



## Prevention and Control of Chagas Disease – An Overview

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### Authors' contributions

All authors had substantial contribution to the literature review, conception of the work, revised the work critically for important intellectual content, approved the final version and agree to be accountable for all aspects.

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### ABSTRACT

Chagas disease is the main cause of heart failure and sudden death in the Western Hemisphere. The literature of the last decades reported on the changing epidemiological profiles of Chagas disease, which now threatens the human population in the cities. The exodus of the Latin America people to the Northern Hemisphere explains the growing concern in countries where the transmission of *Trypanosoma cruzi* was accidental or transferred from a mother to her offspring. Herein, we present the evidence of the possible acquisition of the *T. cruzi* infection by sex. The staggering demonstration of the transmission of the *T. cruzi* infections from males and females to naïve mates by intercourse introduces substantial changes in the surveillance of the Chagas disease. Notably, the sexual transmission of the *T. cruzi* introduces changes in the concepts of medical care, prevention and control; specifically, the risk for the vertical transfer of the parasite-induced kDNA mutations, underpinning the genetically driven autoimmunity, inheritance, and pathogenesis associated with multifaceted clinical manifestations of Chagas disease with high

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ratios of morbidity and mortality. In this regard, the endemics require much paradigm research with new approaches and innovation technologies, aiming at its control. For example, the recent knowledge anticipates useful measures for preventing the potential forthcoming pandemic Chagas parasites. A long-lasting multicenter research program is needed for creative, drug discovery for curtailment of Chagas disease. Meanwhile, the prevention shall rely on the education, information, and communication program for health.

**Keywords:** *Trypanosoma cruzi*; sexual transmission; pathogenesis; drug development; prevention; education.

## ACHRONYMS

*ATPase* : adenyltriphosphatase.

*Benznidazole and Nifurtimox* : nitroderivatives used for the treatment of Chagas disease.

*CCD* : chronic Chagas disease.

*CSB* : kinetoplast minicircle constant sequence block.

*CYP5128–30* : fenarimol herbicide azole inhibitor of *T. cruzi*.

*EICH* : education, information, communication for health.

*eIF2a* : elongation factor 2a.

*ELISA* : enzyme-linked immunosorbent assay.

*IFI* : immunofluorescence indirect.

*ik2* : eukaryote kinase subunit 2.

*EMBL* : European molecular biology laboratory.

*ERV* : endogenous retro virus.

*LINE-1* : long interspersed nuclear element-1.

*MAL-R* : long terminal repeats.

*NAT* : nucleic acid test.

*nDNA* : *T. cruzi* nuclear DNA.

*kDNA* : *T. cruzi* mitochondrion kinetoplast DNA;

*PI3K* : phosphate inositol 3 kinase.

*PCR* : polymerase chain reaction.

<sup>32</sup>P-dATP : P<sup>-</sup>(32)P-labeled; 2'-deoxyadenosine triphosphate.

## 1. INTRODUCTION

The Kinetoplastid flagellates in the Family Trypanosomatidae (from greek; *trypanon*, auger; *soma*, body) include *Trypanosoma cruzi*, the agent of the American trypanosomiasis [1,2]. The *T. cruzi* belongs to the Stercorarian group of flagellates that accomplishes its life cycle in the gut of the invertebrate host; the insect sucks in the blood trypomastigote, which transforms into epimastigote in the foregut and reverts to infective metacyclic trypomastigote in the hind gut. The requirement of an invertebrate host for completion of the protozoa lifecycle ascribes to the beginning of the *T. cruzi* enzootic infection of mammals that dwell in the American Continent [3-5]. The enzootics include reservoirs of the orders Marsupialia, Edentata, Chiroptera, Carnivora, Artiodactyla, Rodentia and Primates, upon whom triatomine bugs prey and transmit *T. cruzi* [5-7]. The spreading of the *T. cruzi* infections requires a broad diversity of triatomine vectors and mammalian hosts [5-10].

In past decades, the epidemiologic profiles of American trypanosomiasis and Chagas disease underwent drastic changes due to the rural exodus and, therefore, now they become an emerging urban problem [6-18]. The changes of the profiles follow pattern of the migration of *T. cruzi*-infected people from the South America continent and their settlement in the Northern Hemisphere [18].

Herein, we review the literature that describes the recent change of epidemiologic profiles of the Chagas disease. The review integrates the available data and information in men and in laboratory animals with growing concern about new technologies for preventing Chagas disease that threatens the people worldwide [7-19]. With this respect, this review article presents up-to-date parasitological, clinic, cell biology, immunologic, genetic, pathology and the scanty epidemiologic information for the attention of the scientific community and for the health

authorities. The aim of this review is to spurring a long-lasting program, aiming at a new drug to treat the carrier and to eradicate the endemic *T. cruzi* infections and control of the sexual transmission of the *T. cruzi* [18-20]. This review anticipates that the curtailment of Chagas disease needs international solidarity and multicenter research groups to undertake efficacious, creative, prevention paradigm achievements. In this regard, a mass-media Education, Information, and Communication for Health (EICH) program to prevent Chagas disease in the forthcoming decades can no longer be neglected.

## 2. TRANSMISSION ROUTES

The main public health problem initiated, possibly, with the accidental triatomine bug-transmission of the *T. cruzi* infections to human [21-23]. However, the route for the bug hindgut's trypomastigote entry into the host's body and parasitic cell growth, is cumbersome: *i*) in the absence of systematic information, it is assumed that most acute *T. cruzi* infections stem from triatomine bug's bite, supposedly, in hinterland endemic areas; *ii*) incomplete knowledge about the bug's feces *T. cruzi* contamination ratios in countless ecotypes of the America continent [24-30]; *iii*) triatomine bug's night habits, frequency of accomplishing a full meal and ratios of *T. cruzi* contamination, is mostly unknown [27-29]; *iv*) quick feeding ability to obtaining a full blood meal, distension of the abdomen and bug immediate defecation, remains to be determined [27-29]; *v*) triatomine bite site scratch-induced allergic reactions and vase dilation promotes bug's proboscis cannulating and suck-in a full blood meal, defecation and spread of the excreta, and the *T. cruzi* contaminates the human body [27-29]; nonetheless, in the absence of an allergic reaction and vase dilation the prey does not aware about the annoyance that incites the scratching, which renders much difficult a full completion of the time consuming blood meal, defecation and possible contamination of the host's body [24-31]; *vi*) the frequency of the hypersensitivity lesion at the port of entry of the parasite in the skin (Chagoma) or in the eye conjunctiva (Romaña's sign) is, possibly, less than circa 1 out of 1000 acute infection [5,6].

The spectrum of drawbacks translates an array of difficulties towards collecting family information about bug's transmitted *T. cruzi* infections to the human population. However, the early

recognition of the vector-transmitted *T. cruzi* infections posited first in the rank among routes of the *T. cruzi* contamination of humans [18,23]. In this regard, further investigation is needed to determine whether the ratios of *T. cruzi*-positive triatomine infestations correlate to the prevalence of Chagas disease in the cities.

The acquisition of the *T. cruzi* infections *per os* is the most ancient route for the *T. cruzi* contaminating the insectivorous mammals' reservoirs in the wilderness [31-33]. A few epidemiologic studies refer to the outbreaks of acute Chagas disease in humans, possibly, by imprudent ingestion of food contaminated with the triatomine bug's feces [2,32,33]. Next, the acquisition of the *T. cruzi* infections by blood transfusion is reported; lately this route of the infection acquisition is considered, carefully, as well in several countries of the Northern Hemisphere [34-36]. Occasionally, the *T. cruzi* infections are acquired by accident in the laboratory and by organ transplantation [36].

The transmission of the *T. cruzi* from the Chagas mother to her offspring is an important route of the infections in humans [36-38]. The congenital transmission of Chagas disease refers to the in uterus infection acquisition by the offspring. However, pregnancy depends upon partnership and male contribution to the *in utero* *T. cruzi* infections awaits investigation. In the course of the pregnancy, the chagasic woman may undergo early embryo resorption, miscarriage, death birth, or neonatal death; live births can either be *T. cruzi*-infected or infection-free, healthy baby. Additionally, the infection-free, healthy progeny can inherit the *T. cruzi* mitochondrion minicircle kDNA sequences into the genome [7].

## 3. CHAGAS DISEASE

The routes of acquisition of the *T. cruzi* infections impose either the silent American trypanosomiasis or the clinically manifested Chagas disease. Fair accounts of the prevalence of those patients are missing and, therefore, the epidemiologic reports on morbidity and mortality due to the *T. cruzi* infections are rather inconclusive [6]. In the absence of disease manifestation, chronically infected humans are intermediate-phase carriers or reservoirs of the *T. cruzi* infections [7,37,38]. A majority of chronically infected individuals remains in the intermediate phase and their life expectancy is similar for non-infected individuals [7].

The field studies show that some chronically infected individuals develop chronic Chagas disease (CCD) usually over ~30 years after the acquisition of the *T. cruzi*: 94.5% are cases affected by heart trouble, among which 38.5% die suddenly, and 56% succumb to heart failure [6,7]. Sudden deaths are associated with arrhythmias and heart rate turbulence. In addition, megaesophagus and megacolon are revealed in 5.5% of chronically infected people. A gamut of neurologic syndromes and clinical dysfunctions are manifestations of Chagas disease, yet, scarcely studied [7-10]. Moreover, CCD clinic and pathologic features, which are indistinguishable from idiopathic inflammatory dilated cardiomyopathy, have the differential diagnosis set by the NAT-nDNA test [6,7].

## 4. DIAGNOSIS

### 4.1 *Trypanosoma cruzi* in the Blood

The direct microscopic demonstration of *T. cruzi* in the blood is recorded in the early phase of the infection. Also, the parasite is detected by concentration methods, such as blood culture and xenodiagnoses (having a clean bug to feed upon the patient's blood). For the epidemiologic surveys, however, these diagnostics approaches are out of reach by a majority of the *T. cruzi* intermediate phase chronically infected population, in a lack of late clinical CCD manifestations.

### 4.2 Immune Diagnosis

In the absence of a direct demonstration of the *T. cruzi* in patient's blood the diagnosis relies on searches for the specific antibody. The acutely infected people are recognized by detecting IgM antibody class or IgG serum conversion usually after the second week of infection. Currently, highly sensitive, but relatively less specific indirect hemagglutination, enzyme-linked immunosorbent assay (ELISA), and indirect immunofluorescence (IFI) tests are routinely used [41-42]. However, none of these assays affords complete sensitivity and specificity, because the host's immune system sees a plethora of parasitic antigens and clonal selection of immune responses [6,7,41,42]. A word of caution is required because a grey zone of inconclusive assays that yield cross-reactive antibodies in the serum of patients with *Leishmania* sp., *Mycobacterium leprae* and *M. tuberculosis*, *Treponema pallidum*, or autoimmune diseases [7,42]. Largely, the

employment of recombinant or synthetic peptide antigens diminishes the sensitivity of the assays, and these concerns bear practical consequences [38,39]. With this respect, caution is required in order to avoid an accidental *T. cruzi*-contaminated blood transfusion. The health systems should no longer rely solely on testing the humoral immunity specific *T. cruzi* antibody [7,41-47]. Nonetheless, the electrocardiograph recordings in series of patients from endemic regions show cases with the positive serum anti-*T. cruzi* IgG, increased heart size in the chest X-rays, and arrhythmias with features of chronic Chagas disease [48-50].

### 4.3 Nucleic Acids Test (NAT)

The accuracy of the diagnoses of the *T. cruzi* infections to the point-of-care for the assessment and curtailment of the epidemics, and as well for the health care delivery to thousands of people aggravated by the yet incurable Chagas disease, is fundamental [43-47,51,52]. Therefore, the diagnosis of the sexually transmitted infection requires the flagellate protozoa footprint [38-40, 43-47], because the specific IgG antibody is absent in the immune tolerant progeny of the *T. cruzi*-infected parents [38,51-54]. The immune tolerance showed in the chicken model system refractory to the *T. cruzi* infection after the 10<sup>th</sup> day of incubation [38,47,51-54]. The chicks hatched from *T. cruzi*-inoculated eggs, which grow to adult life, do not raise specific antibody (Y immunoglobulin) after challenge with the formalin killed parasite [53,54].

The NATs detect *T. cruzi* nuclear DNA (nDNA) and its kinetoplast DNA (kDNA) in somatic cells, and as well in germ cell line of the reproduction systems immune privilege organs [38,43-47,51-54]. Moreover, the NAT-nDNA test is an indispensable condition for the diagnosis of *T. cruzi* infection in immune-tolerant patients, in the absence of the specific antibody. The development of throughput digital PCR platforms [47] needed to testing blood donor candidates, so as deferral of those NAT-nDNA positive blood donors, and to surveying accurate prevalence and correct information on the epidemiology of American trypanosomiasis and Chagas disease.

In so far, the NAT polymerase chain reaction (PCR) is the basis for research laboratories diagnosis of the *T. cruzi* infection and detection of congenital Chagas disease at birth [38,43-46]. Its employment ensures tidy laboratory practices to achieve a specific diagnosis of the highly

repetitive sequences in the flagellate protozoa, showing homology with human chromosomes; and throughput platform required for large-scale epidemiologic surveys. A gamut of nDNA primer sets is available for in-house PCR diagnosis of the *T. cruzi* infections with variable sensitivity and specificity [38, 44,47]. The Tcz1/2 primers set that targets a 188-nt telomere DNA repeat in the *T. cruzi* genome [48] are used in the family-based studies [38-51]. The amplicons resolved in agarose gels and transfer to positively charged nylon membranes hybridize to the 188-nt  $\alpha^{32}$ p-dATP labeled probe, subsequently subjected to X-Rays film exposure during variable periods. The positive amplicons are subjected to cloning and sequencing. The bioinformatics analyses reveal the 188-nt nDNA footprint in the blood and in the semen samples. This in-house NAT is the point-of-care diagnosis of an active *T. cruzi* infection [38, 43-46]. Therefore, gold standard NATs are required to guarantee integrity, purity, and adequacy of blood bank supply donor deferral and pathogen inactivation of blood components. These efforts should be supported by the health care networks for prevention of an emerging *T. cruzi* threat; laboratory procedures quality control assessment by skilled personnel are needed to monitor all steps prior to the blood transfusion and for delivery of the health care. Additionally, the importance of the NAT tests for diagnosis of the *T. cruzi* infection and CCD cannot be underemphasized because the cross-section epidemiological surveys based on the IFI serological assessments of the specific antibody determine neither the accurate prevalence of the *T. cruzi* infections nor the ratios of the parasite sexual transmission in the human population [38, 41-46].

## 5. TRANSFER OF KINETOPLAST DNA (kDNA) FROM AMERICAN TRYPANOSOMES TO HUMANS

The studies showed transfer of the mitochondrial DNA (kDNA) minicircles sequences from *T. cruzi* to the rabbit's chromosome [56], and it was further showed that the Chagas rabbit progeny retained the protozoan minicircle sequence mutation into the genome [7, 52-56]. The chicken family studies spurred the human families' investigations on the transfer of the kDNA sequences from Chagas disease parents to their progeny [54-56]. The founders of study families showed kDNA minicircle sequences integrated into the Long Interspersed Nuclear Element-1 (LINE-1, equivalent to CR1 in the chicken) of the human genome. In addition, the

kDNA minicircle sequences integrated at various locations, such as structural, cell growth and differentiation, immune responses and at important biochemical pathways checkpoint genes [52-56].

The development of Chagas heart trouble in the chronically infected human population is an ominous sign because the cases succumb to the autoimmune inflammatory cardiomyopathy either suddenly or in the range of six months to two years after the beginning of clinical symptoms and failure, and, therefore, it is unusual to find those cases in a cross sectional field study. Nevertheless, the family study showed the accumulation of a minimum of 4 to 8 kDNA mutations into the retrotransposable LINE-1 located at various chromosomes of CCD cases with clinically manifested heart insufficiency [7, 38, 52-56].

The chagasic parental (F0) vertically transferred the *T. cruzi* kDNA minicircle sequences to the F1 and F2 progeny, which integrated into the genome [38]. Additionally, the families study population showed that somatic cells had the *T. cruzi* NAT-nDNA positive tests in family members and that sperm donors had the active *T. cruzi* infection. These findings suggest that intercourse is a sustainable route for transmission of the *T. cruzi* infection [38]. The clinic and epidemiologic importance of these findings address to the flagellate life-long infection, the overtime accumulation of the mitochondrion kDNA minicircle mutations and Chagas disease multifaceted manifestations, in which parasite-induced mutations underpinning the genetically driven autoimmune pathogenesis, remarkably, features of host-parasite relationships and sprouting human disease.

## 6. SEXUAL TRANSMISSION OF THE AMERICAN TRYPANOSOMES

In the last decades, the growing number of Chagas disease in the Northern Hemisphere inhabitants ever exposed to the insect vectors suggests the possibility of acquisition of the *T. cruzi* infections by the intercourse [7, 38]. In this regard, the *T. cruzi* infections encrypted in immune privilege body niches, in the absence of inflammatory infiltrates, make the grounds for the parasitic growth [57-63] in the reproductive organs (Fig. 1) and, thus expelling the protozoan in the semen (Fig. 2) and in the uterine secretions [38, 57].

## 6.1 Family Study

The timely demonstration of the sexually transmitted *T. cruzi* in families' document the epidemic bursts of the infections in the population that received health care at the Center for Chagas Disease Surveillance. Among individuals in the families, there were cases of the acute *T. cruzi* infections showed by direct demonstration of the blood trypomastigotes. However, the epidemiologic history of the study population revealed the absence of the triatomine-bite transmission of the infections and, also, the other routes of the infection-acquisitions were denied.

In the family study, each acute case treated with the anti-trypansome nitro derivative benznidazole (5 mg/kg/day) for 60 days, and all family members received health assistance for five years. The individuals in the families yielded blood samples at 1, 2, and 3 years set points for serum and DNA collection. Interestingly, the NAT-nDNA test was consistently positive in 76% independent assessments of blood samples collected at three different set points: among these, 28.4% tested IgG positive. This broad discrepancy explained by the immune tolerance, in the absence of the antibody in the majority of NAT-kDNA positive individuals, since the sexually transmitted *T. cruzi* could reach the early embryo before the development of the immune system [38,52-57]. Additionally, the NAT-kDNA test was positive in 92.6% family members (EMBL HG008116 to HG008708). The difference between the results obtained by the NAT-nDNA and the NAT-kDNA approaches addresses to the mutations resulting from the integration of the minicircle DNA sequences into the human genome in the absence of live *T. cruzi* infections. (Fig. 3).

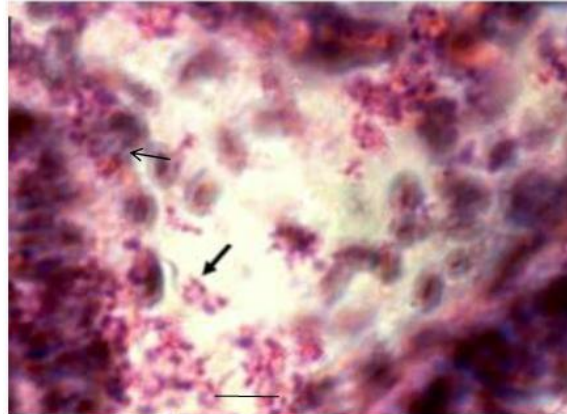
In the absence of the *T. cruzi* antibody, the NAT analyses of the germ cells collected from subjects in the family study confirmed the experimental data [38,52-56] showing that the *T. cruzi* infections acquired during early embryo growth do not bear the specific antibody, whereas those acquired during fetal development do not undergo immune-tolerance and, thus reveal the specific antibody. These analyses showed the *T. cruzi* nDNA- and kDNA-specific bands in the somatic cells of the blood and in the haploid semen samples examined [38, 57-63] (Fig. 4). The data that suggest the sexual transmission of the *T. cruzi* infections confirmed by other experiment, in which the injection of 100

µl aliquot of NAT-nDNA positive Chagas patient's semen into the peritoneal cavity or its infusion into the vagina of mice documented the infectivity of the *T. cruzi* present in the human ejaculate: one month thereafter, the growing *T. cruzi* amastigote nests appeared in the heart and skeletal muscles, and in the lumen of the *vas deferens* and uterine tube (Fig. 4).

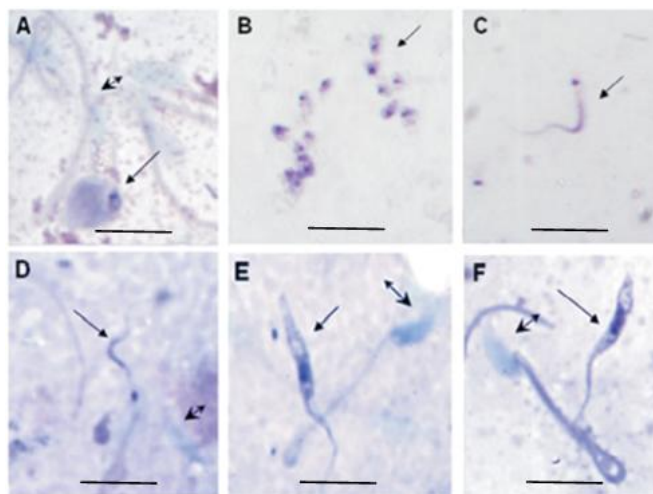
The early epidemiology based serological surveys conducted in the spare individual estimate that the prevalence of the *T. cruzi* infections averages  $7 \pm 3$  percent of the Latin America population [6,49]. The family study NAT assessments revealed much high ratios of the *T. cruzi* infections in male and female family's founders and progeny mates [38,52-56]. However, the so-called congenital transmission of the infection means that the woman only bears for the vertical *T. cruzi* transmission to her baby. Certainly, such prejudice definition is in the lack of scientific understanding, because it omitted the inevitable roles played by both mates during the bi-partisan sexual reproduction [38]. With this respect, further family-based studies at various ecosystems are required to determine the role played by the mate's sexual intercourse in the transmission of the infectious agent retained in the immune privilege reproductive organs. The immune privilege is the absence of destructive inflammatory reactions to microbes' antigen loads in important body structures in the brain, eyes, and reproductive organs, accomplishing sensitive functions, reproduction, and the species survival [64, 65]. The immune privilege sets free the *T. cruzi* growth in the organs of the reproduction, whereas the immune tolerance [64] prevents the immune system rejection of the parasite antigen, because it is recognized as a component of its own body since early embryo life.

## 6.2 Mouse Model System

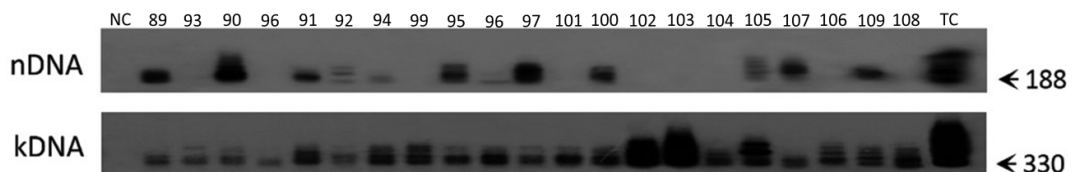
The pathology searches documented a gamut of the *T. cruzi* amastigote nests in the mouse uterine endometrium, ovary theca cells, and testicles' Sertoli cells and in the lumen of the seminiferous tube, and in the epididymis, seminal vesicles, prostate, and ureter [57-63]. Actually, the inoculation of the *T. cruzi* from the hindgut of a triatomine bug incited the replicative flagellates' growth [57] in the recipient mouse ejaculates (Fig. 2). Furthermore, the *T. cruzi* infections were documented upon Chagas patient's semen inoculation or infusion in naïve mice [38].



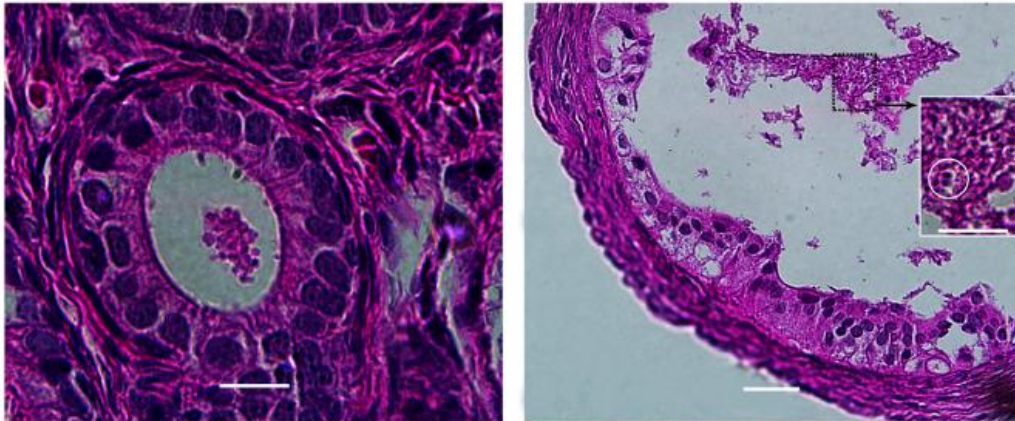
**Fig. 1.** The *Trypanosoma cruzi* infection in the seminiferous tube of a boy with the acute Chagas disease. Notice the round amastigote forms in the gonial blasts and clumps of amastigotes and trypomastigotes in the lumen of the tube (arrows). Bar, 10  $\mu$ m. Microphotograph from Doctor Antonio Teixeira's file, 1970



**Fig. 2.** The *Trypanosoma cruzi* parasitic forms in the semen ejaculate of acutely infected mice. The arrows indicate amastigote (A) within the head of a spermatozoan; free amastigotes (B) and trypomastigotes (C and D); and the epimastigote forms (E and F) in the semen ejaculates (arrows). Giemsa stained smears. Bars, 10  $\mu$ m. Reprinted with permission from the Author and the Publisher [57]



**Fig. 3.** The diagnosis of the *Trypanosoma cruzi* infections made by PCR and Southern hybridizations. Notice the nuclear DNA (nDNA) bands in 11 Chagas patients with the live infections, whereas 21 family members showed the mitochondrial DNA (kDNA) bands. Reprinted with the permission from the Authors and the Publisher [38]



**Fig. 4. The infectivity of the *Trypanosoma cruzi* from the human semen ejaculates upon aliquot instills into the peritoneal or into the vagina of naïve mice. Left) the *T. cruzi* amastigotes clump in the lumen of the vas deferens. Right) the *T. cruzi* amastigotes swarm epithelial cells of the uterine tube (insert, dividing amastigotes). Bars, 10 µm. Reprinted with the permission from the Authors and the Publisher [38]**

Moreover, family studies about the *T. cruzi*-infected male and female mice showed the transmission of the protozoa flagellates to naïve mates upon intercourse. In those experiments, each breeding pair placed in one cage inside a safe box avoided escaping. In experimental group-A, 10 *T. cruzi*-infected males mated with 10 naïve, control females. In experimental group-B, 10 *T. cruzi*-infected females mated with 10 naïve, control males [38]. After breeding, groups A and B mates' yielded NAT-nDNA bands that is, the naïve mates readily acquired the *T. cruzi* after the sexual encounter. The triplicate groups of independent experiments also showed that naïve female or male mouse that sexually mated with a *T. cruzi*-infected male or female acquired the infection. Those nDNA-positive founders (F0) generated progeny F1 and F2 that they raised until six weeks of age. The founders' (F0) transmitted sexually the infections to F1 progeny as shown by the nDNA-positive bands. The breeding of these nDNA-positive mates generated F2 progeny, which showed the nDNA bands that indicate the vertically acquired infections. In these experiments, 58.6% of the progeny had the NAT-positive nDNA assays, and 22% of the progeny had anti-*T. cruzi* antibodies. The results confirmed the absence of the specific antibody in a majority of F1 and F2 mice (78%) that bore vertical *T. cruzi* infections by intercourse. After that, the tissue sections taken for microscopic examination showed the *T. cruzi* in the reproductive system of those mice [38]. In summary, those experiments suggest that few offspring (22%) of chagasic mates had the *T.*

*cruzi*-specific antibody, probably, because the infectious agent reached the fetus with a mature immune system.

## 7. DRUG DEVELOPMENT

The strategic for the treatment of Chagas disease should take into consideration that single infective *T. cruzi* initiates a lifelong infection [7]. Regardless of the route of the infection, after several cycles of division, the *T. cruzi* ends up in the reproduction organs immune privilege sites where it grows [38,64]. The amazing capability of the *T. cruzi* to thrive in the immune privilege mammalian reproductive system, so as to reach out the route to contaminate the host during intercourse; the species survival prevail transmission by sex and warrants the *T. cruzi* anthrozoosis, and, therefore, this new concept shall guide the preventive medicine and public health measures for the control of American trypanosomiasis. This observation makes the absolute need of the parasite eradication in order to accomplish cure of Chagas disease and prevention of the sexually transmitted infections.

The lead drugs used for the treatment of the *T. cruzi* infections are nitro- derivative compounds nifurtimox [4-(5-nitro-phurylideneamino)-tetrahydro-4-4-1, 4-thiazine-1-1-dioxide], and benznidazole [N-benzyl-2-nitro-imidazoleacetamide] [39-40, 49, 50, 81-84]. Both drugs achieve elimination of the *T. cruzi* infections *in vitro* but they fail to eradicate the live



infections in experimental animals and in man [38,40,66,67], possibly, because after-treatment non-phagocyte growing nest sustains the persisting parasite infection. The nitro-derivatives toxicity includes mutagenic, teratogenic, carcinogenic and sterilizing activities [68-76], which relies on the pteridine reductase activity [85-90] upon the lead and release of high-energy electron excited intermediates:  $\text{NO}^2$ ,  $\text{HO}$ ,  $\text{O}^2$ ,  $\text{H}^2\text{O}^2$  that bind to macromolecules and DNA. The drug toxicity targets the parasite and the mammalian host cell [7-79].

## 8. NEW DRUGS FOR PREVENTING CHAGAS DISEASE

The Chagas disease reported in five continents [25-37] has brought much attention from private and public organizations. In this new look, searches for safe, efficacious, low-cost drugs to curtailment of the epidemics needed. Consistently, the *T. cruzi* biochemical pathways checkpoints described herein suggest possible targets for drug development. The clinical trials with the 14 $\alpha$ -demethylase (CYP51) that catalyzes the removal of the 14  $\alpha$ -methyl group from the scaffold revealed that posaconazole and ravuconazole inhibitors of sterol biosynthesis undergo treatment failure above that for benznidazole-treated patients [38,43-50]. The anti-trypanosome activity of lapachone-based 1,2,3-triazole naphthoquinones derivatives presented IC<sub>50</sub>/24 h 80.8 values at 6.8 and 8.2  $\mu\text{M}$  activity which is above that of the anti-*T. cruzi* drug benznidazole. The nitro heterocycles fexinidazole prompted the discovery of the oxazole AN4169 (SCYX-6759) with anti-*T. cruzi* activity in a mouse model. Other leads derivatives of the herbicide fenarimol, which is an azole inhibitor of *T. cruzi* CYP5128–30. Meanwhile, the nitro derivatives benznidazole and nifurtimox registered for treatment of Chagas disease in Latin America [80-82]. In the countries of the Northern hemisphere the prescription of these drugs requires license from the health authorities.

Actually, the sustainable search for compounds from medicinal plants and drug development to kill the *T. cruzi* is needed, because most antibiotics derived from natural metabolites used for defense, reproduction, and survival [82-83]. The use of throughput biotechnologies for the systematic procurement and identification of active molecules in plants from various ecosystems, showing enormous biodiversity shall enhance the chance for obtaining a top lead drug

without toxicity to eradicate the *T. cruzi* for prevention and treatment of Chagas disease. This is a milestone research endeavor.

## 9. PREVENTING AMERICAN TRYPANOSOMIASIS AND CHAGAS DISEASE

Over three decades the World Health Authorities were at odds with the devastating acquired immunodeficiency syndrome (AIDS). The epidemics caused the death of unaccountable people all over the world, in the lack of the specific diagnosis, except for the variety of opportunistic infections in the debilitating immune tolerant organism. Otherwise, International Health Authority Organizations consider Chagas disease minor epidemics that affect the poor people of Latin America, and the local Health Institutions claim that the disease is under control.

Meanwhile, the Chagas disease high morbidity and mortality ratios are growing burden upon the health systems worldwide and, therefore, it should not be neglected anyway (21-37). On the one hand, the Chagas disease unperceived epidemics [84-85] require the attention of the health systems because the potential of spreading the re-emerging transmission routes, including the sexually transmitted *T. cruzi* infections can no longer be underemphasized. On the other, the trophic network that enmeshes the *T. cruzi* in countless ecotypes in different ecosystems [4, 8], including the underground [86], embracing many species of insect vectors, most of which are not used to domiciliation are not susceptible to insecticides, and, therefore, the anthrozoosis cannot be eliminated by the vector control strategy. To approach the magnificent problem the health personnel and scientists agree that time-consuming, unprecedentedly sustainable investigation programs are necessary, in order to create the knowledge new order to combat this threatening epidemic, urgently.

### 9.1 Education Information and Communication for Health (EICH)

The EICH program, aiming at the control of the transmission of the *T. cruzi* infections, is needed. The population is entitled to know about all routes of transmission of the *T. cruzi* infections: a) sexually transmitted; b) accidental contamination of health care workers and of research personnel, and through blood

transfusion not deferred by the antibody assays; c) imprudent ingestion of contaminated food; d) insect-vector transmitted *T. cruzi* infections, which are limited to the American Continent. The a) and b) modes of transmission of the infections are prone to occur in the five continents. The basic EICH program to prevent the transmission of the silent *T. cruzi* infections shall take some experience of from AIDS mass-media guidelines, and the NAT nDNA exam performed early for the detection of the two weeks period in which the acute infections run in the absence of the specific antibodies [38,87,88].

The EICH program should aim at the control of the epidemics through the involvement of the people in the community with the knowledge about the trophic networks that associate the insect vectors reservoirs with over one thousands mammal hosts in the indomitable nature [8]. The information about the several dozens of the insect species (triatomine bugs) transmitters of the *T. cruzi*, thriving in countless ecotypes, hiding away under tree's bark, in the mammals and in the birds' nests, as well under the rocks, and in burrows in the undergrounds, should be promptly delivered to the population [8, 11,17,18]. They should know that a few triatomine bug species are susceptible to domiciliation, and those are main targets for elimination, to assure the family's protection at the household.

The EICH guidelines shall sustain the knowledge, saying that the education tempers the best health agent in the community. Additionally, to inform the people in the community about the genotoxicity of the nitro-derivative pyrethroid pesticide sprayed in the house regardless of the inhabiting infants and adolescents [68,69]. In the case, the health authorities believe it is necessary to spray the pyrethroids an informed consent form with the Family Leader signature is a prerequisite for the insecticide spraying, in the household free of infants and adolescents.

A formidable effort is required for developing the EICH program aiming at the grass roots of the populations under potential exposures to the *T. cruzi* infections. Furthermore, the effective prevention measures shall sustain the concept that the EICH efficacy is a lot more protective of the family in the household than the spray of pesticides that do not reach most blood fasting triatomines dispersed in the hideaway environment. In addition, the citizen under the

benefits of the EICH program shall fight to eliminate the nasty triatomine bug inside his house.

The citizens shall be informed about the constant increasing ratios of the *T. cruzi* infections in Latin America and worldwide. The continuous exodus of the people from Latin America to the Northern Hemisphere partly explains the many cases of the Chagas disease in the regions where the insect-vector does not exist. Meanwhile, the usefulness of the EICH program at school and at social organizations, with emphasis on the sociology, biological and health sciences, and mass-media communication, shall prevail worldwide in order to prevent the sexual transmission of the infections [38,85]. Moreover, unprecedented drug-discovery program needs for the parasite eradication and prevention of the sexually transmitted *T. cruzi* infections in the human population.

## 10. DISCUSSION

The trypanosome survival in invertebrate and in vertebrate animals include fine strategic checkpoints such as hiding away underground and association with countless ecotypes out of reach of skilled health personnel and insecticides. The plethora of countless factors, playing important roles in the multi-factorial chain of events related to transmission of the *T. cruzi* infections requires further studies in the laboratories and in the field. Meanwhile, the complexity of the *T. cruzi* life-cycle does not sustain the belief that currently used strategies are good enough for preventing the *T. cruzi* infections so as to safeguard the human population [8]. Such epistemological definition is essential because not yet defined cumbersome factors have been brought into play by an enormously complex chain of events and the *T. cruzi* encroaches in major ecosystems. The discovery of novel preventive and control strategies should be further investigated by scholars, before propagating the control of the *T. cruzi* infection and Chagas disease is achieved.

Actually, the sexually transmitted *T. cruzi* infection undergoes life-long encryption in the body, and, in the absence of clinic manifestations, the patients do not seek preventive medical care. In the absence of a drug to eradicate the *T. cruzi* infection, the EICH program is the corner stone for preventing the sexual transmission of the American trypanosomiasis and Chagas disease.

## 11. CONCLUDING REMARKS

The EICH actions in the American Continent, concerning the prevention of the triatomines from close contact with the human population should be conducted directly in communities, elementary schools, churches and social clubs, and reinforced by social marketing and mass media communications. The population should be promptly informed about the modes of transmission of the *T. cruzi* infections that pose daily threats to human health. A realistic and affordable control of the sexually transmitted *T. cruzi* infections and the curtailment of Chagas disease rely on a robust EICH program.

The EICH preventive actions to halt the American trypanosomiasis and Chagas disease spread worldwide should be on the time table because the sexual transmissions of the *T. cruzi* infection run simultaneously with the transfer of the parasitic kDNA to the human genome. This frailty introduces a significant change in the concept of public health, and preventive medicine [7,38,67]. The silent parasite-induced, genetically driven autoimmunity [38,64-66,67] associates ubiquitous clinical manifestations with high ratios of morbidity and mortality several decades after the infection acquisition, and, therefore, a conjunction of measures is necessary to control the Chagas disease and its potential to become pandemic:

1. Chagas disease is a global problem that requires international solidarity, consortia, and exchanges of expertise in order to provide the means for protection of the population under risks of acquisition of the *T. cruzi* infections.
2. Education, Information, and Communication mass-media program (EICH) undertaken, soon is the best, in order to obtain full compliance from the people's community, similar to that employed to control HIV-AIDS epidemics.
3. Safe new drugs needed to eradicate the cryptic *T. cruzi* infections: *i)* to treat all the *T. cruzi*-infected people, so as to prevent the sexual and the accidental modes of transmission of the infections. *ii)* To arrest the cardiomyopathy: pre-empt the severe Chagas heart disease case, before the killing of sick bone marrow progenitors of the effector immune lymphocytes before healthy bone marrow transplantation [64,65].
4. Development of throughput digital PCR platforms for assessing the population under

risk of acquisition of the *T. cruzi* infections, so as deferral of NAT-nDNA positive blood donors.

5. The health personnel should share responsibility with the community leaders about the target-directed limited spraying of the pyrethroid insecticide in the triatomine bugs infested households.

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## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

The authors have declared no conflict of interest exists.

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