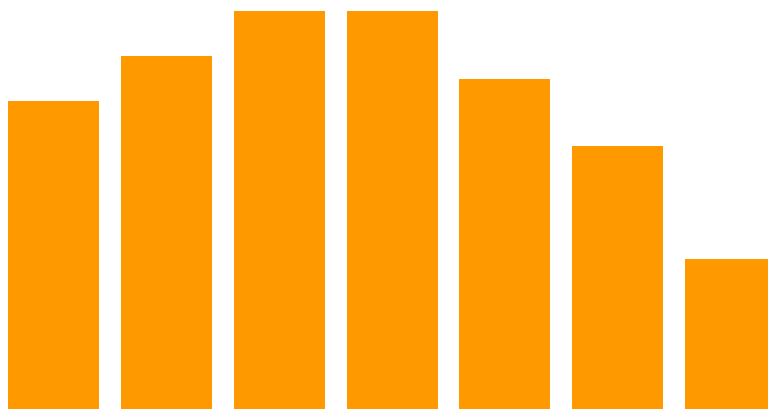# Chagas Disease

# Update on progress
# and future perspectives

# August 2006

José Antonio Ruiz
Unni Karunakara

Médecins sans Frontières
Campaign for Access to Essential Medicines

## Preface

This document is intended to provide an overview of American trypanosomiasis or Chagas' disease. It is meant for both medical and non-medical readers, and seeks to present a comprehensive view on both the current situation and future perspectives for the disease, focusing either on the issues where a global consensus does not exist or where an improvement of the situation is generally considered as needed.

The information presented here is *not* intended to replace or amend current national policies.

We hope that this document will facilitate efforts to guarantee future wider availability of Chagas' disease diagnostic and treatment, and stimulate MSF to continue or even increase its valuable work on one of the most neglected tropical diseases.

## Acknowledgements

## Abbreviations

| | |
|---|---|
| ACE | Angiotensin-converting enzyme |
| AIDS | Acquired Immune Deficiency Syndrome |
| API | Active Pharmaceutical Ingredient |
| BNP | Brain Natriuretic Peptide |
| CsA | Cyclosporin A |
| DHFR | Dihydrofolate reductase |
| DNDi | Drugs for Neglected Diseases initiative |
| ECG | Electrocardiogram |
| ELISA | Enzyme-linked immnunosorbent assay |
| FDA | Food and Drug administration |
| HGPRT | Hypoxanthine-guanine phosphoribosyl transferase |
| HIV | Human Immunodeficiency Virus |
| HVC | Hepatitis Virus C |
| IHA | Indirect Hemagglutination test |
| IIF | Indirect Immunofluorescent test |
| IOWH | Institute for One World Health |
| IRD | Institut de Recherche pour le Developement |
| MoH | Ministry of Health |
| MSF | Médecins Sans Frontières |
| PAHO | Pan American Health Organization |
| PCR | Polymerase chain reaction |
| PEG-PLA | Poly-ethyleneglycol-polylactide |
| TDR | Tropical Disease Research (WHO Special Programme) |
| UNDP | United Nations Development Programme |
| WHO | World Health Organization |

# Table of contents

# 1. Background

In 1909, the Brazilian doctor Carlos Chagas described the clinical picture of the human American trypanosomiasis, the causative parasite and the triatomine vector. Recent studies performed on human mummies from coastal and low valley sites in northern Chile and southern Peru show that the disease is as old as 9,000 years.[i]

## 1.1. Main features of American trypanosomiasis

American trypanosomiasis or Chagas disease is caused by a flagellate protozoan parasite, *Trypanosoma cruzi*, transmitted to humans by triatomine insects (reduviid bugs) known popularly in the different countries as *vinchuca*, *barbeiro* or *chipo*.

In most cases the disease has a chronic and silent course. After an asymptomatic period lasting several years, 10–40% of the patients will develop irreversible and serious lesions affecting internal organs namely the heart, oesophagus and colon and the peripheral nervous system. It causes some 50,000 deaths per year (mainly due to cardiomyopathy).

The risk of infection with Chagas disease is directly related to poverty – the blood-sucking triatomine bug which transmits the parasite finds a favourable habitat in crevices in the walls and roofs of poor houses in rural areas and in the peripheral urban slums. The rural/urban migration movements that occurred in Latin America in the 1970s and 1980s changed the traditional epidemiological pattern of Chagas disease and transformed it into an urban infection that can be transmitted by blood transfusion.

### 1.1.1. Geographical distribution

Chagas disease is endemic in 21 countries, mainly in Latin America, from Mexico to Argentina. A total of 16-18 million people are infected and 40-100 million, i.e. about 10-25% of the population of Latin America are at risk of acquiring Chagas disease. Two countries, Uruguay and Chile have been certified free of vectorial and transfusional transmission of the disease but new cases still occur due to congenital transmission.[ii]

Chagas disease is also found in the USA (an estimated of 100,000 people infected), Canada and Europe due to the large number of immigrants coming from endemic countries. For example, in Spain there are some 600,000 Latin-Americans.[iii] Preliminary results from a *T.cruzi* seroprevalence study carried out in 1,536 Latin-Americans blood donors in Madrid showed 0.8% seropositivity and in Barcelona the seroprevalence rate among 233 Latin-American pregnant women was 0.9%.[iv]

### 1.1.2. The parasite

The causative agent of Chagas disease, *Trypanosoma cruzi,* is an extracellular parasite that multiplies in vertebrates via intracellular stages.

It occurs characteristically in blood films as short "C" or "S" shaped forms with a prominent kinetoplast. These features allow an easy differentiation from *T.rangeli* in stained smears (Plate 1.1.), another species of trypanosome transmitted by reduviid bugs from wild animals to man that appears to be non-pathogenic to humans.[v]

*T.cruzi* strains show different patterns of virulence and tropism during the course of parasitaemia and as it invades different organs. *T.cruzi* strains also exhibit different degrees of sensitivity to chemotherapeutic agents. Two principal strains of *T.cruzi* have been identified: (1) clone 20 (*T.cruzi* I) and (2) clone 39 (*T.cruzi* II). The former mainly encountered in the sylvatic cycle, the latter strongly associated with the domestic cycle (humans).[vi]

A project to characterize the *T.cruzi* genome was launched by UNDP/World Bank/TDR in 1994. The whole-genome sequencing has recently been described[vii]. This may represent an important advance in the application of new technologies to disease prevention and control[viii]. Nevertheless, it must be stressed that much more research is needed to identify a drug or diagnosis targets (D. Steverding personal com.).

**Plate1.1.**

*Trypanosoma cruzi*, blood smear, stained, x1000
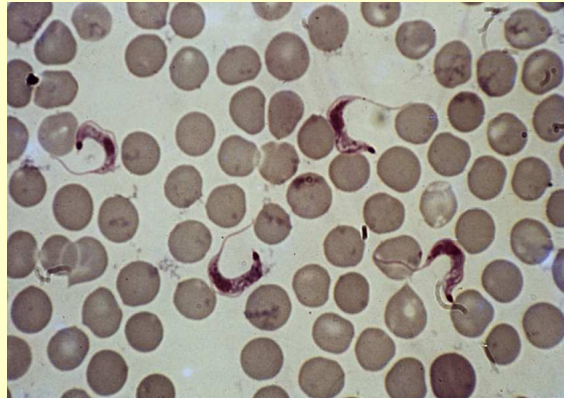


*Trypanosoma rangeli*, blood smear, stained, x1000
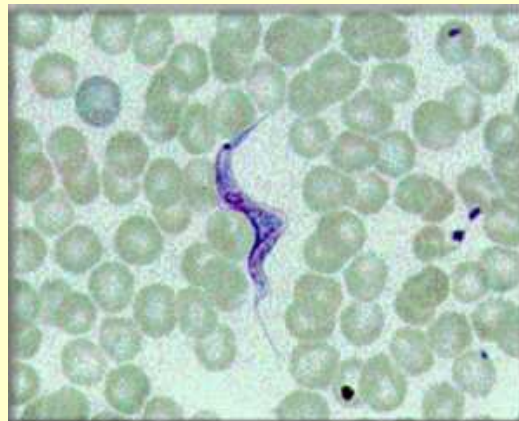


Source:

### 1.1.3. Transmission cycle

Chagas disease can be transmitted by different routes.

1.1.3.1.Vector Transmission

Transmission by insects represents 80-90% of total cases. The vector species epidemiologically linked to Chagas disease are those that have adapted to the human environment: *Rhodnius prolixus* (Central America), *Triatoma dimidiata* (Central America), *T.infestans* (South America), *Panstrongylus megistus* (South America) and *T.brasiliensis* (Brazil) (Plate 1.2.).

Triatomines may feed on humans and domestic animals (domestic cycle) or wild animals (sylvatic cycle). Overall feeding rates in the domestic cycle are 50-91% for humans, 46-81% for dogs and 6-15% for chickens. Humans are the most important domestic reservoir of *T.cruzi* but dogs may be involved in the parasite maintenance as it has been observed in north-eastern Brazil[ix].

**Plate 1.2. Vectors**

*Triatoma infestans*



*Triatoma dimidata*



*Rhodnius prolixus*



Source:

More than 180 species or subspecies of wild mammals have been found to be infected with *T.cruzi*[x]. The most important among these mammals are the opossum (a marsupial), the armadillo (an edentate) and the agouti (a rodent) (see iconography).

The *T.cruzi* life cycle between triatomines and humans is as follows: Metacyclic trypanosomes of *T.cruzi* passed in the faeces of infected triatomine bugs penetrate the skin (due to scratching) or mucous membranes to reach the blood. The parasites enter various muscular tissues (e.g. cardiac, gut, skeletal). Here they transform to amastigotes which divide, producing pseudocysts. Intracellular amastigotes transform into trypomastigotes, then burst out of the cell and enter the bloodstream. Triatomine bug takes a blood meal

and trypomastigotes are ingested and multiply in the midgut (epimastigotes). After 30 days some epimastigotes pass to the hindgut where they transform back to infective metacyclic trypomastigotes (Plate 1.4.).[xi]

**Plate 1.4. Life Cycle of T.cruzi**



Source: http://www.dpd.cdc.gov/dpdx/HTML/TrypanosomiasisAmerican.htm
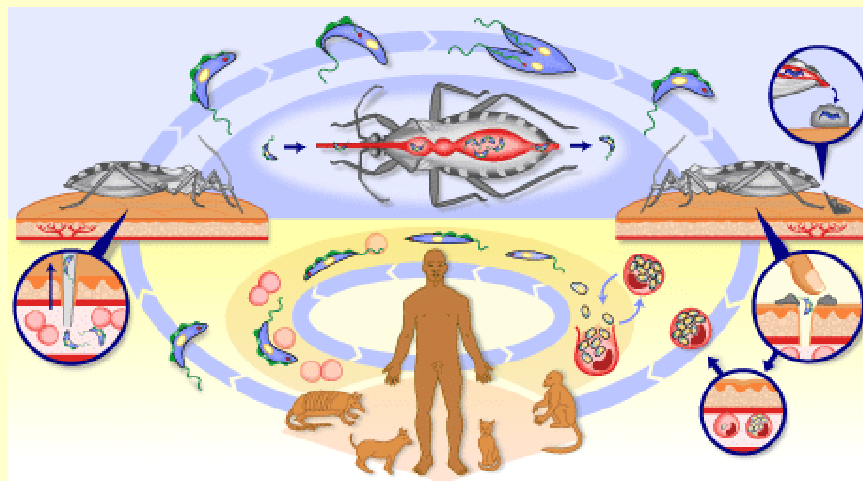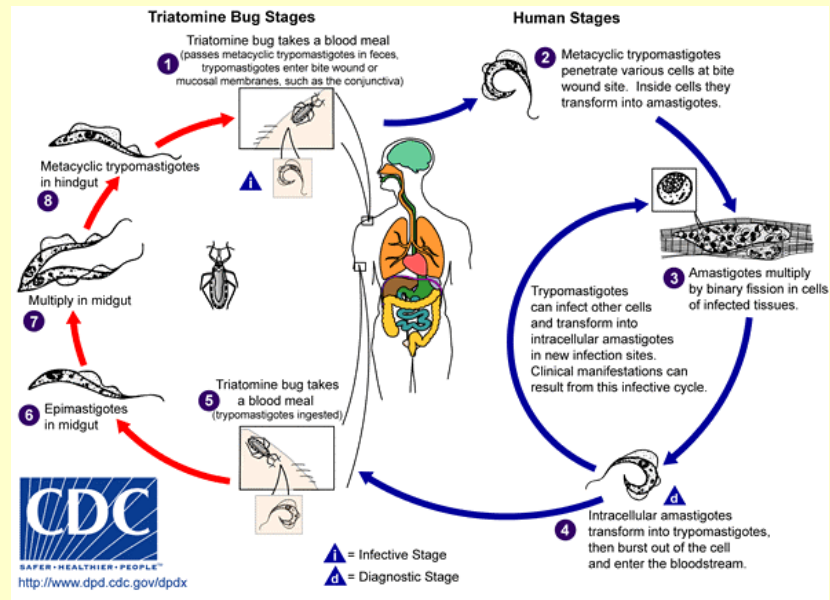
### 1.1.3.2. Blood Transfusion

Transmission by infected blood transfusion represents 5-20% of the total cases. The parasite can be transmitted by means of all blood components and hemoderivatives. *T.cruzi* may survive up to 18 days at 4°C and resists cryopreservation and defrosting. The potential impact of transfusion-transmitted infection of *T.cruzi* is 20%. For *T.cruzi*, the probability that a person may donate blood during the window period (time elapsed between the moment of the infection and the moment when a serological test may show positive) is remote because most infections occur during childhood or adolescence.

The first report predicting transfusion linked infection of *T.cruzi* was made by Pellegrino in 1949.

The WHO recommended only one test, an ELISA assumed to have 99% sensitivity, for blood bank screening in 2002 but no single test has been shown to be sensitive enough to prevent the transmission of *T.cruzi*. Moreover, efficacy estimates for serological tests obtained under optimal conditions (study) may greatly differ from field conditions. An international Latin American performance evaluation program on serological testing from 1997 to 2000 showed false-negative results in 3.22% of 527 samples for *T.cruzi*. A study carried out in a hyperendemic region in Bolivia indicates that blood screening (transfusion) with two tests should be mandatory in settings with high to moderate prevalence of *T.cruzi* infection to avoid false-negative samples. Adding a parallel second test is barely more expensive.[xii]

Nowadays, all Latin American countries have enacted laws that make screening for Chagas infection mandatory on blood donated samples. These laws, decrees, norms and/or regulations appeared in different countries from 1960s till 1990s first because of concerns about transmission of infectious disease such as syphilis and Chagas' disease and later on by worries about hepatitis and HIV. Despite this policy, most countries do not have a well-trained group of inspectors.

A recent review on the status of blood safety in the 17 Latin American continental countries shows that screening coverage in blood banks for Chagas disease may be as low as 25% (Costa Rica), 27% (Mexico) or 34% (Panama). Only 7 countries (Argentina, Brazil, Ecuador, El Salvador, Honduras, Uruguay and Venezuela) reported 100% of donors screened for *T.cruzi*.[xiii]

The number of blood banks also varies enormously between countries. It may be as low as 27 in Honduras or as high as 578 in Argentina, 524 in Mexico and 2,583 in Brazil. By contrast, the number of blood banks in Canada is 14. Larger blood banks are more efficient but efforts to decrease the number of blood banks have generally failed. Bolivia has succeeded in decreasing the number of blood banks from 155 in 2000 to 19 in 2005.

In general, the mean number of blood units collected yearly by Latin American blood banks is around 2,000 and the number of blood donors in 2002 was 6,594,757 but still this does not meet the standards of 50 blood units per 1,000 inhabitants per year (range 6-30 blood units).

On the other hand volunteer donors, who are known to be more healthy than paid or replacement (relatives or friends) donors, represented only 21% (range 2-56%) of the total blood donors in Latin American in 2002.

Moreover, incidents and adverse events related to the administration of blood are not officially reported. Therefore, the potentially negative impact of blood transfusions is not known.

The seroprevalence of *T.cruzi* in blood donors ranged from 0.24% (Mexico) to 10% (Bolivia) in 2002 as reported by Latin American blood banks. Based on data for 2000, it can be estimated that 1,265 cases of *T.cruzi* infection were transmitted by blood transfusion in Mexico.

In 2002, the risk of receiving a *T.cruzi* tainted transfusion unit was estimated from 0.36/10,000 donors in Nicaragua to 138/10,000 donors in Bolivia. Ten years ago, the risk in Bolivia was as high as 1,096 per 10,000 donors.

A recent study in blood donors from Mexico city has shown a *T.cruzi* infection rate of 0.6% (58/9457) in 2003, this being the leading infection when compared with HIV (0.04%) or HVC (0.16%). 29 *T.cruzi* seropositive individuals were further studied and 17% presented an abnormal ECG and 41% a positive parasitological result (PCR-*T.cruzi*).[xiv]

In non-endemic countries in North America or Europe, only Canada, Ireland, France and Spain question blood donors for risk of *T.cruzi* infection and only Spain is considering screening immigrants from Latin America. In the USA, all donors are expected to be screened when the FDA has licensed a screening test.[xv]

It must be pointed out that the French Guyana is geographically located in South America but depends on French legislation. A survey carried out on 1,487 individuals to assess the seroprevalence of *T.cruzi* infection in French Guyana has found 0.5% prevalence. This may

have implications for public health since blood donors are not routinely screened for *T.cruzi* infection in this territory.[xvi]

**Immunosensor**, a technique already used for allergies and *P.falciparum* malaria, has been recently tested for Chagas' disease to facilitate screening in blood banks.[xvii] Preliminary results show that the method is simple, sensitive and quite easy to repeat whenever results are difficult to interpret. For routine analysis, the methodology should be standardized and a detailed comparison of advantages or disadvantages of the electrode method over ELISA will be pursued in the near future. This method may prove important not only for the diagnosis of the presence of antibodies against *T.cruzi* in blood donors, but also to follow antibody decay after treatment of patients.

1.1.3.2. Congenital transmission

It represents 2-10% of the total cases. The risk of acquiring the disease from an infected mother is about 5-6%. It must be kept in mind that second generation congenital transmission is also possible.[xviii]

This route of transmission is of importance in non-endemic countries or in areas where vectorial and transfusional transmission has been interrupted. A seroprevalence study of antibodies to *T.cruzi* in Houston (USA) showed 0.4% prevalence in 2,107 Hispanic pregnant women.[xix]

1.1.3.4. Others

Transmission by organ transplantation (most frequently kidney), accidents (laboratories) and food has also been notified.

A recent outbreak occurred in Santa Catarina (Brazil) due to sugar cane ingestion in February 2005. A total of 25 cases and 3 deaths were registered.[xx]

## 1.2. Clinical features

After the initial period of incubation (on average one week long), Chagas disease goes through two phases:

### 1.2.1. Acute phase

Generally asymptomatic and therefore difficult to diagnose. Only 1-2% of the acute cases are diagnosed. The disease can occur at any age; however, most of the cases are diagnosed before the age of 15.

There may be fever, shivering, headache, anorexia, malaise, lymphadenopathy, mild hepatosplenomegaly and myocarditis. In one out of six newborns diagnosed in Argentina 65% were asymptomatic, 28% presented with hepatosplenomegaly and 11% with hepatitis.[xxi] Local lesions may occur and help to identify the disease: chagoma (an area of red, hardened skin, with a high local temperature) may appear on any part of the body, and lasts around 2-3 months. Romaña's sign may also appear – a painless swelling of the upper and lower eyelids on one eye only - it is accompanied by reddened conjunctiva and moderate oedema of the affected side of the face (see iconography). Death sometimes occurs during this 4-8 week phase, especially in children (usually younger than 2 years old) and young adults.

### 1.2.2. Chronic phase

This can be divided into two forms – the *indeterminate or latent* asymptomatic form and the *chronic* symptomatic form. Around 70% of *T.cruzi* infected people will remain in the indeterminate form their entire life. The infection may be detected through serological or parasitological analyses. The remaining 30% will develop symptoms 10-20 years after *T.cruzi* infection. For these people, average life expectancy is reduced by nine years. Clinical manifestations of the chronic phase are:

- Cardiopathies: in approximately 27% of cases; the most severe manifestations are arrhythmias, bundle branch block, cardiomegaly and heart failure. Chagas disease has not been associated with arterial hypertension.[xxii]

- Digestive tract pathologies (achalasia, megaesophagus and megacolon): in 6% of cases, depending on the geographical area surveyed (mainly observed south of the Equator). They may or may not be associated with cardiac manifestations.

- Autonomous, peripheral and central nervous system (meningoencephalitis) irregularities: in approximately 3% of cases, mainly in immunodepressed patients. Neurological manifestations occur in 75% of patients with both Chagas' disease and AIDS.[xxiii] In 2004, Brazil included Chagas disease reactivation as a condition of AIDS case definition.[xxiv] Neurological lesions must be evaluated carefully, because patients may be misdiagnosed and treated as carriers of 'idiopathic' diseases.[xxv]

- Other: Night blindness can also occur in Chagas' disease. A study conducted on 45 patients with heart problems found 82% presenting trouble seeing with at least one eye. Antibodies geared to attack *T.cruzi* also block rhodopsin in the animal model. Rhodopsin and beta1-adrenergic receptors in heart cells belong to the same class of molecules.[xxvi]

**WAITING FOR Pere's presentation Washington analyses data MSF**

It is noteworthy that in areas **where vectorial transmission has been interrupted clinicians have observed less severe chronic symptomatic forms**. This shows the importance that reinfection may play on the severity of the disease. On the other hand, people infected and treated for another species of trypanosoma (*T.b.gambiense*) have been found to develop immunity against the metacyclic parasite forms and as a consequence their possibility of being reinfected is lower than the general population.[xxvii] Whether a similar situation can be observed for *T.cruzi* infections remains unknown but the fact that this parasite lives only a short period of time extracellularly in the blood may hamper that immunological reaction.

## 1.3. Diagnosis

Good diagnostic methods are necessary to: (1) determine the cause of the disease (very important in congenital cases), (2) evaluate therapeutic efficacy, (3) prevent transmission through blood transfusion (screening) and (4) assess prevalence in the community.

### 1.3.1. Etiologic diagnosis

In order to understand the different methods used and the variability of the results obtained, several concepts must be considered:

- High parasitaemia occurs in the acute phase of the disease. Detection of parasites in the blood is therefore easier during the incubation period of 10-15 days but they may be detected up to 4 months later.

- Parasitaemia depends partly on the age of the patient: children and adults older than 50 years present with detectable parasitaemia. The vast majority of patients between 20 and 50 years old (the majority of those who visit the doctor) present with low parasitaemia.

- In the chronic phase, parasite is hardly detectable in the bloodstream.

The methods for diagnosing the disease are:

**(a) Parasitological methods** (determining the causal agent)

Traditional parasitological tests are especially useful during the parasitaemic stages of infection (acute phase), as they can detect the parasite or its parts. However, these tests are usually laborious or have a low degree of sensitivity.

Direct parasitological methods include:

- **Fresh blood identification of *T.cruzi*:** a drop of fresh blood is placed on a slide and mixed with a drop of isotonic saline solution. An optical microscope is then used to look for trypomastigotes among the blood cells.

- **Thick blood smear identification of *T.cruzi*:** the preparation is stained with Giemsa colouring and searched for presence or absence of characteristic trypomastigotes. It allows differentiation between *T.cruzi* and *T.rangeli*.

- **Strout Method** (identification of *T.cruzi* concentration in serum): after centrifugation, decanting and elimination of the sediment, remaining cells are examined under an optical microscope to identify parasites.

- **Microheamatocrit centrifugation technique:** 4-6 capillary tubes filled in with blood are centrifuged at 12,000 rpm for 3-5 minutes. The plasma sitting just above the buffy coat layer is examined under x10 or x20 for motile trypanosomes. This technique does not allow differentiating *T.cruzi* from *T.rangeli*. Therefore, in areas where both species are present it is convenient to perform a stained blood smear if trypanosomes are observed. Note that the sensitivity of this technique decreases with low parasitaemia.

Indirect methods, although sensitive in acute cases they are mainly used for confirmation of chronic cases but this will depend on availability (e.g. PCR):

- **Xenodiagnosis** (inoculation of human blood into laboratory animals): Forty non-infected reduviid bugs suck the patient's blood through a membrane for thirty minutes. The intestinal contents are examined four weeks later (in case of negative results, examination is done again at 45 and 60 days). A modification of this technique - artificial xenodiagnosis - consists of collecting 10 mL blood from the patient and then the bugs feed on collected blood in a special container (see iconography).

  - *Advantages*: xenodiagnosis is considered a good technique for diagnosing the cause of the disease, selecting patients for treatment, and later, evaluation.

  - *Disadvantages*: requires a time lapse (of up to 4 months), only available in research institutes, not very sensitive (detection of 20-50% of people known to be infected) so it produces false negative results.

- **Haemoculture:** isolation of the causal agent by inoculation in mice, and culture in special media (e.g. NNN, LIT). Sensitivity of blood culture is similar to that of natural xenodiagnosis. The advantage is the possibility of working with larger volumes of blood, which increases the sensitivity. Only available in specialized laboratories.

- **PCR:** This technique relies on the amplification of parasite DNA. Its sensitivity is 1 parasite / 20mL.

  - *Advantages*: it is more sensitive than other parasitological tests. Good technique to assess treatment failure after specific chemotherapy[xxviii].

  - *Disadvantages*: need of specialized laboratories and expensive to perform.

**(b) Serological methods**

During the chronic phase of the disease, parasitemia is reduced to levels undetectable by parasitological methods. During this phase the main method of diagnosis is therefore to detect IgG antibodies against *T.cruzi* in patients' blood using serological methods. In the chronic phase serology is positive in nearly 100% of the untreated cases. Serology can also be used in acute cases to detect IgM. IgM tests are however, not available commercially.

Serological methods are widely used, but it is difficult to standardise a universal reference test for the diagnosis of *T.cruzi* infection. The main reasons are a marked difference in antibody response during different phases of the disease, antigenic complexity of *T.cruzi*, and the variability of evolutionary forms and distribution of different *T.cruzi* lineages in different geographical areas. Field research carried out in different areas also revealed differences in patients' immune response and the degree of clinical manifestations in the chronic phase of the disease.

To conclude that the parasite is present, simultaneous positive result from at least two serological tests are required.

Most antigen extracts used in tests are from the epimastigote (non-infective) form, composed of complex molecules which can cause false positive reactions. They may also cross-react with other diseases, mainly leishmaniasis which often occurs in the same geographical areas as Chagas' so in areas where leishmaniasis is present, caution is therefore recommended when interpreting blood test results. *T. rangeli*, even it is non pathogenic to humans may also interfere serological results[xxix].

The commonest tests used are: Indirect haemagglutination (IHA), Indirect Immunofluorescence (IIF), and the Enzyme-linked immunosorbent assay (ELISA). This three techniques known as "conventional" allow quantification of the antibodies titre, thus may be used for follow-up (table 1).

With the IHA test results are fast (< 2 h), no sophisticated equipment or specialized technical skills are needed and titres are easier to read than ELISA. The sensitivity is in the range of 96-98%, which is lower than that obtained with IIF and ELISA and the test fails to detect 1.6-2.5% of infected individuals (false negatives). The reliability of the result depends on the expertise of the technician and labour required is intensive. The test needs a cold chain. It is commercialized at US$ 0.81/test.

With the IIF test results are relatively fast (2h) but several steps are required such as titration of conjugates. It needs highly trained personnel and expensive equipment (UV light microscope) but once the laboratory is set up then is cheap to operate. The sensitivity is high (99%) but the specificity is lower than IHA. The immunofluorescence is atypical in *T.cruzi* I. This technique is suitable for small laboratories with a low volume of samples to be tested per day (50-100). It needs cold chain.

**Table 1. Main attributes of some serological tests for Chagas disease.**

| | IHA | IIF | ELISA |
|---|---|---|---|
| **Company** | Wiener lab (Chagatest HAI) | Biomanguinhos/FIOCRUZ | Abott laboratorios do Brasil |
| **Time to perform** | 60-90 minutes | 2 hours | 1 - 2 hours |
| **Specialized technical skills/ sophisticated equipment** | No, but reading is subjective | Yes<br>Reading: subjective<br>several steps to perform<br>need of special UV light microscope<br>37ºC stove | Yes<br>Reading: objective (spectrophotometer)<br>It can be automated |
| **Sensitivity** | 96-98% | 99% | 99-100% |
| **Specificity** | 98.8-99% | lower than IHA | 99.6-100% |
| **Commercial test** | Yes | No | Yes |
| **Cost/test** | US$ 0.69 – 0.81 | ? | US$ 1.24-3.51 |
| **Disadvantages** | - Needs cold chain<br>- Patient must fast<br>- false negatives | - Needs cold chain | - No cross reactions tested<br>- Needs cold chain (-20°C for samples, 2-8°C for reagents) |
| | **EIE-Recombinant-Chagas** | **ID-PaGIA** | **TESA-blot** |
| **Company** | Biomanguinhos/FIOCRUZ | DiaMed Brazil, Switzerland | Inst. Med Trop (Sao Paulo) |
| **Time to perform** | 2 hours | 20 minutes | |
| **Specialized technical skills/ sophisticated equipment** | Yes<br>Microplate reader<br>37ºC stove | No<br>It is easy to read (visual) | Yes |

| | | | |
|---|---|---|---|
| Sensitivity | 100% (96.4-100%) | 96.8% (95.7 - 97.4%) | 100% |
| Specificity | 100% (98-100%) | 94% (93.8 – 98.8%) | 100% |
| Commercial test | Yes | ? | No |
| Cost/test | US$ 2 | ? | - |
| Disadvantages | - Needs fridge/freezer (8°C/ -20°C) <br> - *T.rangeli*??? | | |

| | Chagas STAT-PAK | ELISA recombinant v. 3.0 | BIOELISACRUZI |
|---|---|---|---|
| Company | Chembio diagnostic systems, Inc. | Wiener lab | Biolab-Mérieux |
| Time to perform | 15 minutes | 2 hours | 1-2 hours |
| Specialized technical skills/ sophisticated equipment | No | Yes <br> 37°C incubator <br> Spectrophotometer for microtitration plates (optional) | |
| Sensitivity | 98.6-100% | 100% | 98.6% |
| Specificity | 90.5-100% | 97.1-100% | 99.8% |
| Commercial test | Yes | Yes | Yes |
| Cost/test | US$ 1.55 – 3 | US$ 1.40 | US$ 1.59 |
| Disadvantages | - Not differentiates acute and chronic <br> - Only qualitative | Reagents need cold chain | - 25.6% cross reactivity with Leishmania??? <br> - Needs cold chain |

The ELISA technique needs a skilled technician and sophisticated equipment (spectrophotometer), however this has the advantage of avoiding subjectivity when reading the results. The sensitivity is excellent (100%) and specificity is good (99.6-100%). It can be used in large centres for the simultaneous screening of many samples. It provides quantitative and qualitative results that allow follow-up of treatment (paired samples at 12 and 24 months).

Blood sample collection on filter paper can also be done for diagnosis but it is not widely accepted for follow-up yet.

The ideal serological test, which is easy to perform in a single step, fast, cheap, requires no special equipment or refrigeration of reagents, with a sensitivity and specificity of 100%, does not exist (table 1).

With the aim of increasing the specificity of the serological diagnosis and avoiding cross-reactivity with other parasitic diseases, "non-conventional" serology tests based on ELISA techniques but using recombinant proteins, purified antigens, or synthetic peptides as reagents have been developed, some of them (JL8 + MAP) with preliminary results showing both sensitivity and specificity higher than 99%[xxx]. A general advantage of such techniques is their simplicity (one step) and usually the short time required to perform and high specificity. With high specificity, ELISA tests with recombinant proteins are often used as a tie-breaker in multi-test algorithms. The general disadvantage is that their sensitivity might be lower than that of conventional serology and some of the available test kits (that use strips or bears) give only qualitative results.

More recently the **iron superoxide dismutase**, a molecular marker related to the establishment of *T.cruzi* within the host showing high immunogenicity and specificity, has been tested using a Western blot technique compared to the three conventional serological methods with promising results[xxxi].

Some of the existing tests are:

**EIE-Recombinant-Chagas**[xxxii]**:** this test is a direct ELISA which uses two recombinant antigens: CRA and FRA. It has several important advantages:

- Absence of false positive reactions, due to the use of recombinant antigens specific to *T.cruzi*. The test did not produce cross-reactions with the serum of patients infected with cutaneous or visceral leishmaniasis.

- Direct ELISA increases method sensitivity, enabling low-value serum evaluation;

- Handling errors are minimised as undiluted serum samples are used;

- Time required is relatively short (two hours); inexpensive and reliable.

**ID-PaGIA Chagas test:**[xxxiii] based on haemagglutination reactions; the red blood cells are separated in the matrix gel and replaced by polymerous particles containing the target antigen. When these particles are mixed with serum that contains antibodies, they agglutinate. After centrifugation, it is possible to separate the non-agglutinated free particles, which remain in the bottom of the tube, from the agglutinated ones, which are distributed throughout the gel. It can detect acute and chronic infection.

**TESA-blot:**[xxxiv] It is a Western blotting method developed nine years ago and recently tested in inconclusive sera (i.e. discordant results between two tests or doubtful results in at least one test) from blood banks[xxxv]. It uses native fractions of the infective trypomastigote as an antigen including trypomastigote excreted-secreted antigens (TESA), SAPA (Shed Acute Phase Antigen) antigen components and 150-160-kDa protein. It distinguishes acute (IgM) from chronic (IgG) infection.

This test is proposed as confirmatory. It is not yet commercialized and BioMérieux (Brazil) lost interest in doing so due to lack of financial benefit (E. Umezawa, personal com.). TESA-ELISA is appropriate for assaying large number of samples, like in a blood bank.

**Chagas STAT-PAK:**[xxxvi,xxxvii] A quick test based on an immunochromatographic method to detect antibodies against *T.cruzi* in serum, plasma or blood. Recommended for screening. Easy to perform. It requires 10μL of capillary blood extracted using a micropipette (a micropipette costs US$ 200). It is a test adapted to poor health facilities (health posts). Despite its simplicity training must be organized to ensure a correct use (e.g. horizontal position of the device).

Several studies have been carried out yielding a range of different results (table 2).

| Table 2. Studies done on STAT-PAK. | | | | | |
|---|---|---|---|---|---|
| **Author** | **Year** | **n** | **Sample** | **Sp (%)** | **Se (%)** |
| Luquetti et. al.[35] | 2003 | 393 | Serum (coded samples) | 98.5 | 94.8 |
| | | 352 | Serum | 100 | 98.6 |
| | | | Plasma, FP eluates, serum in 50% glycerol | 100 | 98.6 |
| Ponce et. al.[36] | 2005 | 5,998 | Serum | 99.6 | 99.9 |
| IRD & MSF | 2004 | 163 | Serum | 98.2 | 100 |
| IRD & MSF | 2004 | 112 | Whole blood | 90.5 | 100 |
| MSF | 2005 | 549 | Whole blood | 94.9 | 100 |
| Sp: Specificity    Se: Sensitivity | | | | | |

### 1.3.2. Diagnosis of other conditions

Diagnosis of mainly cardiac and digestive abnormalities related to Chagas' disease is crucial. Even if these conditions will not appear in all infected individuals, the fact that they may cause serious problems, including death, and the possibility of managing them with the appropriate treatment make their diagnostic mandatory. Some of the diseases, as

achalasia (a disorder presenting an inadequate relaxation of the lower esophageal sphincter), may be rare worldwide (incidence of 0.03-1.1 cases/100.000 individuals) but in Brazil Chagas' disease is responsible for up to 90% of achalasia cases. The non-specificity of the associated symptoms (dysphagia, regurgitation, weight loss, chest pain or cough) may be responsible for the delay in the diagnosis for more than ten years.

For the clinician to be able to perform his duty some recommendations can be made according to the facility where he is based[xxxviii]. There is a need for a good quality referral system between the different levels.

- First level (health centre): Anamnesis, clinical examination, X-ray and ECG. This level should be able to perform etiological and syndromic diagnosis.

- Second level (hospitals): Specialists will carry out more specific tests for arrhythmias and severe cardiac congestive failure (Holter, echocardiography, ergometry, endoscopies, etc.).

- Third level (hospitals): In charge of most serious cases and surgery.

## 1.4. Treatment

In Chagas' disease both the etiological and syndromic treatment are possible and deserve special attention.

### 1.4.1. Syndromic treatment

Cardiac arrhythmias and cardiac failure can and should be treated. At first they can be managed medically with amiodarone (not included in the 14th WHO model list 2005 but will be considered at the next meeting of the expert committee), digoxin, hydrochlorothiazide, etc. More advanced stages may need pacemaker or heart transplantation.

The availability of cardiac treatment is most related to the financial possibilities of the patients. It must be stressed that the treatment is life-saving and many patients will not be able to afford such expenditures. In that sense it is very important the Brazilian experience where a "basic package" of drugs has been proposed for the management of this cases at all health levels (table 3).[xxxix] (**FOR FREE - LOW COST??**) **WAITING FOR THE PAPER**

**Table 3. Drugs included in the "basic package" for cardiac Chagasic symptoms in Brazil.**

| Group | Drugs |
|---|---|
| Diuretics | Hydrochlorothiziade, furosemide, spironolactone |
| Antiarrhythmic | Amiodarone, digoxine, |
| ACE inhibitors | Enalapril, captopril |
| Beta-blockers | Carvedilol |
| Anticoagulants | Warfarin |
| Antithrombotic | Acetylsalicylic acid |
| Antianginal | Isosorbide dinitrate |
| Antihypertensive | Hydralazine, losartan |

Digestive manifestations of Chagas' disease also deserve specific treatment and in many cases they are associated with cardiopathy, thus indicating a high risk for surgical approach to the treatment of chagasic megaesophagus and megacolon mainly in the geriatric population[xl]. Oesophageal achalasia may be treated by means of pneumatic dilatation which is cheaper than surgery, $US 968 and $US 1144 (in 1997) respectively, but with similar results (67-95%), morbidity (2-9.5%) and mortality (0.7-1%). Chagas' disease is not a predictor of the dilatation outcome, the only significant predictor of a good result is a low oesophageal sphincter pressure post-dilatation (<10 mmHg). It must be pointed out that **no achalasia treatment cures the disease.**[xli]

### 1.4.2. Etiologic treatment

The two drugs currently available for clinical use in Chagas' disease, nifurtimox and benznidazole, are included in the WHO's 14th Essential Drugs list (March 2005). Despite that, their availability in the endemic countries is at present very difficult and a matter of major concern for NGOs, National Programs (MoH) and PAHO/WHO.

There is consensus about the benefit of treating acute cases, regardless of the transmission mode and the clinical manifestations[xlii]. However, there is still a debate about the benefit of treating chronic or indeterminate cases despite recommendations made by PAHO/WHO in 1998 to treat them. Recent studies based on PCR, histopathology and clinical features show the relevance of parasite persistence in the progression of chronic Chagas heart disease[xliii]. Parasitological treatment shows 60-80%[xliv] cure rates in acute phase but only 10 – 20% in late chronic phase. The disease treated in newborns is almost 100% curable.

Some considerations are important to bear in mind with regard to the efficacy or the need for an etiological treatment in the chronic cases: The evidence accumulated so far is encouraging, but still far from being conclusive. There is no data on the clinical efficacy in patients included in five clinical, randomized and controlled trials carried out up to now[xlv]. The fact that the progress of the disease is very slow makes difficult to evaluate the response to the therapy. In a data meta-analysis from more than 2,000 adults in studies published up to 2004, a 45% reduction in mortality was observed after 4 years in groups that received trypanocidal treatment.[xlvi] More recently, the follow-up of one of those cohorts has shown that those treated with benznidazole had a 60% reduction on the "clinical progress" of the disease[xlvii]. Even though these data can be considered interesting and promising there are several points to be discussed. Firstly, these studies compared selected treated individuals to not-treated individuals, and therefore, there is no certainty as to the group's prognosis being the same at the beginning of the treatment. The study should be randomized and longitudinal. Secondly, the results of the analysis from the five mentioned studies refer to all-cause of mortality, not only to cardiac mortality, which means that besides the problem of selection bias on the treated population, there may be many other reasons to explain the results, which are also unknown. Thirdly, the results from the first analysis are not statistically significant. Even though Viotti *et al*. obtained statistically significant results, those are not consistent with the results from other studies (including some that found a higher relative mortality on treated individuals), suggesting that the results can not be generalized. Finally, the lost of patients during the follow-up in several studies is high and it is even higher than the events themselves, which weakens the conclusions that can be obtained from that. In conclusion, the question about the efficacy of the etiologic treatment in chronic cases deserves further studies. (JC Villar, personal com.).

Different mode of actions apply for benznidazole (covalent bond to parasite components as DNA, RNA and proteins) and nifurtimox (nitroanion radicals causing oxidative damage)[xlviii] but they have not been used in combination nor after a treatment failure with one of them.

### 1.4.3. Benznidazole

- International non-propietary name: benznidazole
- Date of discovery: 1974
- Commericial names: Rochagan ®, Radanil®, Rodanil®,Ragonil®
- Produced by: F Hoffmann-La Roche, Brazil
- Pharmaceutical form and presentation: 100mg split tablets; 100 unit packs
- Active compound: N-benzyl-2-nitro-1-imidazo-lactemide
- Shelf life: 5 years after manufacturing date
- Therapeutic action: trypanocide active against *Trypanosoma cruzi*

It is recommended for the treatment of *T.cruzi* acute infection occurring at any age and for the late chronic phase (>10 years) the treatment must be elective. Endemic countries have different treatment protocols to determine the age group to be systematically treated. The

estimated need for benznidazole is some 1.5 million tablets/year taking only into account data from 14 out of 21 endemic countries and treatment in children (< 5 years).

It is better tolerated in children that in adults. It must be pointed out that there are no paediatric formulations in the market despite the fact that majority of patients treated are children. In Bolivia, 874 children aged less than 5 years old were recently treated with benznidazole. The treatment was discontinued only in 0.34% (3/874) due to severe side effects (allergy).[xlix]

Roche decided to transfer technology to manufacture Benznidazole in March 2002. In April 2003, Roche transferred the license and rights to the drug to Brazil's Acre state. This was followed by an agreement between Roche and Acre state to subcontract manufacturing and marketing to a public Brazilian agency—Laboratorio Farmaceutico Do Estado de Pernambuco (LAFEPE)—in July 2003. A private Brazilian company (LABOGEN) was also identified for the production of API. This arrangement fall through as LABOGEN files for bankruptcy.

In August 2004, LAFEPE received enough API from Roche to produce three batches of benznidazole, needed for registration of the drug at ANVISA (national health surveillance agency of Brazil). In March 2006, LAFEPE submitted the benznidazole dossier to ANVISA and registration is expected in November 2006.

A private Brazilian company Nortec Química (?) has now been identified to produce the API necessary for the production of Benznidzole. To bridge any shortfall in API, Roche produced a stock of API (1,500 kg.) in June 2005 and has signed an agreement between Acre state and LAFEPE for the provision of a maximum of 1,500 kg. and a minimum of 500 kg. of API. For 2007, LAFEPE is requesting Roche for 750 kg. (enough for 7 million tablets) of API for the production of the first batch of tablets. Nortec Química is expected to provide API once the Roche stock is exhausted.

Under the technology transfer agreement, Acre state will own the license for benznidazole and LAFEPE will assume liability and be the sole producer of the drug. While LAFEPE will market the drug in Brazil, Roche Argentina will distribute benznidazole, under its generic name, throughout Latin America.(J Jannin, personal communication)

MSF and the Drugs for Neglected Diseases Initiative (DNDi) have been advocating for the production of a paediatric formulation as the current formulation is not ideal for children. Though LAFEPE has expressed interest, they are not ready to cover development costs.

While progress, albeit with delays, has taken place in the technology transfer process, real concerns exist about the availability and the price of drugs. For instance, an order for 200,000 tablets placed by MSF in October 2005 took four months to fill. Roche eventually delivered 50,000 tablets citing problems with the API. With a new manufacturer, it is expected that the registration process in several countries may delay drug availability in many countries. Another problem related to access is the huge difference among prices varying from 6.47 US$ to 172 US$/100 tablets, in Brazil (MSF) and Bolivia (private sector), respectively.[l]

PAHO/WHO dosage recommendations are[li]:

Acute, intermediate and chronic stage treatment
- Adults – 7.5mg/kg/day for 30-60days if weight <40kg and 5mg/kg/day for 30-60 days if weight >40kg.
- Children – up to 10mg/kg/day for 60 days

Late chronic phase treatment
- 5mg/kg/day for 60 days

Congenital infection treatment
- 10mg/kg/day. In low-weight cases, initial dosage should be halved. If there is no evidence of leucopenia or platelet reduction after 72 hours then use 10mg/kg/day.

Contraindications
- Early pregnancy

### 1.4.4. Nifurtimox

- International non-propietary name: nifurtimox
- Date of discovery: 1960 (by veterinary research)
- Commericial names: Lampit ®
- Produced by: Bayer AG, Germany
- Pharmaceutical form and presentation: 30mg, 120mg, 250mg; 100 unit packs
- Active compound: 5-nitrofurane
- Shelf life: 5 years after manufacturing date
- Therapeutic action: trypanocide active against *Trypanosoma cruzi*

Nifurtimox is currently registered in several Latin American countries for oral use in the treatment of Chagas disease. It has been in use since the 1970s and was approved for use since 1984. Nifurtimox was included in WHO's 11th Essential Drug List, one of only thirteen drugs for tropical diseases approved between 1975 and 1999.

Citing lack of market, Bayer discontinued the production of nifurtimox in 1977. As a result, most countries shifted to benznidazole, the only drug in the market for Chagas disease, as their drug-of-choice. Following public pressure, Bayer re-started production of nifurtimox, mainly for use in sleeping sickness.

With nifurtimox now available for use in a disease (sleeping sickness) that it is not registered for, stocks were running dangerously low for the treatment of Chagas disease. After long negotiations, Bayer and the WHO signed an agreement in July 2004 for a donation of 250,000 tablets that covered the estimated demand for a year. In addition Bayer signalled their willingness to distribute an additional 250,000 tablets in Latin America if needed, once the donation had been exhausted. The donation aimed to, among other things to ensure production and to estimate the needs for future negotiations on price and production. Non-utilization of the donation ran the risk of Bayer opting to stop production of Nifurtimox, one of only two drugs for the treatment of Chagas disease and increasingly important in the treatment of sleeping sickness.

PAHO/WHO dosage recommendations are[lii]:
Acute, intermediate and chronic stage treatment
- 10-12mg/kg/day if weight <40kg and 8mg/kg/day if weight >40kg.
Chronic phase treatment
- 8-10mg/kg/day for 60-90 days
Congenital infection treatment
- 10-15mg/kg/day. In low-weight cases, initial dosage should be halved. If there is no evidence of leucopenia or platelet reduction the complete dose should be administered.
Contraindications
- Early pregnancy

It has been extremely difficult to estimate the consumption and therefore the need for nifurtimox. Of the initial 200,000 tablets that were shipped out from PAHO Honduras, as of May 2005; Honduras received 160,000 tablets and treated 566 patients, Bolivia received 20,000 tablets and treated none, Nicaragua received 10,000 tablets and treated 54 (2,000 in stock), Guatemala received 7,000 tablets and treated 35, Argentina treated a few patients in several provinces (no reporting) with the 4,000 tablets received, and Brazil received 600 tablets and did not report usage. So far, only 87,500 tablets have been used with some 700 patients treated (J Jannin, personal communication). NUMBERS DON'T ADD UP! With benznidazole available and preferred by most physicians, consumption of nifurtimox has been very low.

In January 2006, with remaining stocks expiring in July 2006, an additional 100,000 tablets were sent to Argentina, 10,000 to Colombia and another 10,000 to Venezuela. There is no utilization data available. Currently, there is a stock of 250,000 tablets (delivered by Bayer

early 2006) for use in Chagas disease expiring in July 2009. A new donation that will cover a period of 3 years is currently being negotiated by WHO and Bayer (J Jannin, personal communication).

As for benznidazole there is no paediatric formulation. Some paediatricians prefer nifurtimox as there is a risk of children developing Lyell syndrome (1/100,000) with benznidazole (H Freilij, personal communication).

## 1.5. Evaluation of cure

Evaluation of cure after trypanosomicidal treatment with either nifurtimox or benznidazole relies on conventional serological tests. The criteria to declare a patient cured, for both acute or chronic disease is a negative result on the previous positive serological tests. A negative result on a parasitological test is considered to be a prove for a satisfactory treatment outcome but not a criterion of cure because as already mentioned, parasitemia is low in Chagas' disease thus the possibility of assessing a treatment failure based on parasite detection is difficult. The only situation where parasitaemia can be high when reactivation of chronic cases occurs is in immunodepressed patients (HIV or organ transplant).

In the acute form specific antibodies usually take less than 12 months (range 2-24 months) to become undetectable.

In the chronic form specific antibodies may take several years (up to 10-20 or more) before dropping to undetectable levels even if the treatment eliminated the parasites.

It mus be pointed out that the decrease of the antibodies titre is not linear and they can even increase for a certain period of time in a patient that later on will be declared cured.

Earlier the treatment is given (newborns and children) earlier the observation of serological negativity.

On the other hand, the slow progress of the associated symptoms (cardiac or digestive) is not of any help in identifying a treatment failure.

## 1.6. Control

Control strategy for Chagas' disease is based on the interruption of vectorial and blood transfusion transmission. Vector control consists of insecticide spraying of the targeted houses. The main factors leading to failure of vector control have been identified as (1) inappropriate selection of the insecticide, (2) inappropriate application, (3) inaccessibility of the reservoir and (4) insect resistance.

Brazil spent $516,682,000 in prevention and control activities for Chagas' disease from 1975 to 1995. Of this amount, 18.5% was devoted to haematological services and blood banks and 81.5% to vector control activities. The cost-benefit analysis demonstrated that for each dollar spent in vector control, there were $2 in savings in treatment??[liii].

The budget of the National Program in Argentina was 5,102,893 US$ in 2001-2002[liv].

The cost for a 10 years control program in Ecuador is estimated at around US$ 30 million (table 4).

For control results to be assessed epidemiological surveillance must be implemented, this including:

- vectorial surveillance (e.g. infestation, colonization)
- non-vectorial surveillance (e.g. screening in children and pregnant women, blood banks)

Control program evaluation is mainly based on seroprevalence screening of the population this allowing a certain area to be certified as "transmission interrupted".[lv]

At present there is no vaccine to prevent *T.cruzi* infection.

Table 4. Annual budget in US$ (thousands) for a 10-year Chagas disease control program in Manabí, Guayas, El Oro and Loja, Ecuador (5 million at risk and 700,000 dwellings).

| Component | Year 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Vector Control | 6,200 | 7,750 | 1,600 | 160 | 160 | 160 | 160 | 160 | 160 | 160 | 16,670 |
| Serology | 4,000 | 4,000 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 2,500 | 10,640 |
| Reference Lab | 66.5 | 29 | 29 | 29 | 29 | 29 | 29 | 29 | 29 | 29 | 327.5 |
| Entomology Surveillance | 126 | 18.2 | 18.2 | 18.2 | 18.2 | 18.2 | 18.2 | 18.2 | 18.2 | 18.2 | 29.8 |
| General Coordination | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 120 |
| Benznidazole | 25 | 150 | 140 | 0.6 | 0.4 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 316.6 |
| Patient Care | 150 | 500 | 400 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 1,750 |
| Total | 10,579.5 | 12,459.2 | 2,219.2 | 339.8 | 339.8 | 339.6 | 339.4 | 339.3 | 339.3 | 2,819.3 | 30,113.9 |

Source: Report of the "VI Reunion de la Iniciativa Andina para el Control de la Enfermedad de Chagas. Bogota, Colombia, 2-6 mayo 2005".

## 1.6. Social aspects

In some countries it is obligatory to have a serological test on Chagas' disease done to get a job contract[lvi]. This has lead to **discrimination** against individuals with a positive result despite a single serological test neither implies infection in all cases nor disability.

A survey carried out in Sao Paulo (Brazil) on 27,081 urban workers from a mechanical metallurgical industry showed that *T.cruzi* **positive serological individuals had a much lower educational level**. Workers infected with *T.cruzi* had precocious ECG alterations when compared with seronegative workers. These alterations are of particular importance since they are, frequently, the first manifestations of Chagas' disease, although they do not lead to clinical symptoms. In this situation, healthy urban workers infected with *T.cruzi* could be unaware of their clinical status, especially the cardiac. Exposure to heavy and dangerous activity at work could worsen the course of the disease. These findings strengthen the necessity of providing medical assistance to workers without any kind of discrimination[lvii].

## 1.7. The MSF response

### 1.7.1. MSF in the past

TO CHECK WITH OSCAR BERNAL

MSF has been involved in Chagas' disease since 1999. MSF carried out three projects in Honduras and Nicaragua. In Honduras, MSF successfully treated over 200 patients under 14 years old and followed them up over two years. In Nicaragua, out of 3500 patients tested, 66 received treatment and follow up. In 2004 MSF conducted a study in Mexico that showed a 2.3% prevalence of Chagas in children under 14 years and subsequently urged the government to tackle the problem.

### 1.7.2. MSF at present

MSF is currently implementing three programs, in Bolivia (Tarija and Sucre) and Guatemala (Olopa). The results obtained so far can be considered satisfactory (see table 5) but a number of constraints still exist. **TO ADD INFORMATION ABOUT THE PROBLEMS ON BLOOD BANK (TARIJA), LAB AND COLD CHAIN ISSUES (TARIJA, OLOPA),**

**TO ADD INFORMATION ON THE TREATMENT OUTCOME**

The cure has been verified by parasitological and serological methods. In Tarija n/n 0.89% were parasite negative and n/n 79% had lower titre of antibodies)

All patients in Bolivia and Guatemala are currently being followed-up.

| Table 5. Diagnosis and treatment results in MSF projects. | | | | | | | |
|---|---|---|---|---|---|---|---|
| Location | Period | Screening | | No of positive cases treated[1] | | Side effects[1] | |
| | | N | % | N | % | N | % |
| Tarija (Bolivia) | 01/2003-09/2005 | N | 73.6 | 1,041 | 79.5 | 214 | 10.6 |
| Sucre (Bolivia) | 03/2005-09/2005 | N | 90.0 | 34 | 56.6 | 1 | 3.0 |
| Olopa (Guatemala) | 00/0000-09/2005 | N | 53.8 | 58 | 50.0 | 3 | 5.0 |
| [1] Includes only the patients who have completed treatment | | | | | | | |

A part from the programs directly addressed to people living in endemic areas, MSF is advocating for awareness and more attention to be paid to people already infected. In that sense the "Regional Consultation on the Organization and Structure of Health Care for the Sick or Infected by *Trypanosoma cruzi* (Chagas' disease)" held in Montevideo on 13 - 14 October 2005, co-organized by PAHO / MSF is aiming at improving the situation of the many people who remain undiagnosed or without an appropriate treatment (both etiological and syndromic).

## 2. Overview of the current situation

### 2.1. Global epidemiology

The current epidemiological situation of Chagas' disease is mostly unknown at a general level and only data from certain foci are available (see annex 1).

#### 2.1.1. Southern Cone Initiative

The Southern Cone Initiative for the interruption of vector and blood transfusion transmission of Chagas' disease was launched in 1991. It includes 6 countries, Bolivia, Argentina, Chile, Uruguay, Paraguay and Brazil. Uruguay was certified free of transmission in 1997 and Chile in 1999.

There are 164 million people living in this huge region and 11 million infected with *T.cruzi*. This represents 70% of the Chagas' burden in the continent.

As an overall, **successful progress has been made on the interruption of the disease transmission** by eliminating the main domestic vector, *T. infestans*.

Transmission of Chagas' disease was expected to be interrupted by 2005 in those countries[lviii]. Information is not yet available to assess the achievement of that objective in the whole region. Brazil had 8 out of 12 endemic states certified free of transmission in 2000 and currently it has 11 states with vector transmission interrupted (P. Albajar, personal com.) **PERE, DO YOU WANT US TO QUOTE YOUR NAME HERE?**

Argentina is planning a national survey in 2006 to update the figures about the epidemiological situation. An average of 16 acute cases a year has officially been reported from 1996 to 2004 (C. Spillmann personal com.).

Despite the progress achieved on vector control, recent challenges have been identified which could jeopardize control efforts. Wild *T. infestans* are much more widespread throughout Bolivia than previously thought. In some areas the silvatic sites are just a few metres away from the nearest huts and both the hosts and insect vectors presented high level of natural infection (>60%) with the *T.cruzi* parasite. As *T. infestans* underwent a domestication process in the past, it is possible that it could happen again in the future. Moreover, Bolivia is in the middle of the Southern Cone region so wild *T. infestans* could spread to neighbouring regions of northern Paraguay and Argentina[lix].

#### 2.1.2. Andean Pact Initiative

This initiative was launched in 1997 following the good results obtained in the Southern Cone Initiative. It includes 4 countries, Peru, Colombia, Ecuador and Venezuela. The deadline to achieve the interruption of vector and blood transfusion transmission of Chagas' disease has been set for 2010 (2006 for Peru)[lx].

It has been estimated that domestic vector elimination would represent an 80% decrease on the risk of acquiring the disease in the region. Assuming that there are still some 540,000 dwellings to be fumigated this would cost around US$ 31 millions (US$ 58/dwelling). The economical benefit of preventing 70,000 new human infections per year would be around US$ 41 millions annually.

The budget of the National Program in Ecuador is US$ 250,000 for 2005.

This country has estimated the cost of initial serological screening of 2 millions children under 15 years and pregnant women for two years in

US$ 8 millions (US$ 4/ test).

In Peru there is a big gap between the estimated number of cases and the officially reported (see annex 1).

Venezuela is the country where most progress has been reported with a 90% decrease of the infection rate (age group 0-4 years) from 1991 to 2001[lxi].

In Colombia, a national entomological and serological survey was carried out between 1998 and 2001 to know the current distribution of the parasite to target the control activities efficiently. 41,971 houses in 3,375 villages and 51,482 were screened. A total of 127 of the 539 surveyed municipalities were assigned high risk. Several departments that were believed initially to be endemic have been shown to have extremely low levels of risk, enabling control resources to be allocated for other purposes[lxii].

### 2.1.3. Central American Initiative

Also launched in 1997 it includes 4 countries, El Salvador, Guatemala, Honduras and Nicaragua.

Costa Rica and Panama are not carrying out routine vector-control activities and blood donors are poorly screened (<10%).

The deadline to achieve the interruption of vector and blood transfusion transmission of Chagas' disease has been set for 2010.

Reports on this Initiative also contain information about Mexico even though that country does not specifically belong to it. A national survey screening 70,000 people between 1987 and 1989 found Chiapas the most affected state with 5% prevalence

In 2003 three out of the four countries admitted a deficiency on the diagnosis and/or management of the human cases[lxiii].

Up to now there are no epidemiological data to evaluate the impact of the vector control programs on the reduction of Chagas' disease in the region.

### 2.1.4. Amazonian Initiative

The specificity, complexity and scarce epidemiological information on Chagas' disease in the Amazonian basin region led to the creation of the Amazonian Countries Initiative for the Surveillance and Control of Chagas' disease – AMCHA - in September 2004. It includes 9 countries, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guiana, Peru, Suriname and Venezuela.

The main objective of the AMCHA Initiative is to prevent the establishment of a large scale vectorial transmission of Chagas' disease in the Amazonian region[lxiv].

To illustrate the specificity of Chagas' disease in this region there are relevant data from the Brazilian Amazon. Briefly, 205 cases have been notified between 1968 and 2000, 178 of them being acute cases. Even if reservoir hosts of *T.cruzi* and triatomine vectors were identified in the early 1900s in this region, the first human case was reported only in 1969. At least 33 species of wild animals and 10 species of triatomines have been found positive to *T.cruzi*. Unlike other endemic areas in South America, *T.cruzi* I is the predominant lineage and this may be linked to the low morbidity and low parasitemias (indeterminate form) observed in this particular region. 111 of the 178 acute cases were attributable to microepidemics of orally transmitted infection from contaminated food. In 1997, a survey carried out on 2,254 individuals found 13.2% seropositivity with IIF, but this dropped to 5% when additional tests were done (IHA, ELISA, Western blot). *T. rangeli* is widely distributed in the Amazon region and may contribute to cross-reactions[lxv].

In Suriname from 1996 up to date there is only one acute case declared and patients living at the French Guyana border are treated in that country (H. Hiwat personal com.). From January to November 2005 seven cases (five acute and two chronic) coming from Suriname has been diagnosed and treated in French Guyana (E. Chauvet personal com.).

### 2.1.5. Other projects

The "Chagas Disease Intervention Activities - CDIA" is project aiming at promoting research and control to support Chagas' disease National Programs for three regional initiatives (Southern Cone, Central American and Andean) for the period 2004-2007.

## 2.2. Issues with diagnosis, case management and control

### 2.2.1. Diagnosis

The use of rapid diagnostic tests (Stat-Pak) is a major advantage in poor health settings. It must be pointed out that differences in sensitivity and specificity may be observed depending on the patients' place of residence, sample used (whole blood or serum) and the prevalence of the disease in that particular area. A study carried out by MSF in 2004 in Sucre (2,783m above see level) Bolivia, where the prevalence was 5.8% (95/1649) and the test was performed on whole blood (any visible line was considered as positive), showed that the test specificity was 73.1%. When the test was performed on serum the specificity increased to 98.2%. **TO CHECK WITH OSCAR BERNAL IF THIS INFORMATION CAN BE MENTIONED.**

Due to the satisfactory results obtained with Stat-Pak and its adaptability to the field conditions MSF modified the diagnostic tree used for the screening of the disease. This has yielded an increased screening capacity of mobile teams, has reduced the time elapsed between screening and treatment to 1-2 weeks and has reduced the number of ELISA tests performed (see diagnostic tree before and after July 2005 below).

Bolivian diagnostic tree before July 2005

```
                    ┌─────────────┐
                    │  ELISA/IHA  │
                    │─────────────│
                    │    (FP)     │
                    └──────┬──────┘
            ┌──────────────┴──────────────┐
            ▼                              ▼
      ┌──────────┐                   ┌──────────┐
      │ negative │                   │ positive │
      └────┬─────┘                   └────┬─────┘
           ▼                              ▼
       ┌────────┐                  ┌─────────────┐
       │  home  │                  │  ELISA/IHA  │
       └────────┘                  │─────────────│
                                   │    (VB)     │
                                   └──────┬──────┘
            ┌─────────────────────────────┴──────┐
            ▼                                     ▼
      ┌──────────┐                          ┌──────────┐
      │ negative │                          │ positive │
      └────┬─────┘                          └────┬─────┘
           ▼                                     ▼
       ┌────────┐                             ◇ treat ◇
       │  IIF   │
       └────┬───┘
     ┌──────┴────────────────────────┐
     ▼                               ▼
┌──────────┐                   ┌──────────┐
│ positive │                   │ negative │
└────┬─────┘                   └────┬─────┘
     ▼                              ▼
  ◇ treat ◇                    ┌────────┐
                               │  home  │
                               └────────┘
```
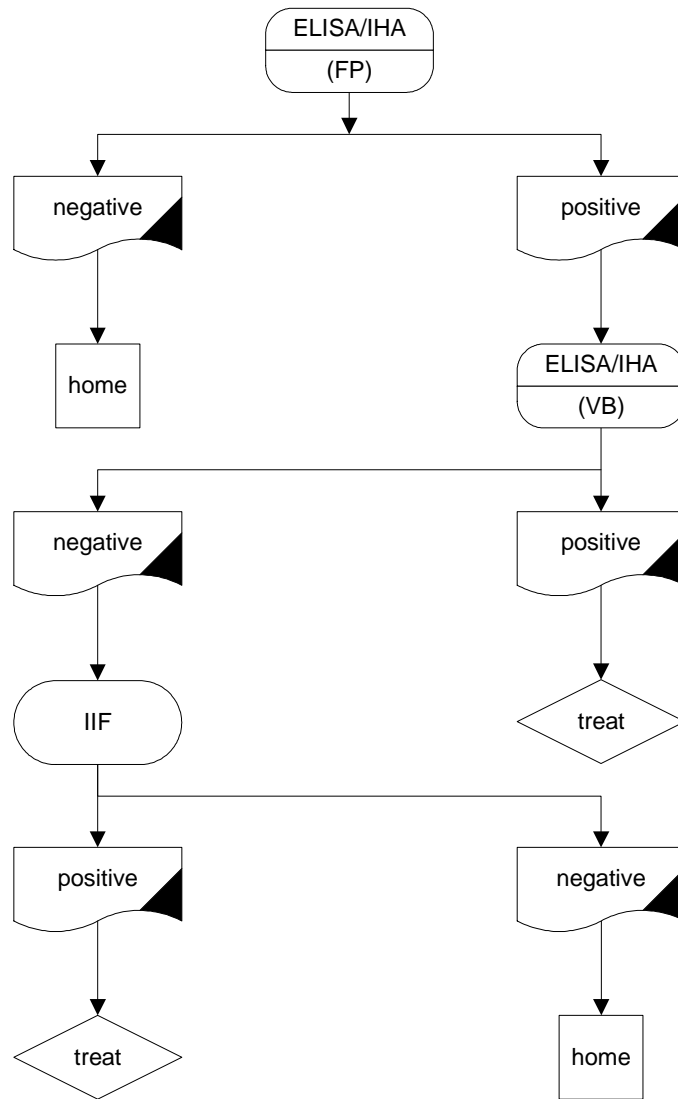
MSF project diagnostic tree after July 2005

```
                          ┌─────────────┐
                          │  Stat-Pak   │◄──────────┐
                          └──────┬──────┘           │
            ┌────────────────────┼────────────┐     │
            ▼                    ▼             ▼     │
      ┌──────────┐        ┌──────────┐   ┌──────────┐│
      │ negative │        │ positive │   │indistinct││
      └────┬─────┘        └────┬─────┘   └──────────┘
           ▼                   ▼
       ┌───────┐         ┌──────────┐
       │ home  │         │ ELISA G1 │
       └───────┘         ├──────────┤
                         │   IHA    │
                         └────┬─────┘
            ┌────────────────┼────────────────┐
            ▼                ▼                 ▼
      ┌──────────┐    ┌────────────┐    ┌──────────┐
      │ negative │    │discordance │    │ positive │
      └────┬─────┘    └─────┬──────┘    └────┬─────┘
           ▼                ▼                ▼
       ┌───────┐      ┌──────────┐      ◇ treat ◇
       │ home  │      │ ELISA G3 │
       └───────┘      └────┬─────┘
            ┌──────────────┴──────────────┐
            ▼                             ▼
      ┌──────────┐                  ┌──────────┐
      │ negative │                  │ positive │
      └────┬─────┘                  └────┬─────┘
           ▼                             ▼
       ┌───────┐                    ◇ treat ◇
       │ home  │
       └───────┘
```
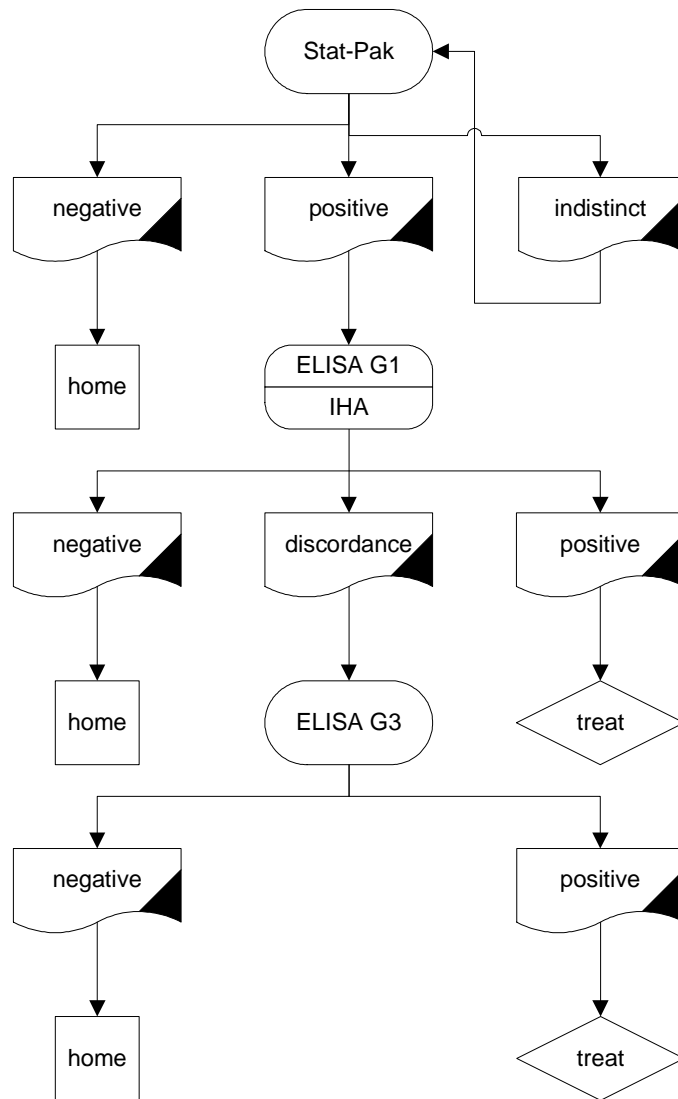
It must be pointed out that quality control is an important aspect in diagnosis methods. This component is not widely applied in all programs in this may have an influence on result reliability.

An important issue concerning diagnostic tests is the difference in prices depending on the country and the distributor.

MSF is currently using blood sample collection on filter paper for follow-up. Depending on the results obtained this method may be widely extended to poor health settings or remote areas thus being of great value.

Some of the new tests using recombinant proteins JL8 +MAP have not yet found companies interested in their development despite their good performance (E. Umezawa, personal com.)

**There are several issues regarding the diagnostic tree we should talk about. Problems with quality control etc.**

### 2.2.2. Case management

A study is currently ongoing to provide more data about the controversial issue of treating chronic cases (see BENEFIT study below).

There is a lack of rapid cure markers in adults. Moreover, treatment response can vary according to different *T.cruzi* strains. Some promising serological markers (antibodies anti F2/3) able to predict cure earlier than serological conventional tests has been identified and are currently under study (H. Freilij personal com.).

From the clinical point of view the criteria based on serological negativity is not enough to assert that the infection will not cause damage some years later. Long-term studies are consequently needed, which could also identify disease progression markers. Efforts are being made to find prognostic markers for Chagas' disease. The brain natriuretic peptide, recently associated to cardiac fibrosis, has been found a good marker even in *T.cruzi* infected patients without cardiac dysfunction in a study involving 90 patients and 78 controls. Therefore, according to these findings it could be periodically measured to screen for incipient ventricular dysfunction[lxvi]. Another study involving 25 patients have only found elevated BNP levels in cases with cardiac impairment[lxvii]. In a trial with 227 patients the combination BNP/ECG was found the best strategy to detect left ventricular dysfunction, the main predictor of mortality in Chagas' disease[lxviii]. The BNP can be easily measured with a simple stick using one drop blood and values obtained within two minutes (T. Walther, personal com.). There is a commercial kit already available from several companies. Roche has different types of kits and the price is around 20 € / test, negotiable (M. Villarino, personal com.)[lxix].

### 2.2.3. Control

Activities to control Chagas' disease are focused on vector control and screening in blood banks. Vector control needs to be sustained in some areas whereas in other needs to be improved. Screening in blood banks has been improved in the recent years and must be sustained.

No mass screening is regularly done in people living in endemic areas. To identify and promptly treat *T.cruzi* infected individuals, especially in recent infected cases, would be an additional factor to stop the transmission cycle in a community level as wells as level to prevent the progression of the disease in a individual level.

Research is currently being done to find out a vaccine for the disease. Parasite specific proteins have been identified as potential vaccine candidates when delivering some genes as **DNA vaccine** in the mouse model. The surface expression of these antigens in infective and intracellular stages is being evaluated to determine their potential to induce protective immunity against *T.cruzi* infection and disease[lxx].

Recently, the use of six different recombinant proteins has provided remarkable immunity, consistently protecting 100% of mice. This strong protective immunity against *T.cruzi* was

CD8(+)-T-cell-dependent[lxxi].

Several years are still needed before a vaccine can be available for humans (A. Osuna, personal com.).

# 3.    Perspectives for the Future

## 3.1.    Epidemiological evolution

The lack of data at a general level makes difficult to assess what is the trend from the epidemiologic point of view. Regular monitoring is needed to better evaluate what is the impact of the activities currently being implemented or to assess the consequences of a lack of intensive control activities.

## 3.2.    New tools for improved diagnosis and treatment

### 3.2.1.    Progress in development of diagnostic tests

MARTINE?

### 3.2.2.    New use or development of new drugs

Since it has been shown that elimination of *T.cruzi* from infected individuals is required to prevent the development of the late-stage lesions[lxxii], the specific anti-parasitic treatment has become relevant and new drugs are needed to find an alternative to the currently available chemotherapy, nifurtimox and benznidazole which show very low anti-parasitic activity in the chronic form (< 20%).

Several drugs already used in other human diseases are currently being tested against *T.cruzi* with promising results. Moreover new compounds are in an experimental phase which may lead to new candidate drugs for testing. Several of them have completed pre-clinical studies and are ready for clinical trials in the next decade[lxxiii].

The **technology transfer** from Roche to Lafepe to produce benznidazole does not seem to represent a handicap to register the drug in Latin American countries. According to the current data on bioequivalence the drug will most likely be qualified by WHO and thus easily registered by countries. On the other hand, benznidazole studies are also being carried out by Lafepe to develop a **paediatric formulation** (suspension) (C. Zackiewicz, personal com.).

There is one ongoing clinical trial attempting to answer the question of efficacy of **benznidazole**. The **BENEFIT study** aims at assessing benznidazole efficacy in chronic Chagasic cardiopathy[lxxiv]. The study started in November 2004 in Brazil. Its pilot phase intends to recruit a total of 600 patients from 30 treatment centres in Argentina, Colombia and Brazil. Inclusion criteria are patients aged between 18-65 years old with at least abnormal ECG, increased cardiothoracic ratio or abnormal 2D-Echo. Currently 15 centres have started the study in Brazil and 218 patients have randomly been enrolled. Argentina and Colombia are expected to start recruitment in two months. The study is coordinated by the Population Health Research Institute at McMaster University (Canada). The study is being sponsored by the University and more funds are still needed to complete the study (C.A. Morillo, personal com.).

Another promising study, the "Cardiovascular Health Investigation and Collaboration to Assess the Markers and Outcomes of Chagas disease" (**CHICAMOCHA**) is aiming at testing a number of compounds already in the market for other indications, and with trypanocidal activity *in vitro* and *in vivo*, but not tested yet for Chagas' disease. Other objectives of this study are (J.C. Villar personal com.):

- To test the reliability of the classification of clinically healthy individuals using conventional *T.cruzi* serology and electrocardiogram.

- To identify the extent of subclinical cardiac findings (i.e. subclinical Chronic chagasic cardiomyopathy - CCC) in seropositive population in terms echocardiography and autonomic function indices and to generate a working definition of subclinical CCC for future longitudinal studies of asymptomatic carriers of infection.

- To identify factors associated with Chagas' cardiomyopathy and to generate algorithms to screen for subclinical CCC among *T.cruzi* infected population.

Allopurinol, an inhibitor of the HGPRT enzyme, was shown to be active in murine models of acute Chagas' disease but there have been conflicting reports of the therapeutic efficacy of allopurinol in humans. It was able to reverse in 49% of the cases or prevent (75% of the cases) the development of electrocardiographic abnormalities after a nine –year follow-up. It must be emphasized that no parasitological cures could be demonstrated according to serological criteria[lxxv]. In 1992 a multicentric trial conducted in Brazil, Argentina and Bolivia on chronic cases was terminated due to the manifest treatment inefficacy[lxxvi].

Eight new selective HGPRT inhibitors were capable of blocking the proliferation of intracellular *T.cruzi* amastigotes *in vitro*, thus also have potential as anti-*T.cruzi* agents[lxxvii]. Due to the limited preclinical development, the clinical development of the new selective HGPRT inhibitors for the treatment of Chagas' disease can only be expected in the long term (ten to fifteen years) (J.A. Urbina personal com.).


Clomipramine, a tryciclic antidepressant used in obsessive-compulsive disorders and panic attacks, has recently been found effective in *T.cruzi* infected mice[lxxviii]. At present the researchers are waiting for funds to start phase II trials (H.W. Rivarola, personal com.).

This drug is contraindicated in children and in case of cardiac arrhythmias (especially heart block) which may limit its clinical use in Chagas' disease, namely in those countries where the cardiac chronic form predominates (Central America).

Moreover, the fact that this drug is not only active on parasite biochemical targets may hinder the long use required for Chagas' disease (J.A. Urbina personal com.).

This drug is included in the 14th WHO model list of essential medicines 2005.


Metronidazole, an antibacterial and antiprotozoal agent widely used in humans, was tried in Chagasic patients with encouraging results in 1993[lxxix]. No other experiences are reported in the scientific literature.


BENZOFUROXAN… > Dr. Hugo Cerecetto: hcerecet@fq.edu.uy

HE IS TRAVELLING… HE WILL REPLY LATER ON…


NITROIMIDAZOLE Edson Ferreira- edsonf@far.fiocruz.br

WAITING FOR FEED-BACK


Trimetrexate is a potent inhibitor of *T.cruzi* DHFR enzyme activity and is also highly effective in killing *T.cruzi* parasites *in vitro* including amastigotes[lxxx]. Trimetrexate is an FDA-approved drug for the treatment of *Pneumocystis carinii* infection in AIDS patients. However, as trimetrexate is also a good inhibitor of human DHFR, further improvement of the selectivity of this drug would be preferable.

The investigators of this novel approach are at present facing financial constraints to carry out further studies, including difficulties in obtaining the molecule from the company (D. Chattopadhyay, personal com.).


Risedronate, a bisphosphonate, has been found effective against *T.cruzi* in several studies carried out in mice[lxxxi,lxxxii,lxxxiii]. It reduces the parasitemia but does not completely eradicate the infection. Moreover, it is ineffective when used against the virulent Tulahuen strain.

As bisphosphonate-containing drugs are FDA-approved for the treatment of bone resorption disorders, their potential innocuousness makes them good candidates to control tropical diseases. They have also shown anti-parasitic activity against *T. brucei, L. donovani, T. gondii* and *P. falciparum*[lxxxiv].

One of the main limitations may be the contraindication of its use in young children, due to the action on bone turnover.

Bisphophonates could also be used in combination with other anti-parasitic drugs or calcium-channel blockers such as verapamil. These studies are currently underway.

The fact that there may be a need for new formulations or to find more selective compounds against the parasite makes the clinical development of bisphosphonates for the treatment of Chagas' disease not likely before ten years (mid term) (J.A. Urbina personal com.).

Statins, widely used in humans to lower low-density lipoprotein cholesterol levels, have shown good activity against *T.cruzi*. Studies with lovastatin have shown that the drug is more effective when administered in combination with other sterol inhibitors[lxxxv].

New cholesterol and triglyceride-lowering agents currently in development in humans, as E5700, have shown activity against *T.cruzi* in animal models – acute form – but not resulting in a complete cure as indicated by the presence of low but detectable levels of parasites after the discontinuation of treatment[lxxxvi]. New experiments exploring different dosing as well as combination therapy are under way.

The fact that this drug inhibits the squalene synthase also crucial for some organs in the host (synthesis of sexual hormones in the testicles), will most likely make necessary to develop more selective compounds against the parasite enzyme and thus the clinical development for the treatment of Chagas' disease can only be expected in the mid term (ten years) (J.A. Urbina personal com.).

2-propen 1-amine derivatives have a remarkable trypanocidal activity *in vitro* and *in vivo* (mice)[lxxxvii]. They inhibit the biosynthesis of ergosterol, an important metabolic route in the survival of *T.cruzi*.

New triazole derivatives are also powerful inhibitors of protozoan and fungal sterol biosynthesis and some of them are the first compounds reported to have curative activity against the acute and chronic forms of Chagas' disease, including nifurtimox and benznidazole resistant *T.cruzi* strains from infected mice. There are several compounds in this group: posaconazole (SCH 56592) was tested in 1998 and 2000 with promising results in a *T.cruzi* murine infected model[lxxxviii,lxxxix]. It has recently been tested in humans with promising results as a systemic antifungal agent[xc]. The company producing the drug, Shering-Plough, is not interested in the clinical development of the compound for Chagas' disease[xci]. This situation could be overcome by conducting clinical trials "off-label" once the drug is registered in the USA as systemic antifungal (expected in 2006).

Posaconazole has been found to act synergistically with amiodarone, which has shown direct activity against *T.cruzi*, both *in vitro* and *in vivo*. Amiodarone disrupts the parasites' Ca (2+) homeostasis and also blocks ergosterol biosynthesis. These results open up the possibility of novel, combination therapy approaches to the treatment of Chagas' disease using currently approved drugs[xcii].

D0870, was tested in 2000 and 2001 in a murine model showing encouraging results. It was effective in both acutely and chronically infected animals (70-100% and 30-45% parasitological cure, respectively) including *T.cruzi* resistant strains[xciii]. To improve the bioavailability due to poor solubility another trial was carried out by means of incorporating long-circulating PEG-PLA nanospheres showing cure rates of 60-90%[xciv]. The development of this compound was terminated by Zeneca Pharmaceuticals in 1995 due to an adverse event in a patient – cardiac arrest – and some unpredictable pharmacokinetic properties in humans[xcv].

Ravuconazole, has shown very potent in vitro anti-*T.cruzi* activity. In murine models of acute Chagas' disease high levels of parasitological cures have been reached, but only when given twice a day. It must be stressed that it shows no curative activity in animals infected

with drug-resistant strains and no curative activity occurred in a chronic model of the disease[xcvi]. Thus, *in vivo* activity in mice is limited, probably due to its unfavourable pharmacokinetic properties in this animal model. However, these results do not necessarily rule out the potential utility of ravuconazole in the treatment of human *T.cruzi* infections. Studies in a canine model seems to provide promising results that could lead to human trials (J.A. Urbina personal com.)

Albaconazole (UR-9825), showed a very potent effect against *T.cruzi*, both epimastigote and amastigote forms, when tested *in vitro*[xcvii]. This led to further studies in animal models (dog) where it has been found 100% parasitologically curative when administered for 90 days in partially resistant strains. One of its drawbacks was the resistance found in nifurtimox and benznidazole susceptible *T.cruzi* strains even when given for 150 days[xcviii].

TAK-187, an experimental antifungal triazole with a long terminal half-life in several experimental animals, yielded high levels (80-100%) of parasitological cures, including resistant strains, in both acute and chronic murine models[xcix]. In recent trials TAK-187 was more effective than benznidazole in preventing *Trypanosoma cruzi*-induced cardiac damage in experimental animals[c].

Depending on legal and economical agreements with the companies producing all those new triazole derivatives, clinical development for the treatment of Chagas' disease can be expected in the short term (five years) (J.A. Urbina personal com.)

Another group of sterol inhibitors, azasterols, have been reported to have an excellent activity against *T.cruzi*[ci].

Miltefosine, a lysophospholipid analogue, has shown satisfactory preliminary results in an experimental mouse model of acute Chagas' disease[cii]. It has promoted survival and reduced the parasitemia, including cases caused by resistant strains of *T.cruzi*. *In vitro* studies show that lysophospholipid analogues appear to be more effective in combination with other drugs such as ketoconazole[ciii]. Additional studies are under way with murine models of both acute and chronic disease. This drug is currently being used for visceral leishmaniasis.

K777 (or CRA-3316), a cysteine protease inhibitor, is at present in preclinical development by the IOWH. Cysteine protease inhibitors have shown a potent activity against *T.cruzi* in model animals and have the advantage of being inexpensive to produce. In February 2002, One World Health announced it was granted all rights to develop and commercialize the compound K777 as an antiparasitic agent by Celera Genomics (Rockville, MD)[civ]. Whether or not this oral drug will proceed into clinical development has not yet been determined (A. Strosberg personal com.).

According to the present available data in the scientific literature the clinical development for the treatment of Chagas' disease can only be expected in the mid term (ten years) (J.A. Urbina personal com.).

Inhibitors of the trypanothione metabolism, as thioridazine, have been shown to increase survival and prevent cardiac damage in murine models of acute Chagas' disease but do not show parasitological cure[cv]. Moreover, the selectivity for the trypanothione enzyme has not been fully demonstrated. So, due to the limited preclinical development, the clinical development of these compounds for the treatment of Chagas' disease can only be expected in the long term (ten to fifteen years) (J.A. Urbina personal com.)

Megazole, another nitroimidazole derivative with promising antitrypanosomal activity has recently been abandoned due to its genotoxicity[cvi]. However, research on non-mutagenic megazole analogues is currently being done and *in vitro* studies have identified compounds which could eventually improve the therapeutic profile and safety of megazol[cvii].

Nifurtimox analogues are currently being synthesized and tested *in vitro* with promising results. They are easy and cheap to produce and appear to be more potent than Nifurtimox[cviii].

Cyclosporine A analogues have recently been tested *in vitro* and show to be interesting compounds in the search for new anti-trypanosomal drugs. Unlike CsA these analogues have less immunosuppressive activity since immunosupression would interfere their anti-parasitic

effect[cix]. *In vivo* studies on a murine model also show good results. At present, toxicity studies on mouse hepatocytes are being carried out to asses how the detoxification process is done in a mammal host. Furthermore, the therapeutic targets of these drugs – ciclophylines genes – are also currently under study (J. Búa, personal com.)

Quinolines have also been synthesized an evaluated *in vitro* with promising activity against *T.cruzi*. Further studies are now under way to evaluate their *in vivo* activity[cx].

There are also natural products (black pepper[cxi], snake venoms[cxii]) containing compounds with demonstrated activity against *T.cruzi*.

Current studies on the snake venom have identified the trypanocidal compound. Unfortunately, its toxic effect is not parasite specific and affects all kind of cells. Thus it is unlikely to obtain a drug to be used for Chagas' disease from that venom (R. DaMatta, personal com.).

Studies are being carried out to find a further utilization of piperine.

Other possibilities currently under investigation to treat Chagas' disease include stem cells transplantation from the spinal cord into the patient's hearts.

## 4.    Final Thoughts

The currently available tests for Chagas' disease have excellent performance but no mass screening is routinely done in endemic areas. The features of those areas, mostly accessible and socially stable, are a factor that should encourage decisions makers to increase active diagnosis for this neglected disease.

Lack of diagnosis has dire consequences as infected individuals do not receive treatment, this causing the progression of the disease. From a public health point of view the lack of diagnosis and treatment hides the real dimension of the situation and does not allow resources to be invested for the disease.

As the drug development process is long term and expensive, the use and/or combination of existing drugs may be the best choice to new antitrypanosomal drugs in the short term.

The requirements of an ideal new drug are: parasitologically cures both acute and chronic Chagas' disease, b) is effective in few doses, c) has few side effects, d) is available without hospitalization, e) does not induce resistance, and f) is available at low cost.

The wide range of therapeutic targets under investigation should hasten the discovery of novel trypanocidal compounds that can be made into effective, widely available drugs[cxiii,cxiv].

**We need to elaborate this section a bit more but can be done later.**

## 5.  Recommendations

1) To advocate for the decrease of blood banks.

2) To advocate for haemovigilance strengthening after blood transfusion.

3) To encourage voluntary repeat donors.

4) To advocate for active case detection.

5) To support studies assessing performance of serological tests under field conditions at different settings.

6) To ensure quality control in laboratory procedures.

7) To advocate for better prices (distribution) in diagnostic tests in collaboration with PAHO/WHO.

8) To make syndromic treatment (cardiac, digestive) available for all patients in need of it.

9) To make etiological treatment available and affordable in collaboration with PAHO/WHO.

10) To advocate for clinical trials in *T.cruzi* infected patients using new drugs or combinations shown to be effective and safe (e.g. posaconazole).

11) To promote studies for clinical prognostic markers (e.g. BNP).

12) To promote studies for new cure markers.

13) To advocate for a centralized data base to ensure updated and prompt access to the information about the situation of the disease in all endemic countries (e.g. n° of people at risk, n° of people infected and/or treated)

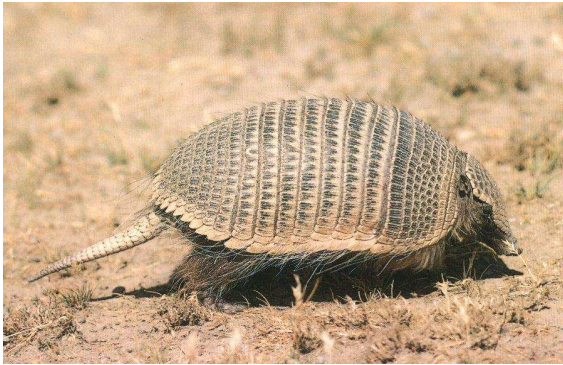# 1. Annex 1: Epidemiological data of human *T.cruzi* infection in Latin America, 1983/2004.

**SEE ATTACHED FILE (Epidemiology countries.xls) (WILL BE INCLUDED HERE IN THE FINAL VERSION)**

Countries where MSF is currently working BOLIVIA, GUATEMALA.

Countries where MSF was working in the past HONDURAS NICARAGUA.
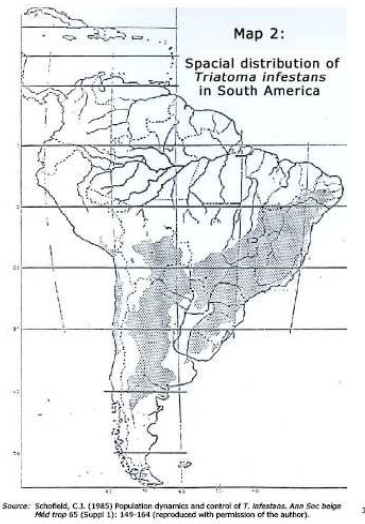
# 2. ICONOGRAPHY

Armadillo



Agouti



Opposum

Vector distribution in Central America (2004). Source PAHO



**Distribution of**
*Rhodnius prolixus & Triatoma dimidiata*
(Central America, 2004)

Honduras

Mexico

Guatemala

Nicaragua

El Salvador

Costa Rica

Panama

Colombia

T. dimidiata

R. prolixus

0    250    500    1,000
Kilometers

Distribution of *T. infestans* in South America.



Southern Cone areas where interruption of vectorial transmission have been achieved.

Xenodiagnosis: Laboratory-bred, clean reduviid bufs are fed on patients suspected of having trypanosomiasis.



Romaña's sign

# References

[i] Aufderheide AC et al. A 9,000-year record of Chagas' disease. *Proc Natl Acad Sci U S A*, 2004, 101(7):2034-9.

[ii] Salvatella R et al. [Seroprevalence of T.cruzi infection in 6- and 12- year-old school children from three Uruguayan endemic departments]. *Bol Chil Parasitol*, 1999, 54(3-4):51-6.

[iii] Gascon J. [Diagnosis and treatment of imported Chagas' disease]. *Med Clin (Barc)*, 2005, 125(6):230-5.

[iv] Muñoz J and Gascon J. [Chagas' disease imported]. *Enf Emerg*, 2005, 7(3):134-8.

[v] Peters W and Gilles HM. Chagas' disease. In: Peters W and Pilles HM, eds. *A colour atlas of tropical medicine and parasitology*. 3rd ed. English Language Book Society/Wolfe Publishing, 1989:48-52.

[vi] World Health Organization. Parasitology. In: *Control of Chagas disease*. WHO Technical Report Series nº 905, Geneva, 2002:35-40.

[vii] El-Sayed NM et al. The genome sequence of *Trypanosoma cruzi*, the etiologic agent of Chagas disease. *Science*, 2005, 309(5733):409-15.

[viii] Huete-Perez JA et al. Genomic and proteomic approaches for Chagas' disease: critical analysis of diagnostic methods. *Expert Rev Mol Diagn*, 2005, 5(4):521-30.

[ix] Herrera L. et al. *Trypanosoma cruzi* infection in wild mammals of the National Park 'Serra da Capivara' and its surroundings (Piaui, Brazil), an area endemic for Chagas disease. *Trans R Trop Med Hyg*, 2005, 99:379-88.

[x] World Health Organization. Natural reservoirs. In: *Control of Chagas disease*. WHO Technical Report Series nº 905, Geneva, 2002:55-8.

[xi] http://www.dpd.cdc.gov/DPDx/HTML/ImageLibrary/TrypanosomiasisAmerican_il.htm (accessed on 26-9-2005).

[xii] Pirard M et al. The validity of serologic tests for *Trypanosoma cruzi* and the effectiveness of transfusional screening strategies in a hyperendemic region. *Transfusion*, 2005, 45:554-61.

[xiii] Schmunis G.A. and Cruz J.R. Safety of the blood supply in Latin America. *Clin Microbiol Rev*, 2005, 18(1): 12-29.

[xiv] Hernandez-Becerril N et al. Blood transfusion and iatrogenic risks in Mexico city. Anti-*Trypanosoma cruzi* seroprevalence in 43,048 blood donors, evaluation of parasitemia, and electrocardiogram findings in seropositive. *Mem Inst Oswaldo Cruz*, 2005, 100(2):111-6.

[xv] Reesink HW. European strategies against the parasite transfusion risk. *Transfusion Clinique et Biologique*, 2005, 12:1-4.

[xvi] Aznar C et al. Seroprevalence of *Trypanosoma cruzi* infection in French Guiana. *Mem Inst Oswaldo Cruz*, 2004, 99(8):805-8.

[xvii] Ferreira AAP et al. Immunosensor for the diagnosis of Chagas' disease. *Biosens Bioelectron*, 2005, 21:175-81.

[xviii] Schenone H et al. Congenital Chagas disease of second generation in Santiago, Chile. Report of two cases. *Rev Inst Med Trop S Paulo*, 2001, 43(4):231-2.

[xix] Di Pentima M.C. et al. Prevalence of antibody to *Trypanosoma cruzi* in pregnant Hispanic women in Houston. *Clin Infect Dis*, 1999, 28(6):1281-5.

[xx] http://dtr2001.saude.gov.br/svs/destaques/chagas0404.htm (accessed on 20-10-2005)

[xxi] Freilij H. [Clinical findings on 176 children with congenital Chagas]. Consulta Técnica Regional OPS/MSF sobre Organización y Estructura de la Atención Médica del Enfermo e Infectado por *T.cruzi* /Enfermedad de Chagas. Montevideo, 13 y 14 octubre 2005

[xxii] Gurgel CB et al. Frequency of hypertension in chronic Chagas' disease: retrospective clinical study. *Arq Bras Cardiol*, 2003, 81(6):545-8.

[xxiii] Harms G and Feldmeier H. The impact of HIV infection on tropical diseases. *Infect Dis Clin North Am*, 2005, 19(1):121-35.

[xxiv] Ramos Jr AN. [Inclusion of Chagas' disease reactivation as a condition for AIDS case definition to epidemiological surveillance in Brazil]. *Rev Soc Bras Med Trop*, 2004, 37(2):192-3.

[xxv] Leon-Sarmiento FE et al. [Neurological effects of American trypanosomiasis: clinical aspects]. *Biomedica*, 2003, 23(4):462-75.

[xxvi] Levin MJ and Paveto C. A case of mistaken molecular identity. http://www.eurekalert.org/pub_releases/2006-02/hhmi-aco022406.php (accessed on 28-2-2006)

[xxvii] Fischer A et al. Increased trypanolytic activity in sera of sleeping sickness patients after chemotherapy. *Trop Med Int Health*, 2001, 6(12):1070-4.

[xxviii] Galvao LMC et al. PCR assay for monitoring *Trypanosoma cruzi* parasitemia in childhood after specific chemotherapy. *J Clin Microbiol*, 2003, 41(11):5066-70.

[xxix] Vasquez JE et al. Serological diagnosis of *Trypanosoma rangeli* infected patients. A comparison of different methods and its implications for the diagnosis of Chagas' disease. *Scand J Immunol*, 1997, 45:322-30.

[xxx] Umezawa ES et al. Serodiagnosis of chronic and acute Chagas' disease with *Trypanosoma cruzi* recombinant proteins: results of a collaborative study in six American countries. *J Clin Microbiol*, 2004, 42(1):449-52.

[xxxi] Villagran ME et al. Use of an iron superoxide dismutase excreted by *Trypanosoma cruzi* in the diagnosis of Chagas' disease: seroprevalence in rural zones of the state of Queretaro, Mexico. *Am J Trop Med Hyg*, 2005, 73(3):510-6.

[xxxii] Gomes YM et al. Serodiagnosis of Chronic Chagas Infection by Using EIE-Recombinant-Chagas-Biomanguinhos Kit. *Mem Inst Oswaldo Cruz*, 2001, 96(4):497

[xxxiii] Rabello A et al. Serodiagnosis of *Trypanosoma cruzi* Infection Using the New Particle Gel Immunoassay-ID-PaGIA Chagas. *Mem Inst Oswaldo Cruz*. 1999;Vol94; Suppl. I, 77-82

[xxxiv] Umezawa ES et al. Immunoblot Assay Using Excreted-Secreted Antigens of *Trypanosoma cruzi* in Serodiagnosis of Congenital, Acute, and Chronic Chagas' Disease. J Clin Microbiol, 1996, 34(9):2143–7.

[xxxv] Silveira-Lacerda E.P. et al. Chagas' disease: application of TESA-blot in inconclusive sera from a Brazilian blood bank. *Vox Sang*, 2004, 87:204-7.

[xxxvi] Luquetti AO et al. Chagas' disease diagnosis: a multicentric evaluation of Chagas Stat-Pak, a rapid immunochromatographic assay with recombinant proteins of *Trypanosoma cruzi*. *Diagn Microbiol Infect Dis*, 2003, 46(4):265-71.

xxxvii Ponce C et al. Validation of a Rapid and Reliable Test for Diagnosis of Chagas' Disease by Detection of Trypanosoma cruzi-Specific Antibodies in Blood of Donors and Patients in Central America. *J Clin Microbiol*, 2005, 43(10):5065-8.

xxxviii Pinto Dias JC. [Management of the *Trypanosoma cruzi* infected: conceptual frameworks]. Consulta Técnica Regional OPS/MSF sobre Organización y Estructura de la Atención Médica del Enfermo e
Infectado por *T.cruzi* /Enfermedad de Chagas. Montevideo, 13 y 14 octubre 2005

xxxix Brazilian Consensus on Chagas disease. *Rev Soc Bras Med Trop.* 2005;38 Suppl 3:7-29.

xl Kamiji MM and de Oliveira RB. [Features of Chagas' disease patients with emphasis on digestive form, in a tertiary hospital of Ribeirao Preto, SP]. *Rev Soc Bras Med Trop*, 2005, 38(4):305-9.

xli Salis GB et al. Pneumatic dilatation in achalasia of the esophagus: a report from Argentina. *Dis Esopha*, 2004, 17:124-8.

xlii Coura JR and de Castro SL. A critical review on Chagas disease chemotherapy. *Mem Inst Oswaldo Cruz*, 2002, 97(1):3-24.

xliii Schijman AG et al. *Trypanosoma cruzi* DNA in cardiac lesions of Argentinean patients with end-stage chronic Chagas heart disease. *Am J Trop Med Hyg*, 2004, 70(2):210-20.

xliv Andrade AL et al. Short report: benznidazole efficacy among *Trypanosoma cruzi*-infected adolescents after a six-year follow-up. *Am J Trop Med Hyg.* 2004, 71(5):594-7.

xlv Villar JC et al. Trypanocidal drugs for chronic asymptomatic *Trypanosoma cruzi* infection. Cochrane Database Syst Rev 2002; CD003463.

xlvi Villar JC. Desenlaces clínicos de sujetos con infección crónica por *Trypanosoma cruzi* tratados o no con agentes tripanocidas: Un metaanálisis de estudios observacionales. MEDUNAB 2002; 5:166-173.

xlvii Viotti R et al. Clinical Predictors of Chronic Chagasic Myocarditis Progression. *Rev Esp Cardiol* 2005; 58(9):1037-1044.

xlviii Docampo R. Sensitivity of parasites to free radical damage by antiparasitic drugs. *Chem Biol Interactions*, 1990, 73:1-27.

xlix Torrico F. [Treatment of chagasic infected children under 5 years: drug adverse reactions 2001-2004]. Consulta Técnica Regional OPS/MSF sobre Organización y Estructura de la Atención Médica del Enfermo e Infectado por *T.cruzi* /Enfermedad de Chagas. Montevideo, 13 y 14 octubre 2005.

l Morote S. [Access to diagnosis and treatment for Chagas' disease]. Consulta Técnica Regional OPS/MSF sobre Organización y Estructura de la Atención Médica del Enfermo e Infectado por *T.cruzi* /Enfermedad de Chagas. Montevideo, 13 y 14 octubre 2005.

li PAHO/WHO. [Etiological treatment of Chagas' disease]. Conclusiones de una consulta tecnica. OPC/HCP/HCT/140/99, 32 pp. (published in *Rev Patol Trop* 28:247-79, 1999).

lii PAHO/WHO. [Etiological treatment of Chagas' disease]. Conclusiones de una consulta tecnica. OPC/HCP/HCT/140/99, 32 pp. (published in *Rev Patol Trop* 28:247-79, 1999).

liii Akhavan D. Analise custo-efetividade do programa de controle da doença de Chagas no Brasil. Organizaçao Pan-americana da Saude, Brasilia, Brazil, 2000, p.7-9

liv XIIa. Reunión Intergubernamental INCOSUR/Chagas, Santiago, Chile, marzo de 2003, pp. 11-34.

lv Organización Panamericana de la Salud. Taller del Cono Sur sobre Enfermedad de Chagas: Conceptualización de la vigilancia epidemiológica (Bs.As., Argentina, septiembre 2003)

lvi Law 22.360, art. 7, in Argentina.

lvii Goldbaum M et al. American trypanosomiasis and electrocardiographic alterations among industrial workers in Sao Paulo, Brazil. *Rev. Inst. Med. Trop. S. Paulo,* 2004, 46(6):299-302.

lviii World Health Organization. Epidemiology and incidence trends. In: *Control of Chagas disease*. WHO Technical Report Series n° 905, Geneva, 2002:59-69.

lix Noireau F. et al.  Can wild *Triatoma infestans* foci in Bolivia jeopardize Chagas disease control efforts? Trends Parasitol, 2005, 21(1):7-10.

lx Organizacion Panamericana de la Salud. V Reunion de la Comision Intergubernamental de la Iniciativa Andina. Lima, Peru, 6-7 mayo 2004.

lxi Organizacion Panamericana de la Salud. VI Reunion de la Iniciativa Andina para el Control de la Enfermedad de Chagas. Bogota, Colombia, 2-6 mayo 2005.

lxii Guhl F. et al. Lessons from a national survey of Chagas disease transmission risk in Colombia. *Trends Parasitol*, 2005, 21(6):259-62.
lxiii Organizacion Panamericana de la Salud. Reunion Internacional para el Establecimiento de Criterios de Certificacion de la Eliminacion de *Rhodnius prolixus*. Ciudad Guatemala, Guatemala, 5-7 marzo 2003.

lxiv Technical report. [Reuniao internacional sobre vigilancia e prevençao da doença de Chagas na Amazonia. Implementaçao da Iniciativa intergovernamental de Vigilacia e Prevançao da doença de Chagas na Amazonia. *Rev Soc Bras Med Trop*, 2005, 38(1):82-9.

lxv Coura JR et al. Emerging Chagas disease in Amazonian Brazil. *Trends Parasitol*, 2002, 18(4):171-6.

lxvi Heringer-Walther S et al. Brain natriuretic peptide predicts survival in

Chagas' disease more effectively than atrial natriurectic peptide. *Heart*,

2005, 91:385-7

lxvii Melo RB et al. [Measurement of human brain natriuretic peptide in patients with Chagas' disease]. *Arq Bras Cardiol*, 2005, 84(2):137-40.

lxviii Ribeiro AL et al. Brain natriuretic peptide based strategy to detect left ventricular dysfunction in Chagas disease: A comparison with the conventional approach. *Int J Cardiol*, 2005 Jul 13.

lxix http://www.encuentraprobnp.com

lxx Bhatia V et al. Utility of the *Trypanosoma cruzi* Sequence

Database for Identification of Potential Vaccine Candidates by In Silico

and In Vitro Screening. *Infect Immun*, 2004, 72(11):6245–54.

lxxi Araujo AF. et al. CD8+-T-cell-dependent control of *Trypanosoma cruzi* infection in a highly susceptible mouse strain after immunization with recombinant proteins based on amastigote surface protein 2. *Infect Immun*, 2005, 73(9):6017-25.

lxxii Croft SL et al. Chemotherapy of trypanosomiases and leishmaniasis. *Trends Parasitol*, 2005, 21(11):508-12.

[lxxiii] Urbina JA and Docampo R. Specific chemotherapy of Chagas disease: controversies and advances. *Trends Parasitol*, 2003, 19(11):495-501.

[lxxiv] http://www.clinicaltrials.gov/ct/gui/show/NCT00123916 (accessed on 20-10-2005)

[lxxv] Apt W et al. Itraconazole or allopurinol in the treatment of chronic American trypanosomiasis: the regression and prevention of electrocardiographic abnormalities during 9 years of follow-up. *Ann Trop Med Parasitol*, 2003, 97:23-9.

[lxxvi] World Health Organization. Tropical disease research, Twelfth Programme Report, UNDP - World Bank - World Health Organization Programme for Research and Training in Tropical diseases, 1995, pp. 129-133.

[lxxvii] Freymann DM et al. Efficient incorporation of inhibitors targeting the closed active site conformation of HPRT from *Trypanosoma cruzi*, *Chem Biol*, 2000, 7:957-68.

[lxxviii] Rivarola HW et al. *Trypanosoma cruzi*: Chemotherapeutic effects of clomipramine in mice infected with an isolate obtained from an endemic area. *Exp Parasitol*, 2005, 111(2):80-6.

[lxxix] Blandon R et al. La experiencia clínica en Panamá con metronidazol en el tratamiento de la enfermedad de Chagas en las fases aguda y crónica. [The clinical experience in Panama with metronidazole in treating Chagas' disease in the acute and chronic phases]. *Rev Med Panama*, 1993, 18(2):94-100.

[lxxx] Senkovich O et al. Lipophilic antifolate trimetrexate is a potent inhibitor of *Trypanosoma cruzi*: prospect for chemotherapy of Chagas' disease. *Antimicrob Agents Chemother*, 2005, 49(8):3234-8.

[lxxxi] Garzoni LR et al. Antiparasitic activity of risedronate in a murine model of acute Chagas' disease. *Int J Antimicrob Agents*, 2004, 23(3):286-90.

[lxxxii] Bouzahzah B et al. Risedronate in the treatment of Murine Chagas' disease. *Parasitol Res*, 2005, 96(3):184-7.

[lxxxiii] Szajnman SH et al. Synthesis and biological evaluation of 1-amino-1,1-bisphosphonates derived from fatty acids against *Trypanosoma cruzi* targeting farnesyl pyrophosphate synthase. *Bioorg Med Chem Lett*, 2005, 15:4685-90.

[lxxxiv] Martin MB et al. Bisphosphonates inhibit the growth of *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania donovani*, *Toxoplasma gondii* and *Plasmodium falciparum*: a potencial route to chemotherapy. *J Med Chem*, 2001, 44: 909-916.

[lxxxv] Steverding D and Tyler KM. Novel antitrypanosomal agents. *Expert Opin Investig Drugs*, 2005, 14(8):939-55.

[lxxxvi] Urbina JA et al. In Vitro and In Vivo Activities of E5700 and ER-119884, Two Novel Orally Active Squalene Synthase Inhibitors, against *Trypanosoma cruzi*. *Antimicrob Agents Chemother*, 2004, 48(7):2379–87.

[lxxxvii] Oliveira DA et al. Trypanocidal activity of 2-propen-1-amine derivatives on trypomastigotes culture and in animal model. *Parasitol Res*, 2005, 95:161–6.

[lxxxviii] Urbina JA et al. Antiproliferative Effects and Mechanism of Action of SCH 56592 against *Trypanosoma (Schizotrypanum) cruzi*: In Vitro and In Vivo Studies. *Antimicrob Agents Chemother*, 1998, 42(7):1771–7.

[lxxxix] Molina et al. Activities of the Triazole Derivative SCH 56592 (Posaconazole) against Drug-Resistant Strains of the Protozoan Parasite *Trypanosoma(Schizotrypanum) cruzi* in Immunocompetent and Immunosuppressed Murine Hosts. *Antimicrob Agents Chemother*, 2000, 44(1):150-5.

[xc] Dodds Ashley ES and Alexander BD. Posaconazole. *Drugs Today (Barc)*, 2005, 41(6):393-400.

[xci] Ceaser M. Battling with Chagas disease in South America. *Lancet Infect Dis*, 2005, 5(8):470-1.

[xcii] Benaim G et al. Amiodarone has intrinsic anti-Trypanosoma cruzi activity and acts synergistically with posaconazole. *J Med Chem,* 2006, 49(3):892-9.

[xciii] Molina J et al. In vivo activity of the bis-triazole D0870 and drug resistant strains of the protozoan parasite *Trypanosoma cruzi*. *J Antimicrob Chemother*, 2000, 46:137-40.

[xciv] Molina J et al. Cure of experimental Chagas´disease by the bis-triazole D0870 incorporated into "stealth" polyethyleneglycol-polylactide nanospheres. *J Antimicrob Chemother*, 2001, 47:101-4.

[xcv] Williams KJ and Denning DW. Termination of development of D0870. *J Antimicrob Chemother*, 2001, 47(5):720-1.

[xcvi] Urbina JA et al. In vitro and in vivo activities of ravuconazole on *Trypanosoma cruzi*, the causative agent of Chagas disease. *Int J Antimicrob Agents*, 2003, 21(1):27-38.

[xcvii] Urbina JA et al. In Vitro Antiproliferative Effects and Mechanism of Action of the New Triazole Derivative UR-9825 against the Protozoan Parasite *Trypanosoma* (*Schizotrypanum*) *cruzi*. *Antimicrob Agents Chemother*, 2000, 44(9):2498–2502.

[xcviii] Da Matta Guedes PM et al. Activity of the New Triazole derivative Albaconazole against*Trypanosoma* (*Schizotrypanum*) *cruzi* in Dog Hosts. *Antimicrob Agents Chemother*, 2004, 48(11):4286-92.

[xcix] Urbina JA et al. Parasitological cure of acute and chronic experimental Chagas disease using the long-acting experimental triazole TAK-187. Activity against drug-resistant *Trypanosoma cruzi* strains. *Int J Antimicrob Agents*, 2003, 21(1):39-48.
[c] Corrales M et al. Comparative efficacies of TAK-187, a long-lasting ergosterol biosynthesis inhibitor, and benznidazole in preventing cardiac damage in a murine model of Chagas' disease. *Antimicrob Agents Chemother*, 2005, 49(4):1556-60.

[ci] Orenes Lorente S et al. Novel azasterols as potential agents for treatment of leishmaniasis and trypanosomiasis. *Antimicrob Agents Chemother*, 2004, 48(8):2937-50.

[cii] Saraiva VB et al. (2002) Proinflammatory and cytotoxic effects of hexadecylphosphocholine (miltefosine) against drug-resistant strains of *Trypanosoma cruzi*. *Antimicrob Agents Chemother*, 2002, 46:3472–7

[ciii] Santa-Rita RM et al. Anti-proliferative synergy of lysophospholipid analogues and ketoconazole against *Trypanosoma cruzi* (Kinetoplastida: *Trypanosomatidae*): cellular and ultrastructural analysis. *J Antimicrob Chemother*, 2005, 55(5):780-4.

[civ] http://washington.bizjournals.com/washington/stories/2002/02/25/story8.html (accessed on 30-9-2005)

[cv] Lo Presti M.S. et al. Thioridazine treatment prevents cardiopathy in *Trypanosoma cruzi* infected mice. *Int J Antimicrob Agents*, 2004, 23(6):634-6.

[cvi] Nesslany F et al. In vitro and in vivo chromosomal aberrations induced by megazol. *Mutat Res*, 2004, 560: 147-158.

[cvii] Carvalho SA et al. Synthesis and antitrypanosomal profile of new functionalized 1,3,4-thiadiazole-2-arylhydrazone derivatives, designed as non-mutagenic megazol analogues. *Bioorg Med Chem Lett*, 2004, 14:5967-70.

[cviii] Pozas R et al. Synthesis and *in vitro* antitrypanosomal activity of novel Nifurtimox analogues. *Bioorg Med Chem Lett*, 2005, 15(5):1417-21.

[cix] Bua J et al. In vitro anti-parasitic activity of Cyclsoporin A analogs on *Trypanosoma cruzi. Bioorg Med Chem Lett*, 2004, 14:4633-7.

[cx] Franck X et al. Biological evaluation of substituted quinolines. *Bioorg Med Chem Lett*, 2005, 14:3635-8.

[cxi] Ribeiro TS et al. Toxic effects of natural piperine and its derivatives on epimastigotes and amastigotes of *Trypanosoma cruzi*. *Bioorg Med Chem Lett*, 2004, 14:3555–8.

[cxii] Goncalves AR et al. Ultrastructural alterations and growth inhibition of *Trypanosoma cruzi* and *Leishmania major* induced by *Bothrops jararaca* venom. *Parasitol Res*, 2002, 88(7):598-602.

[cxiii] Lockman JW and Hamilton AD. Recent developments in the identification of chemotherapeutics for Chagas disease. *Curr Med Chem*, 2005, 12(8):945-59.

[cxiv] Paulino M et al. The chemotherapy of chagas' disease: an overview. *Mini Rev Med Chem*, 2005, 5:499-519.