the gummed paper were carefully detached, with the aid of entomological tweezers and placed in jars covered with mosquito mesh. A piece of filter paper in accordion shape was placed inside the bottle to facilitate the movement of the triatomas and the collection of urine or feces deposited on its surface.

The triatomas are maintained inside the bottles at 25–26°C and 60% humidity (RH) in bacteriological incubator. Triatomas were blood fed directly from a shaved rabbit every 2 weeks allowing them to feed for 20–25 m and then they were placed back in the incubator (**Figure 9**).

2.4. Study of the intestinal content in the triatomine

We use two techniques for collecting intestinal content from triatomine after blood feeding. In the first one, the triatomine is introduced in a 10 x 20 mm tube; normally the bug deposits

Figure 6. The laboratory animal is "any specie of animal that is kept under certain conditions and is used for scientific purposes" [37, 39].

Figure 7. Map of the Mexican Republic. The black stars indicate the capture zones of the triatomas, used in our investigation.

stool or urine in the bottom of the tube and then it is collected with saline solution. The second technique consists in pressing gently the triatomine abdomen, inducing that the rectal blister freely releases the stool (semi-separated blood). Intestinal content is collected in a watch glass and saline solution is added at 37°C. In both techniques, the metacyclic trypomastigote and epimastigote forms are observed fresh, using a microscope with 400 magnifications. The trypomastigotes are counted in a Neubauer chamber. If the count is above 10,000 parasites per cubic centimeter, mice are inoculated as mentioned below.

The same procedure is performed with each one of the strains of species of triatomas captured (**Figure 10**).

2.5. *Trypanosoma cruzi*, inoculation in mice

Male mice of CD-1 strain are used since estrogen in females can stimulate the activity of macrophage phagocytes and, the localized immune response [38].

Using an insulin syringe four groups of 10 mice were inoculated intraperitoneally with $3x10^3$ epimastigote and/or trypomastigote forms of *Trypanosoma cruzi*, isolated from four species of

Figure 8. Traps are placed in the collection site (A). Cage with triatomas stuck to the adhesive tape (B) Photos. Villagrán-Herrera.

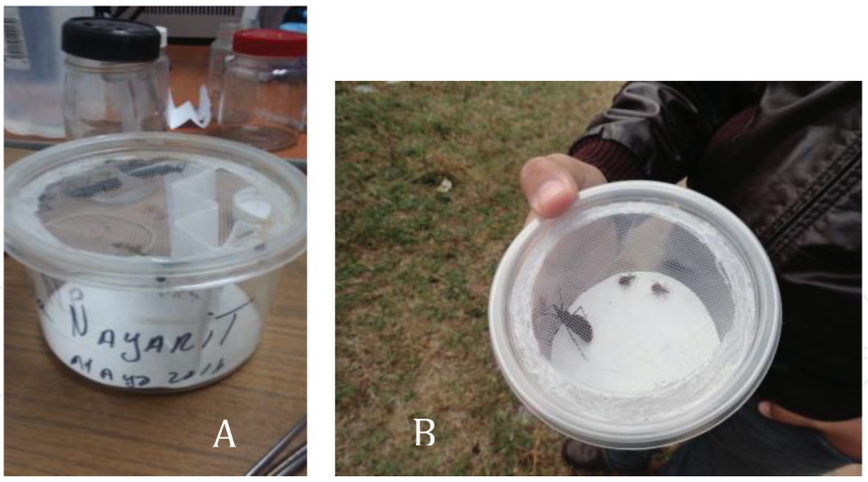

Figure 9. A and B. Transportation and storage of collected triatomas. Photos Villagrán Herrera.

triatomas (*T. mexicana, T. pallidipennis, T. longipennis* and *T. dimidiata*). Mice from the control group were inoculated with saline solution.

2.6. Study of behavior (signs)

After the first day of inoculation, the behavior of the infected murine model was observed, comparing it with an uninfected control.

2.7. Study of the parasitemia

Parasitemia levels are determined in infected mice 5,10,15,20,25 and 30 days post *T. cruzi* inoculation. Blood is obtained from the distal part of the queue 1:4000 EDTA is used as anti-coagulant, in a pipette of leukocyte count. Numbers of parasites per milliliter are calculated from sample observations in Neubauer chamber [37, 38].

2.8. Histopathological analysis

Histological sections of 10 microns are obtained from mouse dissected organs (brain, heart, intestines and skeletal muscle) and stained with H/E. Microscope slide preparations are

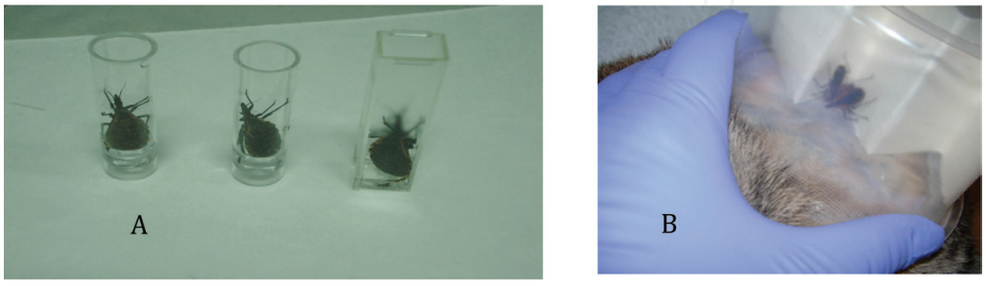

Figure 10. Tube techniques to obtain stool from the Triatoma (10A). Fourth stage Triatoma nymphs feeding on a rabbit. Photos Villagrán-Herrera.

observed at 40X [39]. Tissues from infected mice and the respective control animals are included in the analysis.

2.9. Detection of antibodies in serum (detection of anti-*Trypanosoma cruzi* antibodies in serum)

Blood is obtained by cardiac puncture and centrifuged at 5000 rpm to separate the clot from the serum and maintained at −20°C until it is used to carry out an ELISA (Accutrack Chagas Microelisa Test) in the search of anti-*Trypanosoma cruzi* antibodies.

3. Results

The mice inoculated with all the studied *T. cruzi* strains showed an altered behavior when compared to control animals. The signs presented 24 h post *T. cruzi* inoculation, the mice exhibited hyperactivity, the hair was dull and bristled, the hind legs became intertwined, and it began to drag them away, with great difficulty in moving forward.

However, it was possible to observe some differences in the virulence of each strain according to the geographic area geographic area where they came from (**Figure 11**).

The inoculant obtained from triatomines from Talpa de Allende in Jalisco state and Santa Catarina in Morelos state generated in the corresponding mice a parasitemia of 3: 4 trypanosomes per field, at 14 and 16 days postinoculation, respectively. In both cases, altered movements and physical shape of the mice began at about the same time. By day 20 and 23, respectively, the parasitemia reached the peak, so it was proceeded to sample fresh blood and dissect organs in order to perform the serological and histopathological assays

Parasitemia in mice inoculated with the *Trypanosoma cruzi* from triatomines caught in Jala, in Nayarit State reached the peak 30 days post inoculation. It was possible to detect anti-*T. cruzi* antibodies in 2 mice out of 10 by conventional ELISA test.

Blood parasitemia was undetectable in mice inoculated with *T. cruzi* strains obtained from triatomines collected in San Pablo and Tolimán in Querétaro State. The animal behavior was

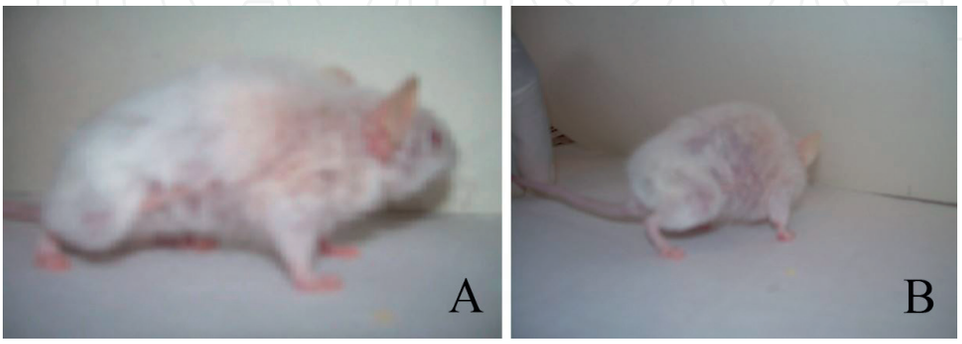

Figure 11. *T. cruzi* infected mice exhibiting hyperactivity, dull and bristled hair and intertwined hind legs. (11A and 11B). Photos Villagran Herrera.

Geographic area	Species of Triatoma	Days in which presents parasitemia	Frizz hair	Difficulty walking	ELISA test	Brain	Heart	Skeletal muscle	Intestine
Talpa de Allende. Jalisco	*T. longipennis*	14	Positive	Positive	Reactive	Negative	Positive	Positive +++	Negative
Jala Nayarit	*T. dimidiata*	30	Positive	Negative	Reactive	Negative	Negative	Positive ++	Negative
San Pablo Tolimán Qro.	*T. mexicana*	It was presented in 90 days	Negative	Negative	Reactive	Negative	Negative	Negative	Negative
Sta. Catarina Morelos	*T. pallidipennis*	16	Positive	Positive	Reactive	Negative	Positive	Positive++	Negative

Table 1. Results of the behavior, parasitemia, serology and histology in the murine model, infected with inoculum of four strains of *Trypanosoma cruzi*, isolated from triatomas captured in different geographical areas.

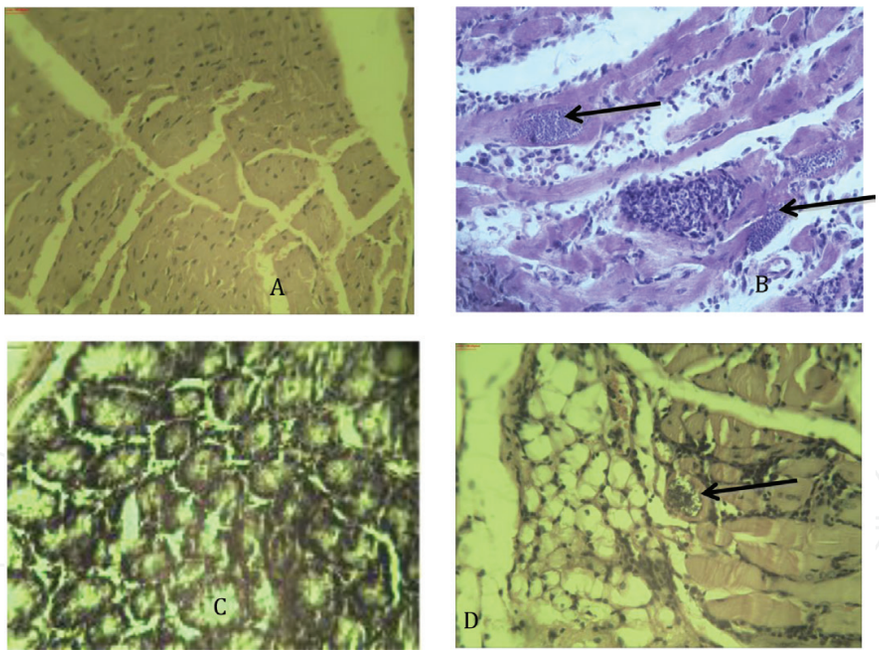

Figure 12. Histopathological analysis of cardiac (A, B) and intestine muscle (C, D) tissues. A and C, control groups. B, cardiac tissue with pseudocysts with high parasitemia and formation of new agglomerations of amastigotes, Jalisco strain. D, intestine muscle tissue with a mild *T. cruzi* parasitemia, Nayarit strain. Photos Villagran-Herrera.

completely normal when comparing to control group. Organs looked slightly bigger, mainly the intestines and heart. It was possible to detect anti-*T. cruzi* antibodies in the serum. Results are summarized in **Table 1**.

Presence of amastigote nests and histopathological damage in heart and intestine muscle showed direct relationship with parasitemia level, which indicates that trypanosomes are installed and recognize different tissues where they reproduce rapidly intracellularly, resulting in a greater number of parasites in blood after they differentiate into trypomastigotes. This sequence is only observed in the most virulent strains such as those from the States of Jalisco, Morelos and Nayarit (**Figure 12**).

4. Conclusions

In the present study, assessment of clinical manifestations, parasitemia levels, histological changes and seropositivity in murine model allowed us to know the behavior of different *T. cruzi* strains found in triatomas from different geographic areas in Mexico. This confirms the existence of genetically different strains that produce a complex called "cruzi" from the pathognomonic and morphophysiological point of view, as previously reported [40].

T. cruzi infection depends on genotype of both the parasite and the mammalian host, which in turn influences tissue tropism and the pathophysiology of infection.

We were able to identify two different *T. cruzi* strains (Tc I) from triatomines from two communities from Queretaro State that exhibited mild virulence when compared to other three strains from triatomines from three different States in México. Isozyme characterization waits to be carried out in order to explain if those observed differences might be attributable to the fact that different species of blood-sucking triatomine insects were used [40].

5. Discussion

Mitie-Nisimura et al., in 2014, were able to induce acute phase inoculating mice by intraperitoneal injection trypomastigote forms of *T. cruzi*. Authors observed the microvascular alterations and oxidative stress in the brain and the formation of pseudocysts full of amastigotes in the heart muscle [41]. Espinoza et al., in 2010 inoculated Balb/c mice with two strains of *T. cruzi* I (Tc I) isolated from patients in Mexico in order to study the immune response [35]. The first case of clinical infection with *T. cruzi* was reported in a horse in South Texas in 2015, observing forms of amastigotes in the spinal cord and cardiac tissue.

Espinoza et al., in 2010, observed contrasting differences between two *T. cruzi* strains isolated from *Triatoma barberi* and *Triatoma mexicana*. In the first case, virulence was clearly observed while in the second one, productive infection and morphological alterations were not observed, and only the anti-*T. cruzi* antibodies were detected.

Author details

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