Review Article/Artigo de Revisão

Chagas disease. What is known and what should be improved: a systemic review

Doença de Chagas. O que é conhecido e o que deve ser melhorado: uma visão sistêmica

José Rodrigues Coura[1] and José Borges-Pereira[1]

ABSTRACT

This study consists of a broad review on what is known and what should be improved regarding knowledge of Chagas disease, not only through analysis on the main studies published on the topics discussed, but to a large extent based on experience of this subject, acquired over the past 50 years (1961-2011). Among the subjects covered, we highlight the pathogenesis and evolution of infection by *Trypanosoma cruzi*, drugs in use and new strategies for treating Chagas disease; the serological tests for the diagnosis and the controls of cure the infection; the regional variations in prevalence, morbidity and response to treatment of the disease; the importance of metacyclogenesis of *T. cruzi* in different species of triatomines and its capacity to transmit Chagas infection; the risks of adaptation of wild triatomines to human dwellings; the morbidity and need for a surveillance and control program for Chagas disease in the Amazon region and the need to prioritize initiatives for controlling Chagas disease in Latin America and Mexico and in non-endemic countries, which is today a major international dilemma. Finally, we raise the need for to create a new initiative for controlling Chagas disease in the Gran Chaco, which involves parts of Argentina, Bolivia and Paraguay.

Keywords: Chagas disesase. Evolutive patterns. *Trypanosoma cruzi* vector interactions. New strategies for treatment. Surveillance and control.

RESUMO

Neste trabalho, fazemos uma ampla revisão sobre o que sabemos e o que deve ser melhorado no conhecimento da doença de Chagas, não somente através da análise dos principais trabalhos publicados sobre os tópicos discutidos, mas em grande parte com base na experiência sobre o assunto, que adquirimos nos últimos 50 anos (1961-2011). Entre os assuntos abordados, destacamos a patogenia e evolução da infecção pelo *Trypanosoma cruzi*, drogas em uso e novas estratégias para o tratamento da doença de Chagas; os testes sorológicos para o diagnóstico e o controle de cura da infecção; as variações regionais da prevalência, morbidade e resposta ao tratamento da doença; a importância da metaciclogênese do *T. cruzi* em diferentes espécies de triatomíneos e sua capacidade de transmissão da infecção chagásica; os riscos de adaptação dos triatomíneos silvestres ao domicílio humano; a morbidade e a necessidade de um programa de vigilância e controle da doença de Chagas na região Amazônica e a necessidade de priorização das Iniciativas de controle da Doença de Chagas na América Latina e México e nos países não endêmicos, hoje um grande dilema internacional. Finalmente, levantamos a necessidade da criação de uma nova iniciativa de controle da doença de Chagas no Gran Chaco, que envolve parte da Argentina, Bolívia e Paraguai.

Palavras-chaves: Doença de Chagas. Padrão evolutivo. Interação *Trypanosoma cruzi*-vetores. Novas estratégias para o tratamento. Vigilância e controle.

INTRODUCTION

A few years ago, we wrote a review article with the title *Chagas disease: what is known and what is needed*[1]. In that article, we discussed the natural history, the origin and distribution of the disease, the determining factors for infection and the pathogenesis of the disease, which we know little about, the phases and clinical forms, and the disease in the Amazon region, where knowledge is also scarce. Finally, we listed the following ten main problems relating to knowledge of Chagas disease that needed to be addressed: A) to explain the pathogenesis and evolutionary pattern of the disease; B) to develop a drug that is efficient for treating the acute and chronic phases; C) to improve the tests for diagnosing the infection, which at that time consisted of *Trypanosoma cruzi* I, II, Z3 and Z1/Z3 hybrids, which are today consensually known as TcI to TcVI[2]; D) to standardize the serological techniques for the diagnosis and for Chagas infection cure control; E) to compare the morbidity of Chagas disease induced in different regions by the various types of *T. cruzi*; F) to study the metacyclogenesis of different strains and clones of *T. cruzi* among different vector species; G) to analyze the risk of wild triatomines adapting to human dwellings; H) to evaluate the morbidity of Chagas disease in the Amazon region; I) to establish a surveillance and control program for Chagas disease in the Amazon region; J) to prioritize disease control programs in Latin America and Mexico, through stimulating the initiatives created by the Pan-American Health Organization.

The objective of this new review was to emphasize what should be improved regarding knowledge of Chagas disease, evaluate some of the progress achieved over the last five years and suggest measures that are necessary for research, technological development and surveillance and control of Chagas disease in Latin America and Mexico and in non-endemic countries. On the other hand, the aim was to raise new issues in relation to the pathogenesis of the disease, regional differences, diagnosis, treatment and perspectives for surveillance and control of the disease.

PATHOGENESIS AND EVOLUTION OF INFECTION BY *TRYPANOSOMA CRUZI*

Since American trypanosomiasis was discovered, numerous studies have been conducted on the pathogenesis of the disease and the evolution of human infection by *T. cruzi* in its acute and chronic phases and the respective clinical[3-6]. Over the past 100 years since the study by Gaspar Vianna[7] on the pathology of the *Illness of Carlos Chagas*, numerous other studies on this theme have been carried out. However, the only finding that remains incontestable is that, as described by that author: *Trypanosoma cruzi* penetrates in the host's cells and multiplies in amastigote form to create *pseudocysts* that break and give rise to an inflammatory reaction that scars as a fibrosis. Through the rupture of the *pseudocysts* new forms of trypomastigotes are released, which circulate in the organism and invade new cells, repeating the cycle and consequently producing new lesions. The second possible mechanism for the pathogenesis of Chagas disease, which is more complex and controversial, is autoimmunity[8,9]. However, Tarleton[10] questioned the autoimmunity mechanism, emphasizing the role of parasite persistence in the pathogenesis of Chagas disease. Teixeira et al.[11] reported in their text: *Questions related to the mechanism by which tissue lesions are formed in the course of T. cruzi infection have long been a matter of contentious debate. Although numerous reviews have proposed a plethora of brilliant hypotheses, the origin of the pathological lesions of Chagas disease remain open to investigation.*

On the other hand Higushi[12] state that sensitivity of TCD4+ and TCD8+ lymphocytes by *T. cruzi*, with development of anti-myocardial cells, associated with migration and activation of macrophages and the release of platelet aggregation factors, there by respectively inducing chronic Chagas myocarditis and myocardial ischemic lesions, may explain the findings encountered in cases of chronic myocardiophaty, but in agreement with Tarleton[10] the pathogenesis of chronic myocarditis in Chagas disease is directly related with the presence of *T. cruzi*). According to Köberle hypothesis[13] megaesopgagus, megacolon and cardiac conduction disturbances in Chagas disease are consequence of the desnervation of the parasympathetic autonomous system. Prata[14], Teixeira et al.[11] and other considerer that the neuron destruction in the heart, esophagus, colon and other hollow viscera may be explained both by direct inflammatory phenomena and by inmunological mechanism that results in the cardiopathy, megaesophagus, megacolon and other visceral alargements seen in Chagas disease.

What is still unknown in the pathogenesis of Chagas disease: A) why some patients infected with *T. cruzi* develop heart disease, others present megaesophagus or megacolon, others remain in an indeterminate form and yet others develop a mixed form with both heart disease and *megas*; B) why some patients present mild heart disease and do not evolve to a severe heart condition, while others do and even die of heart failure or sudden death. Likewise, why some develop an associated form, such as megaesophagus and megacolon, while 80 to 90% do not develop any *mega*; C) why *T. cruzi* does not attack organs like the lungs and kidneys, where it circulates all the time during the acute and chronic phase; D) why there is no megaesophagus and megacolon in the countries with endemic Chagas disease that are located north of the Equator; E) how the regional differences in Chagas disease morbidity can be explained.

Andrade[15,16] and Andrade & Magalhães[17] grouped *T. cruzi* into three groups or biodemes (Types I, II and III) according to its biological characteristics, tissue tropism, morphology, virulence and pathogenicity for mice. *Type I:* macrophage tropism during the initial phase of the infection, with high virulence, maximum parasitemia of 7 to 12 days and 100% mortality of mice within 12 days of infection, with predominance of the thin forms of *T. cruzi*; *Type II:* myotropism with predominance in the myocardium during the acute phase, predominance of wide forms, but with a percentage of thin forms, with maximum parasitemia and mouse mortality in 12 to 20 days; *Type III:* myotropism for skeletal muscle, with predominance of wide forms, maximum parasitemia in 25 to 30 days and low mortality among mice.

Despite the great importance of this classification, it does not explain the variations of human Chagas disease. Macedo & Pena[18] correlated the genetic variations of *T. cruzi* and their implications for the pathogenesis of Chagas disease, according to the clonal histotropic receptors for different strains and clones of the parasite. However, this very logical and attractive theory has not been fully proven in practice. Greater severity in the acute phase, especially among children, observed by Dias[19] in Bambuí, State of Minas Gerais, Brazil, coincided with a greater percentage of severe chronic forms. Similarly, the reinfections in the chronic phase observed by Macedo[20] favored development and evolution of forms of greater severity among patients who remained in endemic areas with great infection pressure from *T. cruzi*. In a study on disease evolution that we carried out in Rio de Janeiro on 510 patients from several states in Brazil[21], we observed that 52.1% had heart disease, 39% had the indeterminate form and 14.3% had megaesophagus and megacolon, with 5.4% presenting an association between *megas* and heart disease. The states with highest frequency of patients with heart disease were Goiás (66.6%), Bahia (65.5%), Minas Gerais (55.7%) and Pernambuco (50.9%).

DRUGS IN USE AND NEW STRATEGIES FOR TREATING CHAGAS DISEASE

The current recommendations for chemotherapy treatment of Chagas disease in Brazil are based on decisions made by specialists who were brought together by the Ministry of Health in 1997, with subsequently revalidation by *experts* coordinated by the Pan-American Health Organization in 1978[22,23], i.e. about 13-14 years ago. These recommendations suggested that acute cases of Chagas disease acquired through vector, oral or congenital transmission, or through laboratory accidents or any other transmission route should be immediately treated with one of the drugs that are currently available: nifurtimox and benznidazole. It was also recommended that children up to the age of 12 years should be treated, as well as cases of recent infection in adults over known periods within the last 10 to 12 years. Coura[24] reviewed those recommendations and suggested that combinations of drugs, such as benznidazole + nifurtimox, and benznidazole or nifurtimox + antifungals that inhibit ergosterol in double or triple associations should be used, as is done with drugs for treating tuberculosis, leprosy and human immunodeficiency virus/acquired mmunodeficiency syndrome (HIV/AIDS). More recently, on the basis on the pathogenesis of the disease and its evolution, Coura & Borges-Pereira[25] recommended that the treatment should be broadened to chronic cases at initial evolutionary stages, such as stage II of the New York Heart Association[26] and up to stage III, at the discretion of the attending doctor and with the patient's agreement.

Since 1912, numerous drugs have been used experimentally and in clinical trials for treating Chagas disease[23-25,27]. However, only when Packachanian[28,29] demonstrated experimentally that nitrofurans were promising for treating *T. cruzi* infection and Brener[30] proved that nitrofurazone acted to cure chronically infected mice, with treatment for 53 days, did clinical treatment of Chagas disease began with some efficiency. Ferreira and Ferreira et al.[31-33] demonstrated *good results* among children treated with nitrofurazone. Coura et al.[34] managed to cure 3/10 chronic cases of the disease among adults treated for 60 days with a nitrofurazone dose of 10mg/kg/day, but with major side effects, especially polyneuropathy.

Currently, only two drugs are in use for treating Chagas disease: nifurtimox at a dose of 7.5 to 10mg/kg/day and benznidazole at a dose of 5 to 7.5mg/kg/day for 60 days. Several authors cited by Coura & Castro, Coura and Coura & Borges Pereira[23-25] also conducted studies with nifurtimox and/or benznidazole in acute and chronic phase of Chagas disease. In a controlled study, was demonstrated that benznidazole was more efficient than nifurtimox for suppressing parasitemia in cases of chronic infection[35]. On the other hand, in a multicenter study, was demonstrated that treatment with benznidazole had the same suppressive action on parasitemia over 30 or 60 days of treatment[36]. Nifurtimox is a nitrofuran with a mechanism of action that (in a simplified manner) involves production of nitro-anion radiation, which in the presence of oxygen impedes the capacity of *T. cruzi* to detoxify the free radicals that act on it[37]. On the other hand, benznidazole acts by binding its metabolites to the nuclear deoxyribonucleic acid (DNA) of the parasite, and to its lipids and proteins[38,39]. Several clinical studies have been conducted and hundreds of patients have been treated with nifurtimox or benznidazole, with variations in the results between the studies and the regions where they were carried out. The average cure rate among acute and recent cases is 80%, while it is less than 20% among chronic cases[23-25]. A pilot study, the Benefit project - Benznidazole Evaluation for Interruping Trypanosomiasis - an international, multicenter, double-bind, placebo-controlled trial of tripanocidal chronic Chagas heart disease is in course[40,41].

Several other drugs have been shown to be effective *in vitro* against *T. cruzi*. Among these are allopurinol, an antiuricemic hypoxanthine that is used to treat gout, and antifungals that inhibit ergosterol, such as ketoconazole, itraconazole and fluconazole[42-46]. These have not been shown to be effective *in vivo*, including during the acute phase of the disease. More recently, posaconazole, another azolic derivate, was shown to be very promising and is now at the clinical trial phase[44].

What we do not know regarding the treatment of Chagas disease: A) why acute and recent cases of *T. cruzi* infection are more frequently cured than the chronic cases; B) why patients in a certain geographical area respond better to treatment than in other areas; C) why the serological parameters of chronic cases of Chagas disease do not become negative immediately after the cure has been achieve, but take years to become negative; D) why has there never been an attempt to use associations of drugs for treating Chagas disease, as is done in cases of tuberculosis, leprosy and HIV/AIDS, which came under control after using combinations of drugs. It is known that certain strains and clones of *T. cruzi* are resistant to treatment, as is the case of the Colombian strain. Cross-resistance to nifurtimox and benznidazole has also been observed.

In the areas where Chagas infection is more easily and frequently cured, this is probably achieved through greater sensitivity of the strains of *T. cruzi* and lower frequency of resistance to chemotherapy. Andrade et al.[47,48] and Portella & Andrade[49] showed that the dendritic cells of animals that had been experimentally treated remained impregnated with *T. cruzi* antigens, thus stimulating the presence of antibodies. This would explain the positive serological tests even after achieving parasitological cure.

SEROLOGICAL TESTS FOR DIAGNOSIS AND CURE VERIFICATION

The conventional serological tests for diagnosing Chagas disease and verifying its cure are indirect hemagglutination (IHA), indirect immunofluorescence (IIF), the immunoenzymatic test or enzyme-linked immunoabsorbent assay (ELISA). Other tests, such as Western blot, complement-mediated trypomastigote lysis and recombinant proteins are less used, mainly because of their high cost or the greater complexity of performing them. An extensive recent review by Luquetti and Schmunis[50], on diagnosis of *Trypanosoma cruzi*, analysed carrefuly all serological tests for diagnosis and cure evaluation of Chagas infection. In this Review they states that the antibodies demand different timing to disappear according to the phase of the disease, and the type of *T. cruzi*. The acute phase and cronically infected with less than 10 years the antibodies desapear between 2 and 10 years[51]. In those countries where *T. cruzi* I predominates the antibody responses evanish by 16 in children[52].

Despite the high specificity and ease of implementation of indirect hemagglutination, its sensitivity presents great variability, according to the origin of its production[53]. Thus, a second confirmatory test is required, especially when the titers range between 1/40 and 1/80. Indirect immunofluorescence, which was initially developed by Fife & Muschell[54] for serological tests on *T. cruzi* infection and was adapted for blood collected on filter paper by Sousa & Camargo[55], is a good test regarding its sensitivity. However, as well as the subjectivity of readings, it frequently presents cross-reactions with other diseases, especially with titers of 1/40 to 1/80. Nonetheless, according to Luquetti et al.[56] it is an excellent test for diagnosing *T. cruzi* infection when the titers are greater than or equal to 1/320. The ELISA test developed by Voller et al.[57] included adaptation for blood collected on filter paper, and it presents sensitivity similar to that of IIF, with the advantage of the possibility of automated reading. This avoids subjectivity and making it possible to apply the test to readings on a great number of samples, as is done in blood banks.

The Western blot test, and particularly the TESA blot using the excretion and secretion antigen of *T. cruzi*, as developed by Umezawa et al.[58], is an excellent confirmatory test that is used especially in research laboratories, since its high cost makes its use in routine diagnosis prohibitive. On the other hand, although complement-mediated trypomastigote lysis, which was developed by Krettli & Brener[59], is a good test for cure verification, its technique is difficult to reproduce in diagnostic laboratories. Some tests with recombinant proteins present high sensitivity and specificity, particularly the mixture of CRA and FRA (*Biomanguinhos*), and the recombinant Wiener ELISA has been applied with good results, but some kits may present false positive reactions, especially when adapted for quick tests, in the same way as synthetic peptides.

The great requirement today is for a quick test with high sensitivity, specificity and stability for fieldwork and blood bank emergencies, in different geographical areas where different strains

and clones of *T. cruzi* (TcI to TcVI) circulate. The tests available, such as Stat-Pack® and Imbios®, present great variation in their sensitivity and specificity according to the geographical area, such as Central America, Bolivia and the Amazon region, and they must always be accompanied by a second confirmatory test[60]. Therefore, it is extremely important and necessary to develop a quick test with high sensivity and specificity.

MORBIDITY DIFFERENCES IN CHAGAS DISEASE ACCORDING TO GEOGRAPHICAL AREA

The regional variations in Chagas disease morbidity were very well evaluated by 32 specialists from nine countries in the Americas (Argentina, Bolivia, Brazil, Chile, Costa Rica, Paraguay, Peru, USA and Venezuela) during a meeting on *Geographical Differences of Chagas disease* in Brasília on August 4 to 5, 1975, coordinated by Prata[61]. Although the determining factors for these variations are not exactly known, they could be related to: A) virulence and pathogenesis of the strains and clones of *T. cruzi* circulating in the area (TcI to TcVI) and their biodemes[15-17]; B) qualities of the vector: anthropophily, capacity for metacyclogenesis to infecting forms of *T. cruzi*, time of evacuation (after or during the meal), number of infecting forms eliminated during excretion[62]; C) initial inoculum of infecting forms of *T. cruzi* and the number of reinfections[19,20]; D) initial and late host immune response, escape mechanisms for *T. cruzi* and its adherence to clonal histotropic receptors[1,11,18]; E) other factors that have still not been determined, such as *adaptation* of wild strains of *T. cruzi* to man, individual genetic factors and others that have not yet been characterized.

In the meeting on the geographical differences of Chagas disease, Professor Aluízio Prata, in his objective style, formulated 52 questions for the participants to answer, of which 48 were answered. These questions were in relation to: epidemiology (12), clinical characteristics (13), anatomopathological characteristics (11), chemotherapy and prevention (12) and not answered (4). The responses showed that there were striking differences in the prevalence and morbidity of Chagas disease, with regard to both heart disease and *megas*, with greater severity of the disease in countries south of the Equator. To the north, heart disease seemed to be less severe, there were practically no *megas* and the response to treatment had been seen to be better. Naturally, there were intraregional variations regarding epidemiological, clinical and anatomopathological factors and in relation to the response to treatment, which have also been observed more recently. On the other hand, there were also variations from one area to another within the same country, as in Brazil.

The evolutionary pattern of Chagas disease is still not completely defined because the morbidity and mortality varies considerably from one area to another. In the study that we carried out in Rio de Janeiro on 510 patients from several states in Brazil[21], 39% were in the indeterminate clinical form, 52.1% in the heart disease form and 14.3% had megaesophagus and/or megacolon. An association between heart disease and *megas* was observed in 5.4% and between megaesophagus and megacolon in 10.9%. The highest heart morbidity was observed among patients in Goiás, Bahia, Minas Gerais and Pernambuco, while the lowest was among patients in Rio de Janeiro and Paraíba.

Over the past 38 years (1973-2011), several cross-sectional and longitudinal case-control studies have been conducted to evaluate

the morbidity of Chagas disease in Brazil by our group over different periods and in different geographical areas of the State of Minas Gerais (Iguatama and Pains in the northwest of the state, Virgem da Lapa and Berilo in the Jequitinhonha valley)[63-67]; in the *Caatinga* (scrublands) of Piauí[68]; in the *Sertão* (semi-arid backcountry) of Paraíba[69-70]; and in areas of the Rio Verde region, Mato Grosso do Sul and the microregion of the middle and upper Negro River, State of Amazonas[71-78], Borges-Pereira et al.[79-88]. **Figure 1** shows the locations of the various field areas that have been or are being studied. There is a great variation of morbidity from one area to other[89-91].
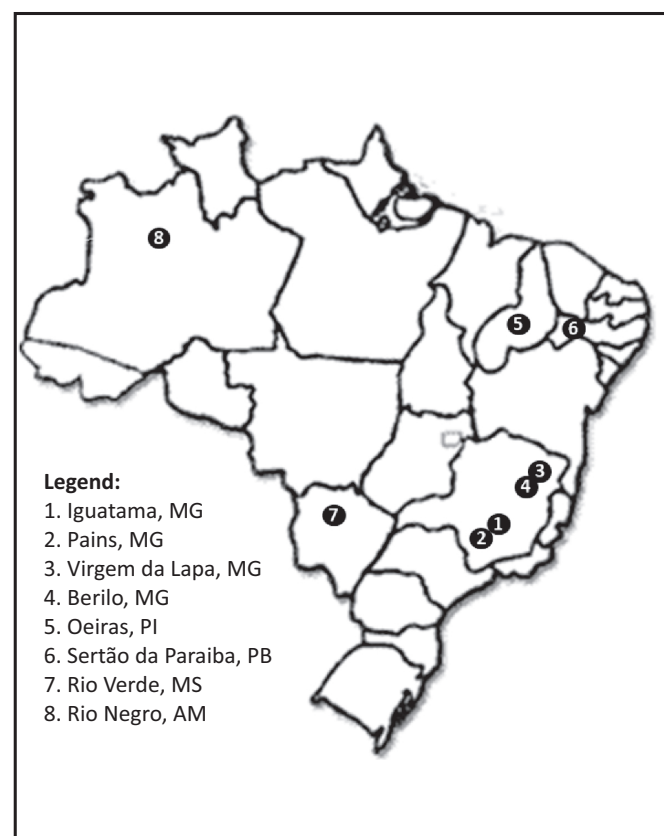

Legend:
1. Iguatama, MG
2. Pains, MG
3. Virgem da Lapa, MG
4. Berilo, MG
5. Oeiras, PI
6. Sertão da Paraiba, PB
7. Rio Verde, MS
8. Rio Negro, AM

FIGURE 1 - **Areas where cross-sectional and longitudinal case-control studies have been conducted to evaluate morbidity and mortality of Chagas disease in the States of Minas Gerais, Piauí, Paraíba, Mato Grosso do Sul an Amazon, Brazil, from 1973 to 2011.**
MG: State of Minas Gerais; **PI:** State of Piauí; **PB:** State of Paraíba; **MS:** State of Mato Grosso do Sul; **AM:** State of Amazon.

From 1999 onwards, we started to classify the areas into two categories: A) areas of high morbidity; and B) areas of low morbidity[92].

Areas of high morbidity

Evolutionary (follow-up) studies were carried out in Iguatama (Area 1) and Pains (Area 2) in the northwest of Minas Gerais for 25 years (1973-1998) among 264 pairs of individuals (initially) of the same age and gender; in Virgem da Lapa (Area 3), among 1,109 pairs of individuals (initially), between 1976 and 2011; and in Berilo (Area 4) in the Jequitinhonha valley, in the north of Minas Gerais, among 100 pairs of individuals (initially). All the pairs of individuals who were investigated in the endemic areas underwent the same types of anamnesis, clinical examinations, electrocardiograms (ECG) with the 12 classical derivations with long D2 and chest X-rays,

emphasizing cardiac and digestive signs and symptoms. Two cross-sectional studies with the same parameters were also carried out in the district of Oeiras (Area 5), in the State of Piauí, northeastern Brazil, separated by an interval of 20 years (1976-1996) among 109 pairs of individuals (initially).

The first cross-sectional study conducted in Iguatama (Area 1) and Pains (Area 2), Virgem da Lapa (Area 3), Berilo (Area 4) and Oeiras (Area 5) showed that the serological prevalence of Chagas infection (from indirect immunofluorescence) was 17.1% for Iguatama, 10.4% for Pains, 12.9% for Virgem da Lapa, 12.7% for Berilo and 12.1% for Oeiras. Heart morbidity evaluated by means of the gradient of electrocardiographic alterations among seropositive and seronegative individuals was, respectively, 23.4% in Iguatama, 18.4% in Pains, 19% in Virgem da Lapa, 22.5% in Berilo and 18.2% in Oeiras. In the evolutionary study, Chagas disease was progressive, by an average of 2.5 to 3% patient/year. Mortality was 5 times higher among Chagas patients and the lethality rate was approximately 2% patient/year, varying naturally with the initial severity of the heart disease[92]. **Figures 2A, B** and **C** shows the gradient of the clinical and electrocardiographic manifestations and the mortality among cases of Chagas disease and their controls, in Virgem da Lapa (Area 3).

Areas of low morbidity

A cross-sectional study carried out in 8 municipalities in the *Sertão* of Paraíba (Area 6) and in 12 municipalities in the Rio Verde region, Mato Grosso do Sul (Area 7), involving respectively 5,137 and 14,700 people, showed that the serological prevalence of Chagas infection in Paraíba was 9.5%, while it was only 1.8% in Mato Grosso do Sul. Heart morbidity, as evaluated according to the electrocardiographic gradient among seropositive and seronegative individuals, was 12.8% among 305 pairs of individuals studied in Paraíba and 15% among 70 pairs of autochthonous individuals in Mato Grosso do Sul. Chagas disease was progressive, at the rate of 1.3% patient/year in Paraíba, although the lethality due to the disease was very low (less than 0.4% patient/year). Mortality and lethality were not evaluated in Mato Grosso do Sul.

In three cross-sectional studies carried out in the municipality of Barcelos (Area 8), in the Negro River microregion, State of Amazonas, respectively in 1991, 1993 and 1997[76], screening serological tests on 2,254 serum samples showed a variation in positivity from 12.5% to 13.7%. However, only 2.8 to 5% were confirmed as positive by means of IIF, ELISA and Western blot (TESA-blot), with a high percentage of cross-reactions. In a recent serological study (2010-2011), IIF on filter paper revealed positivity of 4.5% among 4,880 serum samples from this area. A study on 38 pairs of serologically positive and negative individuals carried out recently by Brum-Soares et al.[93] showed that the electrocardiograph gradient was 15.3% greater among the seropositive individuals, while the echocardiogram was altered among 36.8% of the seropositive individuals and among 18.4% of the seronegative individuals, with an echocardiograph gradient of 13.2%. Taking into considering the small number of patients, it was not possible to safely evaluate the real morbidity of Chagas disease. However, typical and fatal cases of chronic Chagas heart disease have been described in this area[94,95].

Finally, we draw attention to the great regional and personal variation of morbidity among Chagas disease cases. Knowledge of these variations needs to be improved at both national and
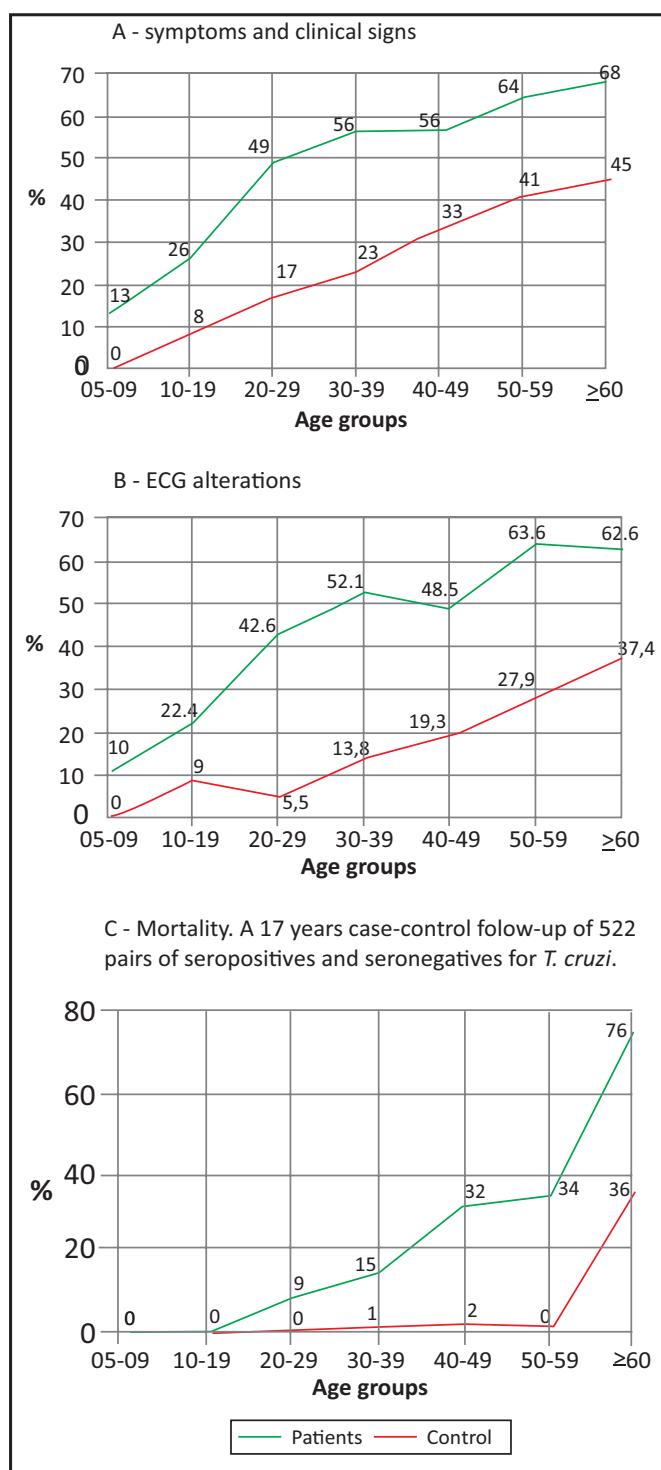

FIGURE 2 - Morbidity and mortality in the Chagas disease: a case-control study of 1.109 pairs, seropositives for *Trypanosoma cruzi* infection, matched with seronegatives of the same sex and age groups. Virgem da Lapa, Minas Gerais. **ECG:** electrocardiogram; *T: Trypanosoma;* Source: Borges-Pereira J. Doctors Thesis. Instituto Oswaldo Cruz, 1997.

international level. Another challenge is the indeterminate form of Chagas disease defined since Chagas[5] and validated by the Brazilian Society of Tropical Medicine[89] as asymptomatic human infection by *T. cruzi* without ECG and Rx abnormality in the heart, esophagus or colon[25]. Even though some abnormalities may appear in the ECG and in the echocardiogram. The indeterminate form of Chagas disease clearly represents a benign condition with a long-term prognosis[90].

METACYCLOGENESIS OF *TRYPANOSOMA CRUZI* IN DIFFERENT SPECIES OF TRIATOMINES AND DIFFERENCES IN INFECTION ACCORDING TO THE PARASITE STRAIN

Metacyclogenesis of *T. cruzi* in different species of triatomines is of great importance in the transmission of Chagasic infection. Some species such as *T. pseudomaculata* can transform *T. cruzi* into infective metacyclic species in little more than 10% of the cases. Thus, this species of triatomine is considered to be a vector of poor efficiency. Other species such as *T. infestans*, *R. prolixus* and *P. megistus* can transform 60 to 70% of the strains of *T. cruzi* into forms that are infective to humans and other vertebrates. This is a very important characteristic in the transmission dynamics and, thus, in the quality of the vector.

Trypanosoma cruzi in the digestive tract of triatomines goes through several transformations from ingested trypomastigotes, to spheromastigotes and epimastigotes, until reaching the infective metacyclic trypanosomes. These are then eliminated through the feces and urine of the vectors[96,97]. This process involves a series of biochemical and immunological mechanisms, in which the enzymes, hemolysins, agglutinins and other antimicrobacterial agents have still not been properly investigated[98]. According to these authors, better understanding of the basic mechanisms of parasite-vector interactions will bring new perspectives for controlling some parasitic diseases.

In an experimental study by Perlowagora-Szunlewicz et al.[99] on the interactions of nine vector species (*R. prolixus*, *R. megistus*, *T. infestans*, *T. braziliensis*, *T. sordida*, *T. pseudomaculata*, *T. rubrovaria* and *T. dimidiata*) that were fed on guinea pigs infected with seven different strains of *Trypanosoma cruzi* (Berenice, Y, FL, CL, São Felipe, Colombiana and Gávea), the following mean triatomine infection rates were demonstrated 30, 60, 90 and 120 days after infection. As can be seen, there was great variation in the triatomine infection rates according to the species and strain of the infecting *T. cruzi* (**Table 1**).

The mean positivity rate among the various species of triatomines ranged from 9.8% (*T. dimidiata*) to 91.4% (*T. pseudomaculata*), with mean positivity of 67.3%. *T. pseudomaculata* (91.4%) *Triatoma sordida* (89.7%), *R. neglectus* (89.7%), *P. megistus* (87.9%) and *T. rubrovaria* (80.8%) were above the mean, while *T. dimidiata* (9.8%), *T. infestans* (45.4%), *R. prolixus* (46%) and *T. braziliensis* (65.4%) were below it. The authors concluded that the domesticated

species of triatomines (*T. dimidiata* and *T. infestans*) were less infected than the wild species, which is true, but they considered that *P. megistus*, *T. sordida* and *T. pseudomaculata* were wild species. In reality, these are not exclusively wild species, but can also be peridomestic and domestic. Among the species studied, *R. neglectus* and *T. rubrovaria* can be considered to be wild. On the other hand, the best transmitters are not always the vectors that were most infected experimentally, because of metacyclogenesis of *T. cruzi* in the digestive tract. This was the case of *T. pseudomaculata*, which was the species most infected in this experiment, but it converts little more than 10% of the ingested *T. cruzi* into the infecting form. In the cited study, it was demonstrated that this species may be best for xenodiagnosis, but not as a vector for *T. cruzi*.

RISKS OF WILD TRIATOMINE ADAPTATION TO HUMAN DWELLINGS

Primitive trypanosomes were monogenetic parasites of non-blood sucking insects that were probably predators. When these insects acquired the habit of sucking blood millions of years ago, they underwent morphological and functional adaptations such that they developed an undulating membrane and flagellum to circulate in the blood of vertebrates, which is the most likely hypothesis for their evolution[100]. Triatomines have been known since the 14th century, but were only described in 1773 by DeGeer, who described *Cimex rubrofasciata* (*Triatoma rubrofasciata*), originally from India[101]. Since then, more than 130 species of 19 genera of the Triatominae subfamily have been described, of which the vast majority are wild triatomines.

The domestication process among wild triatomines is still not completely known. The evolution from predators to blood suckers was associated with a series of morphological, behavioral and demographic modifications relating to three key factors: a) exploitation of vertebrate blood as a food source; b) adaptation to the host's environment; c) progressive dependence on the host as a means of dispersion through passive transportation[102]. This process involves genetic specialization and simplification. The sensory system of triatomines has become progressively simplified with the stability of the domestic habitat[103]. On the other hand, reduction of the size of the insects and sexual dimorphism may have occurred[104]. The high gnetic variability of wild *T. infestans* favours the hipothesis that the Andean were the centre of dispersal of this specie through South America and it adaptation to human dwellings[105].

TABLE 1 - Triatomine infection rates by different strains of *Trypanosoma cruzi.*

Triatomíneos	Berenice		Y		FL		CL		São Felipe		Colombiana		Gávea	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Rhodnius prolixus	29	48.3	28	35.7	26	50.0	31	29.0	23	13.0	31	74.2	32	71.9
Rhodnius neglectus	32	84.4	32	75.0	32	90.6	32	100.0	32	90.6	32	100	32	87.5
Rhodnius megistus	32	93.8	32	96.9	32	90.6	32	96.9	31	93.5	31	77.4	21	66.7
Triatoma infestans	22	50.0	18	55.6	23	52.2	16	81.3	15	66.7	27	63.0	23	30.4
Triatoma brasiliensis	28	64.3	26	84.6	23	43.5	26	53.8	25	92.0	29	55.2	31	64.5
Triatoma sordida	32	87.5	32	93.7	32	96.9	32	93.8	32	93.8	32	81.3	32	81.3
Triatoma pseudomaculata	32	96.9	32	90.6	32	93.8	32	87.5	31	96.8	31	90.3	32	84.4
Triatoma rubrovaria	32	87.5	32	65.6	32	78.1	32	90.6	32	87.5	32	75.0	32	81.3
Triatoma dimidiata	17	11.8	20	15.0	17	17.6	10	0.0	15	6.7	17	17.6	9	0.0

Source: Perlowagora Szunleiwicz et al, Revista de Saude Publica São Paulo 1998.

Wild triatomines approach human peridomestic and domestic areas primarily due to feeding needs, due to deforestation and the distance from their natural sources, which are wild mammals, or when the numbers of these animals become reduced in the natural habitats[106,107]. Sometimes, wild triatomines are attracted by domestic lights or they penetrate the home due to its proximity to palm trees, which occurs frequently in the Amazon region[1,78]. Thus, people may become infected accidently or there may even be epidemic outbreaks due to food contamination and oral transmission[108,109].

The adaptation of wild triatomines to human homes is perhaps the most polemical and uncertain issue among all the knowledge on Chagas disease. It is certainly one of the most important matters, since even if we can control the few domesticated species of triatomines, there will always be the risk of reinvasion of homes by another hundred wild species.

MORBIDITY OF CHAGAS DISEASE IN THE AMAZON REGION

Since Carlos Chagas[110] confirmed as *T. cruzi* trypanosomes isolated from the monkey *Saimiri sciureus*, from Pará, Chagas disease has been considered to be an enzootic disease of wild animals in the Amazon region. Deane[111-115], Deane & Jansen[116] and Deane & Damasceno[117] and several other authors confirmed this concept, describing the presence of *T. cruzi* among 33 species of six orders of wild animals (Marsupialia, Chiroptera, Rodentia, Edentada or Xenarthra, Carnivora and Primata). Floch & Tasqué[118] and Floch & Cormain[119] reported the first cases of humans with acute Chagas disease in French Guiana (Amazon region), and Shaw et al.[120] described the four first acute cases of the disease that occurred in an outbreak in Belém in Pará, Brazil, probably through oral transmission. Since then, more than 60 outbreaks of the acute disease have been described in this region, especially in Pará, Amapá and Amazonas[108,109,121]. Between 1969 and 2008, the Surveillance Department of the Brazilian Ministry of Health notified 761 cases of Chagas disease in the Brazilian Amazon region, and 75% of these (568 cases) were between 2002 and 2008[93].

Over the last 20 years (1991-2011), we have developed seroepidemiological surveys and clinical, radiological and electrocardiographic studies among populations that were seropositive for Chagas infection, in the middle and upper regions of the Negro River, in the State of Amazonas[72-74,76-78]. Several typical and fatal chronic cases of Chagas disease were described[94,95], involving more than 7,000 screening serological reactions and more than 300 confirmatory serological reactions. In three preliminary seroepidemiological surveys conducted in 1991, 1993 and 1997, we carried out 2,254 screening serological reactions using IIF on blood collected on filter paper and found that the mean positivity rate was 13%[76], although only 2.5% to 5% were confirmed by means of serum IIF, ELISA and trypomastigote excreted-secreted antigens (TESA)-blot. More recently, we carried out 4,880 screening serological reactions using IIF on blood samples on filter paper and found a positivity rate of 4.5% for Chagas infection. These cases are now undergoing confirmatory serological reactions.

In a recent seroepidemiological study[93] carried out among 152 individuals living in the municipality of Barcelos, of whom several had already been evaluated previously, serological reactions by means of immunofluorescence, conventional and recombinant ELISA and TESA-blot were used. Thirty-eight (25%) individuals

were considered positive i.e. with at least two positive reactions from different mechanisms, 31 (20.4%) were uncertain with only one positive reaction, and 83 (54.6%) were negative, with the four reactions negative. Positive serological reactions were 10.4 times more frequent among *piaçaba* (*Attalea funifera*) gatherers, and thus Chagas infection was characterized as a professional disease. A total of 86.7% of the seropositive individuals recognized *Rhodnius* as the local vector, while only 34.2% of the seronegative individuals recognized it as such. The 38 seropositive individuals were matched by age and gender with other, seronegative individuals and underwent clinical, electrocardiograph and echocardiograph examinations. Twenty-nine of these pairs underwent chest radiography with contrasted esophagus. The most evident clinical manifestations were palpitations in 31.6% of the seropositive group and 21.8% of the seronegative group and chest pain in, respectively, 28.9% and 15.8% of the seropositive and seronegative groups. The electrocardiogram was altered in 36.8% of the seropositive group and 21.5% of the seronegative group (gradient of 15.3%) and the echocardiogram was altered in 31.6% of the seropositive individuals and 18.4% of the seronegative individuals (gradient of 13.2%). The cardiothoracic rate was greater than 0.5 in four (13.8%) seropositive patients and three (10.3%) seronegative individuals, while the esophagus radiography did not show alterations in either of the groups. It can be concluded that heart morbidity in this area is low and esophagus morbidity is absent, and that it is necessary to increase the number of studied cases for better evaluation of the morbidity of the disease in this area.

Need to establish a surveillance and control program for Chagas disease in the Amazon region

In September of 2004 a meeting was held by the Pan-American Health Organization with the objective of organizing an Initiative for Surveillance and Control of Chagas Disease in Amazon Countries, which was called AMCHA (Amazonas Chagas). Representatives of the governments of the nine countries of the Amazon region and more than 90 researchers from the different countries, including the president of the Brazilian National Research Council (CNPq), participated in this meeting. At the end of the meeting, the following objectives were established: a) To assess the risks of Chagas disease becoming endemic in the Amazon region; b) To define the research needed for surveillance and prevention of the disease in the region; c) To propose surveillance and control measures for the disease in the region; d) To establish a proposal for an international cooperation system among the nine Amazon countries for an integrated surveillance and control system. Three other meetings were held, respectively in Cayenne, French Guiana, in 2005; in Quito, Ecuador, in 2006; and in Caracas, Venezuela, in 2008. However, despite recognition of the risks of the disease becoming endemic in the region, the need to increase knowledge through research on Chagas disease in the region and several proposals for surveillance and prevention, very little has been done up to the present date.

Most cases in the Brazilian Amazon region occur through outbreaks caused by oral transmission[108], while vector transmission occurs mainly among plant product extractive workers, particularly in relation to *piaçaba*[93]. The perspectives for control over the disease in this region are as follows: a) the Surveillance Department of the Ministry of Health has established a surveillance system in the Brazilian Amazon region through compulsory notification of the disease; b) Courses have been conducted to train the malaria

microscopists and capacitate the public laboratory networks (*LACENs*) of the nine Brazilian states in the Amazon region, with support from Doctors Without Borders between 2006 and 2008; c) A manual was prepared for these microscopists and technicians of the public laboratories[122]. Consequently, 100 to 150 new acute cases of Chagas disease are being notified in the Amazon region every year. However, from our perspective, this represents less than 10% of the occurrence of cases, given that most these individuals do not seek the healthcare services because they do not know of the disease, or because of the distance from medical care centers, among other factors.

We also suggest that training should be provided for elementary school teachers and community health agents to work as knowledge multipliers about the disease and its vectors, so as to widen the surveillance and control network for Chagas disease in the Brazilian Amazon region. Teachers have access to children, who are naturally curious and, through familiarity, can locate vectors in the peridomestic and domestic areas. On the other hand, when community health agents are well informed, not only can they recognize the vectors and the disease, but also they can send the insects collected and the suspected patients to the regional health centers, in order to identify the specimens and provide possible treatment for the patients.

PRIORITIZATION OF CONTROL INITIATIVES FOR CHAGAS DISEASE IN LATIN AMERICA AND MEXICO AND IN NON-ENDEMIC COUNTRIES

The control initiatives for Chagas disease in endemic countries, created respectively for the Southern Cone in 1991, Andean Countries in 1997, Central America and Mexico in 1998 and the Amazon region in 2004, received a large boost early on, especially in the Southern Cone, where Uruguay, Chile and Brazil eliminated *T. infestans* and Paraguay and Argentina have been working towards this objective. In South America, the biggest current problems are located in Bolivia and in the Gran Chaco, belonging to Argentina, Bolivia and Paraguay, where the prevalence of Chagas infection is very high in some communities. Several countries in Central America are working towards the aim of eliminating *R. prolixus*. We see these as countries where control measures should be stimulated. Moreover, in Bolivia and Mexico, the problem is significant but the control measures are limited. On the other hand, the Andean countries and the Amazon Initiative need a greater boost.

Several meetings have been held in different countries to discuss the subject. In 2009, a meeting was held in Uberaba, State of Minas Gerais, Brazil[123]. At this meeting, the outcomes from several initiatives were weighed up, with a focus on the following challenges: I) stimulation to reestablish the political priority of controlling Chagas disease; II) reinforcement of the international coordination and evaluation of national programs through the initiatives; III) effective definition and implementation of quality standards for all the preventive actions, giving priority to vector control, blood banks, treatment of acute cases and care for chronic patients. In 2011, another meeting was held in Bogotá, Colombia and another meeting is scheduled to be held in Rio de Janeiro, during the 18th International Congress of Tropical Medicine and Malaria on September 23-27, 2012.

Finally, it is important to highlight the problem of Chagas disease in non-endemic countries, especially in Europe and in the United States[124][125]. Regarding the new initiatives, we suggest that in 2012 international congress, there should be discussion about the need to create a specific control initiative for Chagas disease in the Gran Chaco.

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