“Focus on Neglected Tropical Diseases: Chagas Disease a Public Health Threat in the Americas & Beyond”

FONDATION MERIEUX in Partnership with the WHO
MEETING REPORT

CONFIDENTIAL VERSION

The Chagas Disease meeting organized by Fondation Mérieux was held at “Les Pensieres” Conference Center from May 5 to the 7, 2008 in Veyrier du Lac, France. The meeting brought together foremost international experts from North America, Latin America & Europe, scientific personalities that have performed private and public research investigation on the subject.

The following report summarizes the information provided during the meeting based on abstracts and speaker’s lectures, all procedure specifics are not detailed in this report.

Meeting Reporter: Valentina Picot
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Disclaimer

Information on this report was obtained from the lectures and abstracts given by the speakers as per scientific agenda of the Fondation Mérieux meeting “Chagas Disease a Public Health Threat in the Americas & Beyond” in partnership with the World Health Organization, held in France in May 2008, at “Les Pensieres” conference center in Veyrier du lac France. All graphs, flow charts and images were obtained from the speaker’s presentations to facilitate the comprehension on the subject. The information posted on the articles was authorized as per signed authorization form by the speakers in question. The articles were done for overall meeting reporting information purposes a modified form of vulgarization of this information might require further speaker's authorization.

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I. Meeting Objective

- To consolidate knowledge on the disease novel findings.
- To assess current program strategies and identify needs and gaps where actions can be taken to respond to the entire scope of Chagas disease.
- To foster knowledge and feedback sharing.
- To serve as a nest for the offset of collaborative efforts.
II. Summary of Scientific Agenda Lecture Presentations

The meeting was presented in sessions as follows.

1. Welcome Address & Keynote Presentation

2. Session I: GNChE, Triatominae Vector and Information Systems
   a. Chaired by: Mario Zaidenberg
   b. Lectures Briefings

3. Session II: T.cruzi, Immune Response and Diagnosis in Chagas Disease
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1. Welcome Address & Keynote Presentation

Christophe Longuet, Medical Director of the Mérieux Foundation, welcomed the speakers and participants to “Les Pensières” conference center. He presented the foundation’s mission: to control infectious diseases in developing countries by supporting scientific research, sharing knowledge and supporting health structures, patients and their families. The presentation allowed participants to better understand the scope and role of the Mérieux Foundation in disease control activities, emphasizing that the Advances in Infectious Disease Modelling meeting is one aspect of the foundation’s knowledge sharing activities.

1.1. Keynote Lecture
Project of Intervention in Primary Students Infected with Chagas Disease in Salta City
Hector Freilij, Chief Diseases Centre, Pediatric Hospital Ricardo Gutierrez, Buenos Aires, Argentina

To perform this study, the province of Salta was chosen as it is located in a Chagas problematic zone know as the Chaco. Thanks to the funding received by Fondation Mérieux we were able to screen a great number of children that were not normally fully covered by social medical services with regards of Chagas disease.

Some background regarding Chagas Disease also known as American Trypanosomiasis. Chagas disease is an anthorpozoonosis caused by Trypanosoma cruzi, it was discovered in 1909 by the brilliant parasitologist Carlos Chagas, this is a lifelong chronic bloodstream infection in vetebrate hosts including humans.

The transmission routes can be done via vectorial when hematophagus reduviid bites the host’s skin T. cruzi parasites are deposited with the insecte faeces, and penetrates the host; via blood transfusions from infected donors; via transplacentally, and other less common ways such as transplanted organs, etc.

Triatoma infestans: Principal domiciliary vector in Argentina.
Chagas treatment is very limited as there are not many efficient molecules against the parasite, and they act during a specific period. More than a 100 drugs have been tested and only two have been accepted they are: Nifurtimox and Beznidazole. Treatment have more often being addressed with the Beznidazole, but there is the intention to go for the Nifurtimox in pediatrics as it shows a lot less collateral effects.

Following a map that shows the geographical location of Salta’s province in Argentina:

The following map shows the distribution of the domiciliary vector in Argentina, and related cases of acute Chagas disease.
The map shows the related cases of acute Chagas diagnosed clinically, but for each kid with a manifested acute Chagas there is an important percentage (about 30 to 40%) of kids with acute Chagas without clinical manifestations.

The epidemiologic characteristics of the Salta province in the last 20 years are as follows:

- Low level of domiciliary vector infestation. (< 3 %).
- However, still many areas with major number of infested houses. This situation maintains the population in permanent risk of infection.
- Variable prevalence of human infection.

Some numbers regarding the prevalence of the disease in Salta’s province:

**Rate of Infection**

- In general population: 6.7 %
- In pregnant women: 8 %.
- In Blood banks: 4.2 %.
- Incidence of infected newborn from infected mothers: 5 %.

These numbers are the mix result of:

- The native population with more than 20 years of residence in the area.
A big issue with Chagas disease is the urbanization of the disease, going from rural areas to the cities. For example, Salta is a city free of vectors however there is an important infected population rate. In this case, the disease is mainly transmitted from maternal-fetal transmission, blood transfusion. It’s important to take into account that the existence of Chagas disease in a given population increases the development of cardiac and gastrointestinal pathologies.

Great emphasis has focused on the serological testing and screening of the disease but little in the follow up treatment for the positive cases. Public health screening is indeed important to determine the disease status, and also the impact of vector control activities, etc, but lack of treatment makes irrelevant the finding of positive cases.

The study here described aims to diagnose and treat Chagas disease in the seropositive children, and importantly to sensitize the public health authorities about the fundamental importance for diagnosing and treating children and adolescent infected with *T. cruzi*. This taking into consideration that the rate of disease recovery after treatment in kids accounts for 80 to 90%.

Salta’s province last census in 200: Population: 462,015, Primary scholar population (up to 15 years): 45,300, the study aims to address 20,000 cases in the scholar population in the province as per the following study objectives:

- Determine the prevalence of Chagas disease in children of school age in Salta city and its vicinity.
- Identify infected children and treat them according to national Norms.

**Specific objectives:**
- Perform a serologic diagnosis in children under 15 years which assist to schools in Salta city and its vicinity. To treat the infected children.

The study was performed as per good clinical practices, for regulatory approvals, document compliance and in general as per protocol. Following the study flow chart:
Serology was performed by HAI and Elisa, pk samples were taken in Serokits.

Current status of the study in Salta:
- A total of 29 schools studied
- 14182 enrolled children.
- 127 seropositive cases to the double serology testing.
- Venous blood testing reconfirmation was done to 82 of the 127 sero+, and 78 were confirmed sero+.
- Treatment evolved as follows: 37 finished treatment, 8 still under treatment, 4 abandoned treatment, and 2 did not want to receive treatment.
- Minor adverse events where noted.

The routes of infection of the screened children were as follows:
- Congenital: 80.5%
- Transfusional: 4.8%
- Vectorial: 2.4%
- Unknown: 12.3%

The exams done to the children at the beginning, middle and end of the study as per protocol were:
Biochemical exams (hemogram, creatinin, liver function), and the EKG, these exams were normal.
Due to the congenital transmission of the disease, once a child was detected positive in a given school, his/her brothers or sisters were also included in the study for screening purposes.

A total of 87 siblings were studied, that gave a total of 19 seropositives, and a confirmed total of 19 seropositives after venous sample testing; from this 7 finished treatment, 3 are under treatment and 0 abandoned.

The study prolonged its scope to a more north city than Salta, a city called Taco Pozo.

Status of the advancement of the study in Taco Pozo:

- 7 Schools studied.
- 1489 enrolled children.
- 27 seropositive cases to the double serology testing.
- Venous blood testing reconfirmation was obtained from 25 children out of the 27 sero+.
- Treatment evolved as follows: 21 finished treatment, 8 still under treatment.
- Minor adverse events were noted.
Some pictures to show the living conditions of the habitants in Taco Pozo where the vector finds perfect habitat to breed.

The study in summary
- Number of studied children: 15,947
- Positive by screening: 176
- Positive studied by veined blood: 125
- Children who ended the treatment: 65
- Children under treatment: 18
- 2 children discontinued the treatment
- 2 children decided not to be treated

Some of the obstacles found during the study were: Strikes of the teaching staff, lack of support by the teaching staff, difficulty in receiving the kits, difficulty in meeting the positive screened patients to perform venous blood collection.

To heal a child from Chagas allows:
- Avoiding a cardiopathy.
- Avoiding new cases of congenital Chagas disease.
- Increasing the amount of organ and blood donors.
- Avoiding the discrimination for working and studying.
2. Session I: GNChE, Triatominae Vector and Information Systems

The Globalization of Chagas Disease: A New Challenge for Elimination
Jean Jannin, HIV/AIDS, Tuberculosis, Malaria and Neglected Tropical Diseases (HTM), World Health Organization, Geneva, Switzerland
Neglected Tropical Diseases Control Innovative and Intensified Disease Management

When addressing Chagas disease, the question that rises is What WHO can do and can provide to support Chagas disease control? And from this to determine:
What more can be done to control it? Should we continue to follow the same scheme for progressing?
Now, taking into account the progress made and the feasibility of elimination, can we deduct that there is a need for changing our vision? This considering that the target is to eliminate Chagas disease by 2010, a target objective that is unlikely to be met. A new resolution to extend the target date will be required.

Quotation from Dr Margaret Chan, Director-General of the World Health Organization
4 January 2007 – Addressed to WHO staff

"The neglected tropical diseases provide another example of our solidarity. These diseases do not travel internationally, threaten the health or economies of wealthy countries, or make headline news. Yet they cause immense suffering and disability for millions of people and anchor them in poverty. The world is now paying attention to these diseases and making progress in unprecedented ways, with ambitious goals, excellent interventions, and growing evidence of multiple benefits for health. This attention to long-neglected diseases is a positive sign that health is a responsibility shared by the international community"

In another speech in Bangkok in February 1, 2007, stated “The control of neglected tropical diseases is a pro-poor initiative, a poverty reduction strategy for the masses.”
She ask few questions among them, one that it is key for disease control “what forces need to come together to make this access possible?”

These statements show the acknowledgment by the WHO organization of the importance that neglected diseases has and is taking in today’s public health, and the clear desire to implement programs that will address them.

In terms of Chagas disease, two main questions were access during different meetings:
• How to provide more support and to re-enforce capacities for reaching the goal of complete control/elimination.
• How WHO can provide a framework for strengthening links between all people involved in Chagas control.

In this vision, in February 2007 in Copan a proposal for the establishment of a WHO global network for Chagas disease elimination was settled. This based on the concept of program coordination and networking.
In April 2007, during the NTD Global partner’s meeting, Chagas disease was brought up as part of the list of diseases that are taking a relevant place in the strategy for disease elimination at the WHO.

As for many diseases the control is divided in several parts, in the case of Chagas see graph bellow:

The different groups that create the strategy for disease elimination sometimes can be found in an environment where competition triggered by fund raising or other motives leads to overall negative outcomes. The idea is to integrate all these parts in one sole strategy to target a sustainable elimination strategy.

Proposed steps for Chagas disease elimination:
1. Create the international momentum, which has already started taken place.
3. Mobilize resources.
4. Establish a global consensus for the most adequate strategies.

In a meeting held in 2004 with Bayer, a 2 year agreement for the donation of Nifurtimox tablets was allocated for Sleeping sickness, the presence of experts in Chagas disease during that meeting created first momentum in the effort to obtain also a donation of Nifurtimox for Chagas disease. Other agreements with Bayer took place to expand the initial one which included allocations of Nifurtimox for Chagas disease. Last 5 year agreement was signed in April 2007, this included Nifurtimox donation and a cash donation to expand the program.
A meeting held in 2007 at the WHO, gave birth to a concrete WHO global network for Chagas Disease elimination (GNChE). The network consists of addressing:

- Surveillance (vector – cases – blood screening).
- Consensus for diagnostic tools.
- Consensus for case management.
- Chagas in non endemic countries.

This global network has taken a greater scope as the acknowledgement of the disease spread in non endemic countries is a relevant issue today with this disease.

As expressed by Dr Margaret Chan, WHO Director- general at a speech in Geneva on July 2007. "The establishment of the WHO Global Network to combat Chagas disease occurs in the broader context of the WHO’s renewed fight against neglected tropical diseases. The prospects for reducing the burden caused by these diseases have changed dramatically in the past few years. While Chagas disease is controlled in many countries in the Americas, commitment must be strengthened as elimination of the disease is now attainable. Cases identified in non-endemic countries have demonstrated the need to globalize our efforts."

Following the different Chagas disease initiatives:
The idea of creating the GNChE is to integrate initiatives and to provide a framework for them to:

- Facilitate the integration of their activities.
- Join efforts
- Provide flexibility and avoid further layers of bureaucracy.
- Provide Secretariat, Management support.
- Advocacy
- Resource mobilization
- Dissemination of information, achievement and reports

The GNChE has established Technical Groups (TG) to address the disease, as follows:

1. TG on epidemiological surveillance and information systems: communities, vectors, cases, and other factors relevant to transmission

2. TG on prevention of transfusional and organ transplantation transmission of *T. cruzi* in endemic and non-endemic countries.

3. TG on diagnostic test(s) for screening and diagnosis of *T. cruzi* infections. To identify improvements needed.

4. TG on prevention and control of congenital transmission and case management of congenital and non-congenital infections. Case-finding, diagnosis, and treatment strategies at different health care levels that can be applied in endemic and non-endemic countries.

Regarding Treatment drugs:
It is fundamental to have accurate forecast for drug needs to be able to intervene appropriately before industry to obtain drug allocations that will not signify future waist, and to establish credibility for future demands. Also is important that each distributed stock be follow up in terms of distribution and results to be able to provided accurate reports of how the drugs have been used, and on treatment monitoring system – SAE Monitoring of drug efficacy

In terms of Nifurtimox 2,500,000 tablets for 5 years 2007-2011 provided by Bayer and distributed by the WHO and PAHO.

In terms of Benznidazole in Feb 2008, 600,000 tabs will available for sale, and there is enough raw material to produce 6 million tablets, not stock shortage is anticipated. However, there are many unknowns as to the costs, the distribution network, and issues with registration processes.

Goals for the Chagas Elimination Global Network

- Re-enforce inter-countries vector control initiatives.
- Increase the epidemiological surveillance system (including vectors and cases).
- Increase and harmonize screening methods for blood banks and organs transplants in endemic and non endemic countries.
- Define a strategy for prevention of vertical transmission.
• Improve the standardization and decision value of diagnostic tools.
• Improve the decision tree for treatment.
• Develop new drugs/diagnostic tools and develop pediatric formulations.
• Definition of strategies for ensuring the sustainability of the elimination.

**Phylogeny and Evolution of Triatominae**  
*Chris Schofield, ECLAT Coordinator, LSHTIM (ITD), London, UK*

When speaking about the evolution of Triatominae one of the key features that we have to consider the evolution of blood sucking, and for this is important to know that all Triatominae sub-families are evolving from Hemiptera, Heteroptera and Reduviidae family and from this evolution some of them became predators and some of them blood suckers.

The development of bloodsucking in Hemiptera is very common, and so we can find bloodsucking insects in many different taxonomic groups of this such as the Lygaeidae, in many of the Reduviidae, and the Anthocoridae.

**Known occurrence of haematophagy in Hemiptera**

The evolution of the Triatomines goes from predator to bloodsuckers progressively, the process goes from:

- Free-living Predator
- Nest-dwelling Predator
- Facultative Blood-sucker
- Silvatic Blood-sucker
- Peridomestic +/- Domestic

Predators bugs because they aim to paralyze their host have a very toxic saliva, bite is very painful and can be fatal for some small mammals; the facultative blood sucker have less toxic saliva, bite is painful and produce a high immediate reaction; and the obligate blood-sucker have a saliva with progressively reduce toxicity, and the bite is almost imperceptible.

When observing bite reactions the severity of the bite reactions allow us to determine weather the biting bug is used to or not on feeding on people, if is a more of a domestic or more of a silvatic bug. In general Triatoma bugs bite reactions are very small or none existent, especially with highly domestic bugs. It is important not to confuse bite reactions with chagomas.

Knowing this one can say that bite reactions can help in the surveillance of type of bugs present in an endemic area.

Important to bear in mind that *T. cruzi* can be transmited to a series of hosts, and Triatominae might have played a serious role in the variability of vertebrate hosts that *T. cruzi* can infect. Other conclusion is that this route seems to be common to Hemiptera in general, not only to Reduviidae family, is common in several sub-families of Reduviidae and has resulted in a very large number of species of Triatominae sub-family, 140 species currently known.
We might well conclude that the Triatominae sub-family is not a monophyletic group; its various entities have derived from different origins at different times.

By observing different genetic aspects of the Triatominae evolution is possible to conclude that is polyphyletic, this is important as per the analysis of a given entity in a polyphyletic group is that the results obtain for one specie are not the same for the other in the same sub-family (e.g. results from Triatoma differ from those of Rhodnini).

For example when performing phenograms based on wing geometries is possible to observe clusters that gives an idea of the origins for the different entities within the group.

Although Triatominae have evolved as blood-sucking from different sources at different times, they have not done it for long as many of them still capable of being predators. Some are blood-suckers that can still show predatory capacities as the Triatomae brasiliensis.

Evidence for recent evolution of Haematophagy in Triatominae is based on:

- Biogeography
- Host associations
- Morphological similarity between bloodsuckers Triatominae and predatory Reduviidae
- Persistence of predatory behavior in many Triatominae species
- Lack of a mycetome
- Apparent capacity for rapid adaptation:
  - Genetic drift*
  - Phenetic drift**
  - Inter-sibling competition***

*The genetic diversity in T. cruzi is likely related to the ability of bloodsucking bugs to vector into different vertebrates. There are Triatominae that are morphologically indistingible but genetically quite different.

**This is Triatominaes that are morphologically very different but genetically are the same.

A final process to consider is when bugs become domestic, those bugs instead of living in a low density population in domestic living bugs get a rich supply of blood, are protected from environmental changes, it reproduces more or less through out the whole year increasing its population. Domestic houses are very close environments as a result that bug population cannot grow forever then at some point it plats off. In average at that point the rate of population growth is 1 (Ro= 1), this means each female bug is only having one daughter, if she has more the population will increase if she have less the population will decrease. Each female bug lays about 200 eggs and half of them are females, this means most of then will not reach reproductive age resulting in a very strong inter-sibling competition. Each generation can only give raise to the same number of parents, consequently the reminder do not reach reproductive age.

To conclude Triatomiane are:

- Polyphyletic assemblage of predators, facultative and obligate blood-suckers.
- Evolved from different predatory forms, probably at different times, but most fairly recently
- Evolved by a process of adaptation, genetic drift, and morphological plasticity phenetic drift.
- Evolution in domestic habits involves “Intersibling competition”
- Domestic populations tend to have reduced genetic variability
The main issue that will be addressed in this talk is about the sustainability of the Vector control and other related programs against Chagas disease.

The message in a nutshell is the following:
Current Chagas disease control programs are composed of divorced entities:
- Vector control program: field-based; routine insecticide spraying, bug detection.
- Disease control program: hospital-based; blood bank screening, diagnosis and eventual etiologic treatment, medical care.

The challenge to achieve actual sustainability is to build integrated disease control and management. One way to achieve this is to combine vector control with case detection and treatment to increase the impact and cost effectiveness of the control program, its public acceptance and long-term sustainability; however, disease case detection today is one of the less well perform activities even in the most advance programs, therefore the integration of the full program scope does not exist.

In a general consensus the aim is to:
- Reduce disease burden by reducing prevalence and incidence of human infection with T. cruzi and by reducing the disease specific death rate.
- Eliminate the implicated vector species, if feasible, this means to reach vector abundance to = 0 in a defined geographical region in the absence of control actions.

The final endpoints to be achieved sometimes cannot be that clear:
- If vector elimination is achieved throughout the distribution range of the vector (= eradication), vector surveillance is not necessary; however, this has not been achieved thus vector control, long-term surveillance is indispensable.

To main aspects of vector control is its sustainability and its cost-effectiveness.

Why do we need to do emphasis in sustainability?
Due to the fact that vector elimination has not been achieved and may not be possible to do so in most affected regions, therefore, there is a need to shift toward recurrent bug control actions through an established surveillance system.

Why vector elimination fails? Why regions where programs of sustain vector control have not achieved elimination?
1. "Tools and actors are not perfect", residual insecticides, spray teams and householders, have each their pitfalls and therefore 100% elimination results cannot be obtained.
2. "The world is not flat", the heterogeneity environmental, demographic and spatial-temporal generate hot-spots of infestation, infectiousness and of parasite transmission that cannot always be treated in a standard manner, however, control actions have been traditionally homogeneous applying the same recipe to all zones.
3. "Changes happen, and are hard to anticipate and to deal with", Changes in national, provincial and municipal authorities; recurrent political, social and economic instability; competing diseases (dengue, leish); decentralization of control programs.
Current vector control programs: These programs basically are managed by the health services. The following is a list of relevant actors that are rarely included in a consistent fashion over the whole process:

- Local school system.
- NGO: environmental, ethnic, religious.
- Governmental agencies linked to rural development.
- Universities and research institutes.
- Local lay organizations.
- Local municipalities.
- The affected individuals, households, communities.

Having all these parties involved, the questions not yet fully answered that rise are: Who are the most likely to respond when the problem reappears? and What is the evidence?

Following a graph with the apparent distribution of Triatoma infestans


In the above map can be observed the success in the control of the *Triatoma infestans* in the south cone of South America, but in the core the control has been less positive and the transmission persists. Today this scenario still very similar with certain pluri-annual variability.
The endemic persistant core area earlier described is known as the “Gran Chaco” (see graph left). This area is shared among Argentina (62%), Paraguay (25%), Bolivia (12%) and Brazil (8%), many characteristics of this area promotes disease spreading and prevalence, such as:
- Million km2.
- Million people, <5/km2.
- High poverty levels.
- Large neglected disease burden.
- Under staffed, overburdened health services.

In Brazil in June 2006, during the XV Meeting of the south-cone Inter-governmental commission for the elimination of Triatoma infestans and the interruption of the transmission of Tripanosomiasis transfusional of INCOSUR relevant conclusions were drawn stating that:
Knowledge on factors and mechanisms that determine the persistence of *T. infestans* in the Gran Chaco is a top priority of the Southern Cone Initiative since 2006.
The Gran Chaco is the last frontier in the elimination of *Triatoma infestans*. 

Domestic reinfestation by *Triatoma infestans* without vector surveillance, and with community-based, supervised vector surveillance in the Amamá area, Santiago del Estero, 1985-2004

The above graph first spray shows that if a program of insecticide spraying is maintained vector elimination can almost be reached, however, if a program for the control and surveillance is not continued a vector recuperation phase begins. Within two years of the process of vector re-infestation new cases of Chagas begin to appear.

In the process of spraying and selective control generates a completely different pattern, in this process there is not elimination vectors continue to exist but in low density.

Dynamics of *T. cruzi* infection in *T. infestans*, dogs and humans without and with sustained vector surveillance
In the above graph, when looking at the different host of the Chagas vectors, it is observed that the prevalence changes. In the humans there is a not very high prevalence that gradually goes down in a sustain manner, the sustain control of that prevalence makes it drop to zero. In dogs there is a sudden recuperation after first spray and then decadence towards zero under spraying and control actions, similar for the insects.

Following some graphs that shows the results of insecticide spraying programs and actions: Domestic infestation and annual coverage of selective insecticide sprays (% of all houses treated).

![Domestic infestation and annual coverage of selective insecticide sprays](image1)

![Peridomestic infestation and annual coverage of selective insecticide sprays](image2)
Focus on Neglected Tropical Infectious Diseases: Chagas Disease a Public Health Threat in the Americas & Beyond
Report issued: September 30, 2008

*T. cruzi* infection in domestic *T. infestans* and total domestic bug abundance
T. cruzi infection in peridomestic T. infestans and total peridomestic bug abundance

Age-specific human seroprevalence of T. cruzi before and after intervention (YPI, years post-intervention), 1985-2002. 697 people examined at least once, ~2300 results.

When observing the prevalence curves by population age in the above graph, for the first spraying shows that at seven years not changes were noted, and at 20 years post-intervention the transmission was interrupted.
These values on human incidence were compared to the values of unsupervised programs that were taking place in the peripheral area and the following results were obtained in terms of human incidence:

- Supervised, sustained control area: 0.134 per 100 person-years over 1992-2006 (3 introduced cases, N = 2,246 person-years).
- Unsupervised, sporadic control area: 0.986 per 100 person-years over 1992-2006 (N = 304 person-years).

The results show clearly that control and surveillance is fundamental for a long term positive results and the essential combination of treatment.

Why is essential the combination with treatment, following an evidence as per the data from the program performed at Santiago del Estero.

- 34 children aged < 15 years seropositive for *T. cruzi* were referred for etiologic treatment to Hospital Independencia (Santiago del Estero) between 1992-1994.
- Of 26 children who were re-examined 2-13 yrs post-treatment, 17 (65%) were seronegative for *T. cruzi* using standard serodiagnosis.

Basically a vector control program, beyond the investigation aspect of it, it also have a preventive impact avoiding infections in the human population. How many human infections were avoided by the sustained control program over 10 years?

156-225 new cases prevented at expected incidence rates of 4.3% and 8% per year, respectively. Based on a catalytic irreversible model ($S \rightarrow I$) with age- and time-independent transmission, no serorecovery and no mortality attributable to *T. cruzi*.

This is the principal impact of this program.

Some conclusions

- Before interventions some new cases in humans and dogs occurred at very low domestic bug densities ➔ No tolerance to light domestic infestations.
- With persisting peridomestic infestations and domestic reservoir hosts (dogs and humans), transmission arises focally when the intensity of bug control actions declines.
- Residual foci in peridomestic sites cause early domestic reinfestation.
- The elimination of *T. infestans* requires a greater intensity and quality of control actions than those that have been used in the Argentine Chaco so far.
- Long-term sustainable control of Chagas disease in the Argentine Chaco is feasible.
- Community participation is no spontaneous panacea. It depends on a broad social participation (school, local leaders and other local actors), and needs to be nurtured and supervised in a sustained fashion.
- This process is a long-term construction which includes health promotion, community mobilization and motivation, periodic monitoring of control actions and its effects, and commitment.
- Multifaceted, integrated, intersectored, inter-programmatic actions for sustainable control of neglected diseases in vulnerable populations.

General Hypothesis

- ‘Hot-spots’ occur on a range of scales from the household level to the community and beyond.
- Such heterogeneities are derived to a large extent from the highly uneven contribution of the various determinants of infestation and transmission in space and in time.
- Heterogeneities may be exploited for targeted vector control and disease prevention.
Geographic Analysis as a Support Tool for Triatominae Control Programmes

David Gorla, Centro Regional de Investigaciones Científicas y Transferencia Tecnológica Consejo Nacional de Investigaciones Científicas y Técnicas
Anillaco – La Rioja, Argentina

The zone of the Gran Chaco in the south cone of the America has important remanent of Triatominae infestants, (see bottom graph).

The Gran Chaco region can be divided in zones as: Chaco Humid, Chaco Dry, and Chaco Arid. A work-study is being performed in the south zone in Chaco Arid, this talk will show the different aspects obtained or discovered during the worked performed in this area.

One of the main issues in the control of T. infestants is not really inside the domicile as this one is normally spread out with relative success, the main risk is coming from the peri-domicile areas. The same control tools used in other areas are not as efficient in this area, also because the peri-domicile areas are more complex in nature and offer more shelter for the vector to grow.

Taking the above into consideration a Chagas program was performed in La Rioja, Argentina applying a geographic information system for vector control interventions.

The program included:

- Working area 71.000 sq kms
- 5 vehicles
- 15 well trained field staff
- 1 person selected for training on GPS and GIS use

An important aspect of this program was the integration of satellite navigators to obtain geographical information.
Focus on Neglected Tropical Infectious Diseases:
Chagas Disease a Public Health Threat in the Americas & Beyond
Report issued: September 30, 2008

Geographic database built during 2005 – 2007 by the Programa Chagas La Rioja

Frequency distribution of the number of rural houses per locality

During the years 2005 and 2007 the program staff registered the geographical location of houses in the region. The map above (histogram of frequency) shows the density of housing by location; the concept of location for this study is an aggregate of houses that is distant one another at about 2 km. It is observed that more than 60% of the locations have four or less than four houses which portrays the high density dispersion of the rural population.

Frequency distribution of the number of rural houses per locality. (5045 houses, 827 localities)

It is observed that more than 60% of the locations have four or less than four houses which portrays the density dispersion of the rural population. This is major constraint for the operational applicability of vector control programs.
Infestation of rural houses before the attack phase by Programa Chagas LR. Partial data

**Frequency distribution of the number of rural houses per locality with Intra-Domicile (ID) infestation (1124 houses [22.3%], 464 localities) 464/827= 56.1% dispersion index.
From this data it was observed that 22.3% of the houses have intra-domiciliary T. infestants, and according to the above graph there is great concentration in low density locations.**

**Frequency distribution of the number of rural houses per locality with Peri-Domiciliary infestation (1565 houses [31.0%], 552 localities) 552/827=66.7% dispersion index.
From this data it was observed that 31% of the houses have peri-domiciliary T. infestants, and according to the above graph there is great concentration in low density locations.**
Infestation prevalence is negatively associated with number of houses in a locality as observed in the following graph for the peri and intra domiciliary infestation and totals.

The fact that the lowest populated areas is where infestation have the highest prevalence, makes efforts for vector control more costly as reaching those areas imply in simple terms, longer distances that implies higher human resources, transportation expenses among other logistical costs.

In the infested areas of this zone are hot and cold spots; hot spots can be observed by the red dots (areas 2 and 1), and cold spots (areas 3 and 4) in the following histogram. This is the scenario observed in 2005 before the program for vector control began again after a long period of absence in the implementation of vector control strategies.
Following the same analysis for the peri-domiciliary infestation:

In the following graph shows the status in 2004 of a region named San Martin in terms of Intra-domiciliary infestation before the implementation of vector insecticide spraying strategies.
Following the same graph of the area of San Martin in 2007, after the implementation of vector spraying control strategies.

![Graph showing ID and PD infestation after vector control implementation](image)

Similar data was found for the Peri-domicile in this region. The following graph shows the evolution after the implementation of vector control in the San Martin area in terms of ID and PD.

![Bar chart showing ID and PD evolution](image)

From this information is important to observe that vector control strategies had a relevant impact on the ID but a lot less impact on the PD. This is not related to a lack of will from the personal performing the tasks; it is greatly related to the spraying technique applied, and type of insecticides which does not seem to work well for peri-domiciliary areas. New technique and insecticide components are required for peri-domiciliary areas.
In general we can conclude that the use of satellite navigators as geographic tools for the control of Triatominae vectors can be very positive. Once the quality of data gathered is confirmed to be reliable using GIS, then this data will be:

- Readily usable.
- Comparable over time.
- Usable to assign intervention priorities.
- Easily integrated with other components within the Chagas Program (serology, treatment, patient management) and other primary health care programs.

3. Session II: *T. cruzi*, Immune Response & Diagnosis in Chagas Disease

The *Trypanosoma cruzi* genome project: Comparative genomics and functional characterization of a novel surface protein family

*Nagib El Sayed*, Cell Biology and Molecular Genetics & Center for Bioinformatics and Computational Biology, University of Maryland, College Park

The project main efforts were focusing on sequencing each one of the genomes of *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania major*, and to try to compare them to determine similarities and differences.

While these three pathogens share many general characteristics, each organism presents distinct aspects, including transmission by a different insect, unique life cycle features, different target tissues and immune evasion mechanisms, as well as different disease characteristics in their mammalian host, all of which are reflected by differences in their genome sequences.
Following the Sequencing Strategy: Whole Genome Shotgun (WGS)

**T. cruzi** CL-Brener Genome has the following characteristics:

- Haploid genome size: ~ 50 Mb
- Genome highly repetitive: non-coding repeats, multi-gene families.
- Proteins with repetitive domains
- Highly polymorphic – CL-Brener is a hybrid
- Complex karyotype

**Molecular Karyotype of T. cruzi CL Brener**
(Santos et al, 1997)
In an effort to characterize the core TriTryp proteome as well as genes that are species-specific, we have identified the orthology relationships between individual genes of the three genomes. Clusters of orthologous genes (COGs) were constructed and used to compare gene content as well as genome architecture. Our results show that regions encoding the TriTryp core proteome present a remarkably high degree of synteny while in Trypanosoma, non-syntenic regions have been expanded and harbor many large species-specific gene families, majority of which appear to be surface antigen families. The different numbers of such species-specific genes largely reflect the different strategies of immune evasion used in each organism.

Summary
- Despite having diverged 200-500 mya, the genomes of trypanosomatids are highly syntenic.
- Detailed examination of the synteny breakpoints reveal they are associated with expansions of strand-switch regions, species-restricted gene families, multigene families, retroelements and/or structural RNAs.
- There appears to be a strong selective pressure to maintain the gene order and keep the DGC structure intact.
- Trypanosomatid genomes appear paradoxically composed of rapidly evolving sequences harbored at the edges of a slowly evolving trypanosomatid genome ‘core’.

Defining the Tritypr proteome: The Trityprs share a core proteome of 6,200 genes (see following graph).

Variant Surface Glycoproteins, ESAGs, Hypothetical Proteins (HPs)

A special discovery on T. cruzi is the MASPs, which are the Mucin Associated Surface Proteins, a large family of about 1400 proteins that were completely undetected to date.
General features of the Tritryp genomes:

<table>
<thead>
<tr>
<th></th>
<th>T. brucei</th>
<th>T. cruzi</th>
<th>L. major</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haploid Genome size (Mbp)</td>
<td>25a</td>
<td>55</td>
<td>33</td>
</tr>
<tr>
<td>No. of chromosomes (per haploid genome)</td>
<td>11a</td>
<td>~28b</td>
<td>36</td>
</tr>
<tr>
<td>No. of genes (per haploid genome)</td>
<td>9068c</td>
<td>~12,000d</td>
<td>8,311e</td>
</tr>
<tr>
<td>Syntenic 3-way COGs</td>
<td>5,812</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-syntenic 3-way COGs</td>
<td>346</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total regions with synteny blocks (Mbp)</td>
<td>19.9</td>
<td>Ncf</td>
<td>30.7</td>
</tr>
</tbody>
</table>

The shared genes (in blue) in all parasites occupy different space area, for example in T. cruzi, the same total of genes occupy 19.9 Mbp, and in L. major 30.7 Mbp, this clearly portrait a likely important relevance in the way these genes are distributed.

This can further be observed in the following table comparing Gene size and density in the three tryps:

<table>
<thead>
<tr>
<th></th>
<th>T. cruzi</th>
<th>T. brucei</th>
<th>L. major</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDS length – average (bp)</td>
<td>1,457</td>
<td>1,511</td>
<td>1,731</td>
</tr>
<tr>
<td>CDS length – geometric mean (bp)</td>
<td>1,158</td>
<td>1,187</td>
<td>1,301</td>
</tr>
<tr>
<td>Inter-CDS length –average (bp)</td>
<td>561</td>
<td>721</td>
<td>1,432</td>
</tr>
</tbody>
</table>

Much of the pathological ‘space’ remains to be explored within the T. cruzi lineage and the genetic bases of the diverse pathogenic potentials, clinical outcomes, epidemiology, transmission routes, host range and vector selection have not been investigated. I will report on an effort to sequence 10 additional isolates is about to get underway (in addition to several other tryps including some strategic outgroups) at one of the NIH-NHGRI genome sequencing centers. Those isolates include T. cruzi Silvio X10 (TCI), Can III (TCIIa), Esmeraldo (TCIIb), 3869 (TCIIc), NRc13 (TCIIId) and Tula cl2 (TCIIe).

The second part of the talk will focus on a novel large family of hypervariable proteins in T. cruzi CL Brener that was identified within large non-syntenic (T. cruzi-specific) regions. This family (MASP), encoded by approximately 6% of the T. cruzi diploid genome, is characterized by conserved N- and C-terminal domains that encode a signal peptide and a GPI-anchor addition site, respectively, suggesting a surface location in the parasite. The 1,377 members of the MASP family can be subdivided into different subfamilies based on sequence similarity of the central hypervariable and repetitive region. Northern and Western blot analyses indicate that MASP is expressed preferentially in the bloodstream trypomastigote forms. Transcriptome profiling suggests a limited set of members is expressed at a given time in the parasite population. MASP pattern of expression, surface localization by immuno-fluorescence
analysis and its variable nature suggest a role in host-parasite interactions. We speculate MASP may be involved in host cell attachment and/or invasion or in mechanisms of immune evasion.

**Phylogenetics in T. cruzi: Towards Integrated Strain Profiling and Phylogenetic Character Mapping in Trypanosoma cruzi, the agent of Chagas disease**

Michel Tibayrenc, MD, PhD, IRD Representative in Thailand, IRD Representative Office, French Embasy, Bangkok, Thailand

Unexpectedly, Trypanosoma cruzi is probably the microorganism which intraspecific genetic diversity is the best known. T. cruzi for parasitic protozoa is something what is Escherichia coli for bacteria. Since the 70’s and the pioneering Multilocus Enzyme Electrophoresis (MLEE) studies by Miles et al., many teams have produced abundant crops of data dealing with the agent of Chagas disease genetic polymorphism, with various techniques, including MLEE, Random Primed Amplified Polymorphic DNA (RAPD), kinetoplast DNA Restriction Fragment Length Polymorphism (schizodeme analysis), microsatellites, ribotyping and others. Many of these studies relied on an empirical reading of the polymorphism observed. Our group has long championed a population genetics and evolutionary analysis of T. cruzi molecular diversity. The observation of strong departures from panmictic expectations (mainly a considerable linkage disequilibrium or nonrandom association of genotypes occurring at different loci) led to the hypothesis of a predominant clonal evolution, with only possible occasional bouts of hybridization.

Linkage disequilibrium in Trypanosoma cruzi was observed between:
- Isoenzymes
- RAPD
- Schizodeme polymorphism (RFLP of kinetoplast/mitochondrial DNA)
- Miniexon genes
- Microsatellites
- Polymorphism of expressed genes (RADES)
- Sequences of several genes (Multilocus sequence typing; MLST).

This evolutionary patterns leads to the existence of discrete and stable genetic subdivisions within T. cruzi. However the existence of occasional genetic exchange, confirmed by experiments (Miles et al.), makes it impossible to equate these subdivisions to real clades. We have coined for them the term of “Discrete Typing Units” (DTUs). T. cruzi is subdivided into 2 main DTUs, DTU I and II, which correspond to the so-called “T. cruzi I and II”. DTU II is itself shared into 5 lesser subdivisions, DTU 2a to e.

In this talk, I will recall these main results obtained by our group and others, I will expose what is the epidemiological, ecological and medical relevance of these genetic subdivisions. I will then introduce recent studies of our group (polymorphism of expressed sequences, multilocus gene sequencing, proteomic analysis) and will analyze the relevance of the concepts of Integrated strain profiling and Phylogenetic Character Mapping for the agent of Chagas disease.
Immune Response in Chagas Disease: Protective or Pathogenic?
Manuel Fresno, Center of Molecular Biology “Severo Ochoa” (CSIC-UAM), University Autonoma de Madrid, Madrid, Spain

In terms of immunology, Chagas disease presents many challenges, some of them are:
- Why Chagas’s pathology is mostly clinically relevant in the chronic phase, considering that:
  - Much fewer parasites in the chronic that in acute phase
  - Similar or even greater immune infiltration in the heart. Qualitative changes?

- Why takes 15-20 years to develop?
- Why is only a subsets of patients (30%)?
- Differences in Clinical outcome (digestive, cardiac, nervous system) are relevant for understanding this disease?
- Different T. cruzi strains-different pathology?

Factors influencing clinical outcome in Chagas’ disease
We are not sure whether the acute phase influences chronic phase, but is likely the case.
Many different factors play in the disease as:
- Genetic: Host, Parasite strain,
- Environmental: Immunosuppression, reinfection, other as (Fertile field hypothesis) other infections, heart damage etc.

Objectives to fight Chagas disease include:
To review current program strategies to respond to the entire scope of Chagas’ disease control
- Prevention of transmission
  - Insect control programs
  - Organ transplant and Blood transfusion
  - Vertical transmission
- Chemotherapy
- Immunological control
  - Immunotherapy
  - Vaccines

In terms of immunological control the question that rises is why there is not vaccine for Chagas disease?
The answer is not only due to economical reasons thus Chagas is a neglected disease but is also due to immunological challenges not yet overcome. The host-pathogen interaction of this disease is complex, in occasions the host immune response can trigger the immunopathology whether due to quality factors for quantity factors that apply to this response, (see bottom graph).
Why we do not have a vaccine for Chagas’ disease yet?

**A**

The immune response attenuates parasite replication and disease

Parasite replication

Immune response

Symptoms threshold

Disease

Ineffective immune response

**B**

Inappropriate immune response:

Attenuates parasite replication but induces pathology

Inappropriate strength

Inappropriate type

Time postinfection

- Microorganism
- Host
- Infection
- Virulence
- Erradication
- Immunity
- Immunopathology
- Quality
- Quantity
- Autoimmunity
- Incorrect Th response
- Immunosuppression
- Hypersensitivity
Leading causes that limits vaccine development against *T. cruzi*, this is specially true in a two items in bold as follows:
- Lack of basic knowledge on biology.
- Lack of knowledge of protective immune response
- Sophisticated evasion immune mechanisms.

**- Complex immunoregulatory mechanisms**
- Infection of immunocompetent cells.
- **Lack of immune correlates of protection**

Protective immune response in Chagas’ disease triggers the activation of:
- CD8 T and CD4 Th 1 cells secreting IFNγ.
- IFNγ and TNF activate Macrophages to release Nitric Oxide and become trypanocidals.
- Antibody contributes in a minor extent to protection.

**Strategies Virulence factors used by T. cruzi to evade the host immune responses**
- Anatomic reclusion
- Modification of antigenicity through mimetism with own antigens, molecular mimicry
- Inhibition of complement activity
- Alteration of macrophages phagocyte microbicidal systems
- Modification of the host immune response
  a) Antibody destruction
  b) Induction of suppressor cells

All these virulence factors represent challenges for vaccine development.

Besides the immunological challenges there are other challenges for Chagas vaccine development as:
- An important challenge is that correlate of protection is not known whether is:
  Parasitemia, parasite burden in the heart, heart infiltration, clinical symptoms…
- The need of a good animal model: Mice Susceptible/resistant? , *T. cruzi* strain to use? type I , II ?

As an example: Following the immune response against *T. cruzi* in cytokine deficient mice

<table>
<thead>
<tr>
<th>KO mice</th>
<th>Parasitemia</th>
<th>Parasites in heart</th>
<th>Cardiac inflammation</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4R</td>
<td>=</td>
<td>↓↓</td>
<td>↑↑</td>
<td>=</td>
</tr>
<tr>
<td>IFN-γR</td>
<td>↑↑↑</td>
<td>↑</td>
<td>↓↓</td>
<td>↑↑↑</td>
</tr>
<tr>
<td>TNF</td>
<td>↑↑</td>
<td>↑</td>
<td>↓↓</td>
<td>↑</td>
</tr>
<tr>
<td>IL-10</td>
<td>↓↓</td>
<td>↓↓</td>
<td>↑</td>
<td>↑↑↑</td>
</tr>
</tbody>
</table>

(lack of myeloid suppressor cells)

(TNF shock)

It is observed that the present or absence of a given cytokine triggers different degrees of parasitism, cardiac inflammation, etc as shown above.
Vaccination animal studies of Chagas candidate vaccines, the influence of cytokines in vaccine development against T. cruzi showed following results:

![Graph showing parasitemia over time for different genotypes of IFN-γR-/- and IL-4-/- mice.](image)

These results show that is not only necessary to have a good antigen but also to know what is a good correlate of protection, and understanding how these impacts the host immune response.

**Chagas chronic pathophysiology: Autoimmunity or parasite persistence?**

Contradictory results have been reported on the participation of autoimmunity in experimental infection, and its role in the pathogenesis of Chagas’ disease remains controversial. However, the existence of molecular homologies between parasite and host molecules is indisputable…..

There are several ways in which infectious agents could break self-tolerance

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Effect</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disruption of cell or tissue barrier</td>
<td>Release of sequestered self antigen; activation of nonfolerized cells</td>
<td>Sympathetic ophthalna</td>
</tr>
<tr>
<td>Infection of antigen-presenting cell</td>
<td>Release of inflammatory mediators, notably IFN-α</td>
<td>? SLE</td>
</tr>
<tr>
<td>Binding of pathogen to self protein</td>
<td>Pathogen acts as carrier to allow anti-self response</td>
<td>? Interstitial nephritis</td>
</tr>
<tr>
<td>Molecular mimicry</td>
<td>Production of cross-reactive antibodies or T cells</td>
<td>Rheumatic fever</td>
</tr>
<tr>
<td>Superantigen</td>
<td>Polyclonal activation of autoreactive T cells</td>
<td>? Diabetes</td>
</tr>
</tbody>
</table>

Many of these mechanisms occur in T. cruzi as Molecular mimicry, infection of antigen-presenting cells, binding of pathogen to self protein, as other. These are also reasons why
Chagas disease has been catalogued as autoimmune disease, the question is whether the autoimmunity present in Chagas is relevant to its pathology or not for cause or effect.

Molecular mimicry: similarity between pathogenic antigens and self-antigens causes the generation of auto-reactive T cells

The focus of our studies is to understand in Chagas disease the process of molecular mimicry.

The criteria required for demonstration of the involvement of molecular mimicry in an autoimmune disease.
1. Association of the disease with a particular microorganism.
2. Identification of the culprit microorganism epitope that elicits the cross-reactive response.
3. T or B cell populations against that epitope should be expanded in the infection.
4. Elimination of the cross-reactive epitope from the microorganism should result in non-pathogenic infection
5. Autoreactive T cells should be able to transfer the disease.

Isolation of a chagasic autoantigen “Cha”
Through the investigations the isolation of a novel dominant chagasic autoantigen, called “Cha” was achieved through the Immunoscreening of a human Jurkat cDNA expression library using a pool of sera from chagasic patients.

It was found that the autoantigen Cha have high molecular mimicry with two T.cruzi antigens, (Tenu and Sapa) and there was homology (homologue regions) between human and mouse Cha and T.cruzi antigens.
It was observed in the Chagasic patients sera recognized a short Cha translation product sCha.

![Diagram showing Cha and sCha](image)

It was specially observed, after performing an epitopic mapping, that all human Chagasic sera recognized the mimicry peptide sCha epitope.

![Graph showing epitope mapping](image)

Basically in Chagas is found a Dual Molecular Mimicry (R3, R1) which is case unique in described in the literature.

![Diagram showing molecular mimicry](image)

This protein shows two molecular mimicries with two T.cruzi proteins, one at the level of antibodies and another at the level of T cells.
Myocarditis and antibody responses in Chagasic patients increase with symptomatology and decrease with treatment. This is as per the number 3 criteria required for demonstration of the involvement of molecular mimicry in an autoimmune disease earlier mentioned about the proliferation of T and B cells.

The number 4 criteria, elimination of the cross-reactive epitope from the microorganism should result in non-pathogenic infection is very difficult to comply as not all tools needed are available to eliminate the cross-reactive epitopes.

To achieve the 5 criteria: Autoreactive T cells should be able to transfer the disease, through the following model study performed in mice to determine if the outcome was myocarditis or antibodies.

**SAPA and R1 specific T lymphocytes adoptive transfer**

- T cells purified from lymphatic node from mice immunized with SAPA (S1) or R1 peptides.
- Intraperitoneal transfer (1.5 million lymphocytes)
- CBA/J receptor mouse
- Myocarditis? Antibodies?

The results in histopathology show monocytes and leucocytes infiltration in myocardium, valves, endocardium, vessels showing all the characteristics of a Chagasic cardiopathy.

This implies that yes Chagas disease has a degree of autoimmunity in its pathology processes.

However, Chagas is not only an autoimmune disease, when looking at the following model, in Acute Chagas is observed that is produced T and B cell responses and also cardiac damage that triggers molecular mimicry and bystander activation respectively which leads to the expansion of autoreactive T cells in the early chronic phase of the disease. From this some other mechanism must happen, this is also called “Fertile Field” (a re-infection, or something that damage the tissue in the myocardium) to reactivate the T cells expansion after many years 10 to 30 years to produce a chronic chagasic cardiomyopathy.
Basically depending of the phase of the disease the parasite replication with the years becomes less important in the disease pathology and the autoimmune response begins to be more important in explaining the pathology. In all cases is a combination of both factors and not a strict separation of either or.

The antigen Cha can also be used as a diagnostic tool to differentiate cardiopathy from Chagas of those from other reasons, as a important % of Chagasic sera during trials showed to recognized this antigen.

**Cha as disease marker**

- A) Direct correlation with disease severity
- B) Inverse Correlation with treatment

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**Immune Response & Diagnosis in Chagas Disease: New Serological Diagnostic Tools**  
**Alejandro O. Luquettti**, Associate Professor of Parasitology, Federal University of Goias, Goiania, Brazil

In the diagnosis of Chagas disease the two recognized phases of the disease acute and chronic have a very different diagnostics means of tests employed. Serological diagnosis tests are more applicable to the chronic phase of the disease, for the acute presentation this mean of diagnosis is not very applicable.
Another important point to take into consideration is that serological diagnosis must be accompanied by a context of epidemiology and clinical findings of the disease.

The value of the laboratorial diagnosis

<table>
<thead>
<tr>
<th>ACUTE PHASE: Parasites</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHRONIC PHASE: Antibodies</td>
<td>95-98%</td>
</tr>
</tbody>
</table>

As we observed the presence of antibodies is not found in a 100% of all infected chagasic patients, a small percentage of infected individuals do not develop antibodies.

Unlike other disease as Leishmaniosis, in Chagas the presence of antibodies is a very useful tool to diagnose the disease, also taken into consideration that parasitism is present to less than 50% in the infected individuals. The parasitemia is usually low or absent, inconstant, variable, erratic, and even if there, not necessarily present at the sample.

Before the problem with serological test (ELISA, IIF, IHA) was its lack of consistency in the results due to limitations in the methodology, good quality kits, today these limitations are few and mainly related to specificity with Leishmaniosis. When looking at serology results during the years it’s possible to affirm the greater reliability and accuracy of current tests.

One of the issues with serological diagnosis of T. cruzi is to agree on what results are to judge positive or negative as values differ according to the trade of the kit and laboratory internal control.

Conventional Serological Tests

Values observed in infected and non infected, as observed different values are portrait including inconclusive, due for example to maternofetal transmission of antibodies from mother to child.

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
<th>NEGATIVE</th>
<th>INCONCLUSIVE</th>
<th>POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA O.D./Cut-off</td>
<td>&lt;0,9</td>
<td>0,9-1,1</td>
<td>&gt;1,1</td>
<td></td>
</tr>
<tr>
<td>IIF TITER</td>
<td>&lt; 1/20</td>
<td>1/20-1/40</td>
<td>&gt;1/40</td>
<td></td>
</tr>
<tr>
<td>IHA(2ME) TITER</td>
<td>&lt;1/8-1/10</td>
<td>1/8-1/10</td>
<td>&gt;1/8-1/10</td>
<td></td>
</tr>
</tbody>
</table>

Some history on the evolution of Conventional Tests
- The first test available: CFT (1913).
- Then IHA, DA2ME, IIF, ELISA (1975)
- First test where done in house: so they where very difficult to compare.
- Then industry got interested and they become commercially available, increasing reproductibility.
- Extensively used: specific: IHA.
- High sensibility: IIF, ELISA.
- Better results when using two tests in parallel.

**Inconclusive Results**

As observed by the following facts inclusive results show in a very small % of cases, most are clear results of positives or negatives.

- By simultaneous use of three conventional tests (ELISA, IFI, HAI).
- From 7,849 sera, 4,800 (61,2%) positive and 2,194 negative
- Only 133 (1,7%) inconclusive.
- This means: 1 test on cut-off (+/-10%), 1 negative and 1 positive or 2 – 3 on gray zone
- Possible cause detected in 63 (47,5%) with aid of IIF leishmania, IgM, Reumatoid factor, Montenegro test.
- Leishmaniosis in 26%, treated in 14%, acute phase in 3%, passive transference of Ab in 3% and hepatitis in 1,5%.

A problem in serological diagnosis of T.cruzi is the question of T. cruzi I and II

As known:
T. cruzi I: Homogeneous, in north Amazonas river, isolated humans and silvatic, diaelphis, palm trees, also sylvatic below Amazonas, some humans double infection, only appears on immunosupression or tissues, easy treatment.

T. cruzi II: Homogeneous, isolated in humans in East Brazil, associated megaesophagus, low congenital(<1%), severe cardiopathy, difficult treatment.

The question of T.cruzi I & II and Serology

- T. cruzi II and reagents made from T. cruzi II mark all persons with T.cruzi and the same for T.cruzi I.
- The issue is that patients with T. cruzi II and tested with reagents made from T.cruzi I have not been extensively tested, and also limitations appear from patients with T. cruzi I tested with reagents of T.cruzi II, but in less degree. Further studies are necessary to confirm the potential of this diagnosis.

Observations during the correct use of conventional tests

- Problems of specificity (with IIF & ELISA), and sensibility (with IHA).
- Inconclusive results
- Diminution of costs
- Diminution of time of execution (few steps)
- Lack of equipment for reading (naked eye)
- Possibility of automation in busy laboratories, hemocenters.

Other solutions for identified problems

On specificity: Better antigens
- Purified (gp 90, gp 72, gp 25, gp 50, A&T)
- Recombinants (CRA, FRA, A13, H49, Ag1, JL7, etc)
- Synthetic peptides
On Speed:
- Supports: Gel, Nitrocelulose membranes
- Good equipment: naked eye: colour, beads, bands

An Ideal test will be 100% sensibility and specificity, count with few steps, results will be obtained in minimal time, no equipment eye reading only, possibility of automation, and low costs. But all this together is very difficult to achieve.

To obtain better serology diagnostic results, recommendations:
- Conventional Tests: To use two tests of different principle in parallel for diagnosis

Some examples of non-conventional tests
- Not in the market, Not available.
  - A&T: Requires plate chemoluminometer (cost), purified Ag, non rapid.
  - Flux cytometer (cost), uses trypomastigote forms, difficult + risk.
- On the market, not automated
  - Innolia: No rapid, high cost (> 20US$ by test), Results difficult to interpret.
  - Gador: No rapid, high cost.

Some examples of non-conventional tests
- CHEMBIO (USA): Rapid (10 min), allows storage, sensibility 97-98%, high cost (2US$).
- PaGIA (Switzerland): Rapid (20 min), needs special centrifuge, cost affordable (< 1, US$/test), sensitivity, now 96-97% used to be higher. Specificity, now 99%, used to be lower.
- IMUNOCOMB (Israel): semi-rapid: one hour, few samples (x10), technician dedicated full time, sensitivity and specificity nearly 99%.
- TESA-BLOT (Brazil, Biomerieux): semi-rapid: three hours, few samples, technician dedicated full time, sensitivity and specificity nearly 99% not available, no cost-effective for Biomerieux.

Perhaps a more precise question: What should be diagnosed? What are we looking for?

<table>
<thead>
<tr>
<th>Situation</th>
<th>what we have</th>
<th>what are the needs?</th>
</tr>
</thead>
<tbody>
<tr>
<td>A clinical case (single)</td>
<td>2 tests improve</td>
<td>specificity</td>
</tr>
<tr>
<td>To exclude donors (hundreds)</td>
<td>1 Elisa</td>
<td>nothing for exclude</td>
</tr>
<tr>
<td>To search for congenital tran.</td>
<td>2 tests</td>
<td>develop SAPA?</td>
</tr>
<tr>
<td>To diagnose acute phase</td>
<td>paras.</td>
<td>develop IgM Elisa?</td>
</tr>
<tr>
<td>Epidemiology: search for infected people</td>
<td>a)2 tests (EL+IF)</td>
<td>operational probl.</td>
</tr>
<tr>
<td>(to solve question TcI/II)</td>
<td>b)rapid</td>
<td>filter-paper&gt;conf</td>
</tr>
<tr>
<td>Evidence of cure after treatment</td>
<td>2/4 test</td>
<td>good, to improve</td>
</tr>
</tbody>
</table>

*need new tools
[a fight with BmemoryL, who produce Ab 20 years after Ag dissapear: finally they do, 20-40
years after; mice cannot be used, don´t live so much; perhaps look for what the parasite does
(proteomics)].

The diagnosis from congenital transmission is mainly done by diagnosing the mother, if
results appear positive and determine after 6 months of the child’s birth determine if
antibodies are present.

Do we need new tools? Yes, for the remaining 5% which are not correctly diagnosed by the
conventional ones. Also, for the difficult task of evaluating antibody levels in specifically
treated patients. Much work has been performed in this direction. First, using more specific
antigens, from recombinant to synthetic peptides. Then, the use of other methods, such as
chemiluminiscence, flow cytometry, and western blot.
Two problems have arisen: difficulty to obtain the antigens and the need of sophisticated
devices, not available in developing countries. So, we have the tools, but the application on
the field has been delayed. The industries also, are not stimulated, since the economic return
seems to be low.
We may look at the problem in other way: do we have tests with at least the same
performance as the conventional, but with other advantages, such as quicker, cheaper, easier,
i.e., rapid tests? The answer is partial: we do have, but prices are higher. Perhaps one of the
goals would be to produce these tests at lower cost, in order to use them in the field. We have
a number of them on the market, and some proved to have at least similar results to
conventional serology. Nevertheless, in some contexts, as blood banks, they do not have the
sensitivity required.
Two last points. The confirmation of diagnosis should be done with certainty. The
consequences of an error, both false positive or false negative, may be serious. This question
has been handled by the combination of at least two tests, with success. Second, in our
experience, sera with dubious results in conventional serology, are not always solved by non-
conventional tests. We have no single gold standard test.

Conclusions
-We have not ideal single test, but with two tests we are near the ideal.
-We have no single gold standard, but build it with two tests.
-We have no single gold standard, but build it with two tests.
-Rapid tests should not be used as a single test for diagnosis.
-Trends are to improve performance.
-Difficult to get 100% Specificity and 100% Sensitivity with a single assay.
-To adequate the type of test to the objective.
-With the tools we have, nearly all (98,7%) sera results could be solved as from non infected
or infected individuals.
New Molecular Diagnostics for Chaga’s Disease

Philippe Buscher, Unit of Parasite Diagnostics, Department of Parasitology, Institute of Tropical Medicine Antwerp, Belgium

An accurate diagnosis of Chagas disease is important in, acute/intermediate/chronic disease, congenital transmission, blood bank screening, transplantation, treatment outcome assessment, and epidemiological surveys.

The methods for standard diagnosis of Chagas’ disease include:

Parasite detection
- direct microscopy
- xenodiagnosis
- hemoculture

Immuno diagnosis*
- ELISA
- IFAT
- IHA
- PaGia
- Lateral Flow
- RIPA

*Recommended to do two test in parallel

For the Acute phase is recommended to do parasite detection and immuno diagnosis, Intermediate and Chronic phase immuno diagnosis, Congenital immuno diagnosis (>6 months), Blood bank immuno diagnosis ((= 2 to 3 tests in parallel).

Molecular diagnosis of Chagas’ disease
This type of diagnosis as surrogates parasite detection, because molecular diagnosis for example detecting parasite’s DNA is not really parasite detection.

One method is the PCR based amplification of the parasite’s DNA:
Requirements: Detects only T.cruzi (but all lineages), high copy DNA target, sensitivity PCR is higher than that the sensitivity parasite detection.
Attractive DNA targets: mainly Kinetoplast DNA (kDNA), repetitive nuclear DNA for example ~ 195 bp satellite, or ribosomal gene cluster.

Is been observed in dozens of in-house PCRs, the high sensitivity and specificity of this in-house devices has been claimed.

Applied on: Acute and chronic chagasic patients, congenital transmission, patient follow up after treatment, disease reactivation after transplantation, epidemiological studies.

New molecular diagnostic methods for Chaga’s disease include:
- High-tech approach with real-time PCRs, this method can make utilization easier and can be used as well as a quantitative test, which a normal PCR is not.
- Low-tech approach which a test called T.cruzi OligoC.TesT.
Regarding the High-Tech approach, Real-Time PCR:

- During the PCR reaction you will induce a fluorescence marker molecule as an indicator of amplicon production.
- The advantage is that there is not need for post amplification steps.
- Rapid
- High-throughput technology allowing to do many samples in little time.
- The problem is that requires specific equipment and expensive reagents and skilled personnel.

Follow some list of publications in relation to Real-time PCR for Chagas’ disease:

<table>
<thead>
<tr>
<th>Reference</th>
<th>Target DNA</th>
<th>Detection limit</th>
<th>Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cummings et al. 2003</td>
<td>satellite DNA - kDNA</td>
<td>0.01 - 0.1 parasite</td>
<td>mouse tissue</td>
</tr>
<tr>
<td>Freitas et al. 2005</td>
<td>24 S ribosomal DNA (heminested)</td>
<td>0.1 parasite</td>
<td>mouse tissue - human tissue</td>
</tr>
<tr>
<td>Virreira et al. 2007</td>
<td>kDNA (Cummings et al. 2003)</td>
<td>0.1 parasite</td>
<td>newborn blood</td>
</tr>
<tr>
<td>Piron et al. 2007</td>
<td>satellite DNA</td>
<td>0.01 parasite</td>
<td>human blood</td>
</tr>
</tbody>
</table>

This table shows that this method works, the detection limit observed is quite acceptable.

Regarding the low tech approach, the T.Cruz OligoC – TesT:
This test is being developed in collaboration between - ITM Antwerp ~ Coris BioConcept, Gembloux, Belgium, and the evaluation is done in collaboration with the University of Chile.

This tests also begins with a PCR, (the intention is to change this step for a less high-tech technology). The graph above describes overally the technique involved, publication on this testing technology is under preparation.

The T.cruzi OligoC – TesT, looks to have the following benefits:
Low tech approach, simple and rapid, targets a short sequence within the 195 bp satellite DNA, kit format for PCR and dipstick detection ~ standardisation, PCR internal control, Carry-over contamination proof.

This test claims to detect:
In terms of analytical sensitivity
- 1 fg of DNA per reaction, 1 fg ~ 0.01 parasite (quite acceptable).
- 1 parasite in a 180 μl blood sample.

In terms of specificity
- all 6 T. cruzi lineages can be detected.
- no cross-reaction observed with: Leishmania Donovani, Trypanosoma brucei gambiense, Mycobacterium tuberculosis, Schistosoma mansoni, Plasmodium falciparum.

However there is cross-reaction with 26 Trypanosoma rangeli strains from different regions, as shown in the following table:

<table>
<thead>
<tr>
<th># of strains</th>
<th>Detection limit</th>
<th>Parasites/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>&gt; 200 pg/μl</td>
<td>&gt; 1,000,000</td>
</tr>
<tr>
<td>7</td>
<td>200 pg/μl</td>
<td>1,000,000</td>
</tr>
<tr>
<td>7</td>
<td>20 pg/μl</td>
<td>100,000</td>
</tr>
<tr>
<td>4</td>
<td>2 pg/μl</td>
<td>10,000</td>
</tr>
</tbody>
</table>

However, only four strains have a detection of 2 pg/μl which is equivalent to 10000 parasites/ml which is should not affect importantly the diagnosing of T. cruzi.

Cross-reaction can be explained by the different copy number of the satellite DNA sequence between T. cruzi and T. rangeli (Brenière et al., Mem Inst Oswaldo Cruz, 1993).

The following are the results from a small evaluation of the OligoC-TesT
Sensitivity and specificity on humans, animals and vectors

<table>
<thead>
<tr>
<th>Biological sample</th>
<th>#</th>
<th>Pos OligoC-TesT</th>
<th>Specificity (95% CI)</th>
<th>Sensitivity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-endemic controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAT patients R.D. Congo</td>
<td>20</td>
<td>0</td>
<td>100% (83.9%-100%)</td>
<td></td>
</tr>
<tr>
<td>(Sleeping sickness patients)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAL patients Zambia</td>
<td>20</td>
<td>0</td>
<td>100% (83.9%-100%)</td>
<td></td>
</tr>
<tr>
<td>(Malaria patients)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LEI patients Nepal</td>
<td>20</td>
<td>0</td>
<td>100% (83.9%-100%)</td>
<td></td>
</tr>
<tr>
<td>(Leishmania patients)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Endemic controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood bank Chile</td>
<td>48</td>
<td>0</td>
<td>100% (92.6%-100%)</td>
<td></td>
</tr>
<tr>
<td><strong>Chagasic patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td>6</td>
<td>4</td>
<td>66.7% (30%-90.3%)</td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td>27</td>
<td>27</td>
<td>100 % (87.5%-100%)</td>
<td></td>
</tr>
<tr>
<td><strong>Infected animals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Octodon degus</td>
<td>2</td>
<td>2</td>
<td>100% (34.3%-100%)</td>
<td></td>
</tr>
<tr>
<td>Arbothrix olivaceus</td>
<td>5</td>
<td>5</td>
<td>100% (56.6%-100%)</td>
<td></td>
</tr>
<tr>
<td><strong>Infected vectors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mepraia spinolai</td>
<td>14</td>
<td>14</td>
<td>100% (78.5%-100%)</td>
<td></td>
</tr>
</tbody>
</table>

As seen above table, the OligoC-TesT gave negative Specificity results for the Sleeping sickness, Malaria and Leishmania patients, as well as for the endemic control of the blood banks in Chile, results that are good. Sensitivity showed not too bad results, was compared to the gold standard which for Chagas at this time is parasite detection.

**WHO/PAHO network on standardization PCR for Chagas’ disease**

Co-ordinator: INGEBI-CONICET, Buenos Aires, Argentina.

Funding: WHO/TDR and Pan American Health Organization

Objective: standardization of the use of PCR for diagnosis of Chagas’ disease

Methods:

- 26 participating laboratories (America's and Europe)
- Panel A: 10-fold serial dilutions of *T. cruzi* DNA
- Panel B: 10-fold serial dilutions of parasites in human blood
- Panel C: 45 blood samples from seropositive and seronegative individuals
- Blinded samples to be tested in duplex
- PCR workshop in Buenos Aires, expected in September 2008, on final evaluation of selected PCRs
- Output: "Practical laboratory guidelines for the use of PCR to detect *T. cruzi* in human peripheral blood for use in clinical research settings”.

To implement this project, in Argentina panels of samples are prepared and send out to the network of labs in Latin America, North America and Europe. The labs are requested to test with the own PCR, and anlyzed these panels, afterwards all data is collected and evaluated. The most performed labs will be brought together for a workshop today schedule in Sep 2008. This to make a practical exercise with the PCR that was best evaluated, and the eventually the outcome should be the guidelines on PCR earlier mentioned.

Conclusions

- Molecular parasite detection is promising for diagnosis of Chagas’ disease
- PCR – gel electrophoresis still the most frequently used
- High-tech approach: real-time PCR
- Low-tech approach: *T. cruzi* OligoC-TesT
- WHO/PAHO network aims at standardization of PCR based diagnosis
4. Session III: Latin America, USA and Europe – Blood Banks

Strategy of Blood Banks in Latin America: The Experience in Sao Paulo  
**Ester Cerdeira Sabino, MD, PhD, Fundacao Pro-Sangue/Hemocentro de Sao Paulo. Associate Professor, Department of Hematology and Hemotherapy, University of Sao Paulo, Brazil**

Following a graph that shows the trends in % of Chagas discarded units in relation to the different methods used.

The following graph shows the prevalence (positive cases / 10000 donors) of Chagas disease among first-time blood donors.

What are the difficulties encountered with Chagas Tests

- Initial tests developed for Chagas RFC, IHA and IFI were not easy to be standardized.
- Lack of a gold standard.
- Parasitic tests are not sensitive.
- Cross reaction with other parasitic infections.
- Serological response between individuals.

Taking into consideration these limitations are the reason why is chosen to screen Chagas using 2 tests.
However for Blood Banking when screening with two tests the following problem arrives as shown on following table:

<table>
<thead>
<tr>
<th>Test or combination of tests</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIF</td>
<td>2,809</td>
<td>40.5</td>
</tr>
<tr>
<td>IHA</td>
<td>902</td>
<td>13.0</td>
</tr>
<tr>
<td>ELISA</td>
<td>541</td>
<td>7.8</td>
</tr>
<tr>
<td>IIF+IHA</td>
<td>215</td>
<td>3.1</td>
</tr>
<tr>
<td>IIF+EI A</td>
<td>277</td>
<td>4.0</td>
</tr>
<tr>
<td>IHA+EI A</td>
<td>97</td>
<td>1.4</td>
</tr>
<tr>
<td>IHA+II F + ELISA</td>
<td>2,095</td>
<td>30.2</td>
</tr>
<tr>
<td>Total</td>
<td>6,936</td>
<td>100</td>
</tr>
</tbody>
</table>

Salles et al Transfusion 1996, 36:969.

6,936 units (1.7%) among 411,617 units screened in 1993-94, in this study only 30% of the samples were reactive to the three assays used, and most of the samples were reactive to only one assay. And most of those are probably false positives because the testing was done in a lower prevalence group of people.

The results obtained from the follow up of samples
The reactive patients where screened again an results where obtained as follows.

<table>
<thead>
<tr>
<th>Results at return</th>
<th>Reactive</th>
<th>Negative</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results at screening</td>
<td>3 tests+</td>
<td>2 tests+</td>
<td>1 test+</td>
</tr>
<tr>
<td>3 tests +</td>
<td>370 (95)</td>
<td>16 (4)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>2 tests+</td>
<td>21 (18)</td>
<td>39 (33)</td>
<td>31 (27)</td>
</tr>
<tr>
<td>1 test+</td>
<td>1 (0.1)</td>
<td>29 (4)</td>
<td>192 (25)</td>
</tr>
<tr>
<td>Total</td>
<td>392 (31)</td>
<td>84 (7)</td>
<td>228 (18)</td>
</tr>
</tbody>
</table>

As observed after testing that most gave negative, and some remained inconclusive, so basically the earlier assumption that most were false-positives was true.

Regarding the epidemiological evidence of Chagas infection among inconclusive samples, some questions where done to the individuals to determine epidemiological context as:

<table>
<thead>
<tr>
<th>Serological results at follow up</th>
<th>Yes to one of the 2 questions</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 tests +</td>
<td>141 (74%)</td>
<td>190</td>
</tr>
<tr>
<td>2 tests+</td>
<td>16 (57%)</td>
<td>28</td>
</tr>
<tr>
<td>1 test+</td>
<td>30 (29%)*</td>
<td>103</td>
</tr>
<tr>
<td>Control neg 1</td>
<td>6 (13%)*</td>
<td>45</td>
</tr>
<tr>
<td>Control neg 2</td>
<td>18 (15%)*</td>
<td>119</td>
</tr>
</tbody>
</table>

Does someone in your family have Chagas disease?
Have you ever lived in a house where the Chagas bug was present?
The fact that some of those individuals reacted to only one test and after the questions were performed, then it was necessary to evaluate with a confirmatory test.

The question is how should a confirmatory test should be evaluated?
  - What samples should be used?
  - Only those reactive to all tests? Inconclusive samples?

The INNO-LIA assay was chosen and the following results were obtained:

<table>
<thead>
<tr>
<th>CSS</th>
<th>Negative</th>
<th>Indeterminate</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>566 (98.1)</td>
<td>5 (0.9)</td>
<td>6 (1.0)</td>
<td>577</td>
</tr>
<tr>
<td>1</td>
<td>438 (93.0)</td>
<td>16 (3.4)</td>
<td>17 (3.6)</td>
<td>471</td>
</tr>
<tr>
<td>2</td>
<td>17 (32.1)</td>
<td>1 (1.9)</td>
<td>35 (66.0)</td>
<td>53</td>
</tr>
<tr>
<td>3</td>
<td>3 (0.6)</td>
<td>0 (1.0)</td>
<td>500 (99.4)</td>
<td>503</td>
</tr>
<tr>
<td>Total</td>
<td>1,024 (63.8)</td>
<td>22 (1.4)</td>
<td>558 (34.8)</td>
<td>1,604</td>
</tr>
</tbody>
</table>

As observed in this confirmatory assay the majority of individuals positive reacted positive in three assays, the majority of negatives also reacted negative, however, some questions arise about the negatives as if they were really negative results, and few other differences.

To show the degree of the complexity of this screening process, the following graph
This shows that is indeed difficult to know in often cases which samples are indeed a true positive and which are indeed true negative.

Another way that a group did to define sensitivity in all the assays in Latin America, is the WHO panel Methods summary, as follows:

- 437 samples from: Argentina, Bolivia, Brazil, Colombia, Ecuador, El Salvador, Honduras, Mexico, Nicaragua, Paraguay.
- Different assays used at screening.
- Number of screening kits: 18 (11EIA, 5IHA, 2PA).
- Supplemental test performed: IFI, IB, WB, RIPA.

The results according to number of reactive tests obtained after combining all the data are the following.

257 samples reacted up to three tests, 162 samples that reacted either to 16, 17 and to all kits evaluated, and 18 samples inconclusive.

To interpret these results the following algorithm was used:

Four supplement tests were used, so if the sample was + for the four or three of them was positive, and the opposite for a negative result, and for the other results inclusive.
The Final results according to the sample groups classify by A, B and C

<table>
<thead>
<tr>
<th></th>
<th>IFI</th>
<th></th>
<th></th>
<th></th>
<th>BW</th>
<th></th>
<th></th>
<th>RIPA</th>
<th></th>
<th></th>
<th></th>
<th>Final status</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pos</td>
<td>Inc</td>
<td>Neg</td>
<td>Pos</td>
<td>Inc</td>
<td>Neg</td>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
<td>Inc</td>
<td>Neg</td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td>2</td>
<td>255</td>
<td>0</td>
<td>1</td>
<td>259</td>
<td>5</td>
<td>252</td>
<td>0</td>
<td>257</td>
<td>0</td>
<td>0</td>
<td>257</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>2</td>
<td>9</td>
<td>15</td>
<td>3</td>
<td>7</td>
<td>11</td>
<td>6</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>161</td>
<td>1</td>
<td>0</td>
<td>160</td>
<td>1</td>
<td>1</td>
<td>162</td>
<td>0</td>
<td>162</td>
<td>0</td>
<td>162</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

A relevant aspect of the results is that among the B group – 18 inconclusive samples, we had 5 negative, 6 positive and 7 inconclusive. So there was samples were a consensus was not reached.

According to the results obtained with the different assays we can observed the following in relation to Sensitivity and Specificity:

<table>
<thead>
<tr>
<th>EIA assays</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBK 401 HEMOBO CHAGAS</td>
<td>100 (97.8 – 100)</td>
<td>99.62 (97.9 – 100)</td>
</tr>
<tr>
<td>CHAGAS – ELISA</td>
<td>97.62 (94.0 – 99.3)</td>
<td>97.71 (95.1 – 99.2)</td>
</tr>
<tr>
<td>CHAGATEK ELISA</td>
<td>99.40 (96.7 – 100)</td>
<td>99.24 (97.3 – 99.9)</td>
</tr>
<tr>
<td>Premier® Chagas' IgG ELISA Test</td>
<td>94.04 (89.3 – 97.1)</td>
<td>100 (98.6 – 100)</td>
</tr>
<tr>
<td>TEST ELISA PARA CHAGAS</td>
<td>99.40 (91.2 – 98.1)</td>
<td>99.62 (97.9 – 100)</td>
</tr>
<tr>
<td>BIOELISACRUZI®</td>
<td>98.21 (94.9 – 99.6)</td>
<td>99.24 (97.3 – 99.9)</td>
</tr>
<tr>
<td>ABBOTT CHAGAS ANTICORPOS EIA</td>
<td>99.40 (96.2 – 100)</td>
<td>98.09 (95.6 – 99.4)</td>
</tr>
<tr>
<td>CHAGAS – TEST HCS. método ELISA</td>
<td>97.02 (93.2-99.0)</td>
<td>99.24 (97.3-99.9)</td>
</tr>
<tr>
<td>Chagatest Elisa</td>
<td>98.81 (95.8 – 99.9)</td>
<td>99.62 (97.9 – 100)</td>
</tr>
<tr>
<td>Bioelisa CHAGAS</td>
<td>100 (97.8 – 100)</td>
<td>99.24 (97.3 – 99.9)</td>
</tr>
<tr>
<td>HEMAGEN® ELISA</td>
<td>100 (97.8 – 100)</td>
<td>96.56 (93.6-98.4)</td>
</tr>
</tbody>
</table>
The hemoagglutination is the worst assay in relation to overall specificity and sensibility, some more than others.

What is the clinical significance of low reactive samples?
- Spontaneous parasite clearance?
  - Ab clearance occurs in treated patients as well as in a small proportion of the controls of clinical trials.
- Low antibody response. If this is the case there is a real need to improve the testing kits.
- Cross reactivity with other parasitic infection (Leishmania).

A method to come out of this problem is to develop a good PCR.

Confidential Session Underlined: The following are the results of a study performed by Dr. Ana del Pozo in the Argentinian Chaco region, and is believed to be the gold standard study to determine sensibility and specificity of any assay, as it was directly in the blood donor population.
She tested 8 different assays and as observed there are low reactive samples that might represent almost ten percent in the positives to all assays.

Above the different assays used and shows that ELISA has a much better response in general, and again the HAI has the worst response. In Latin America for blood banking only ELISA should be used.

Quality Control Experience
Positive plasma units collection since 1995
- External Quality Assessment Programs
- Kits Evaluation / Development
  - New products
  - Kits selection at purchase process
  - Batch to batch evaluation
- Low titer internal control sera
- Research

Example of Batch to Batch evaluation
Evaluation of the performance of Blood banking in testing for Chagas disease
(Saez-Alquézar et al. Vox Sang. 1998,74:228)

- 4 programs / 57 Brazilian major blood banks in 1994
- Positive samples with titers higher > 1/160 IHA
- False negative results:
  - EIA 0-3%
  - IIF 1.2-1.9%
  - IHA 2.9-10%

What it was interesting to see on this program is that even with samples with high titles lab missed an important % of positives as see above in the false negative results.
When using ELISA the % of false negative was a lot lower.

Final Considerations
- Chagas screening tests have greatly improved in the past few years. Most of the discrepant results will be solved with the new generation of EIA.
- Low titer samples (difficult samples) will remain and probably represent 5-10% of the total positive samples:
  - Are they infectious?
  - Do they represent self resolved infection?

- Are we still missing infectious units with current EIA test?
  - study with new antigens non previously selected population from highly endemic regions.

- Need more work to improve PCR methodology for Chagas disease and to compare results between centers.

Strategy of Blood Banks in Europe
Azzedine Assal, Biologist, Coordinator of the French Blood Screening Platforms for Immuno-hematology, Serology and Nucleic Acid Testing. Etablissement Français du Sang, EFS, France

Background
Chagas disease is a zoonotic disease caused by the bloodborne parasite: Trypanosoma cruzi.
The disease is endemic in Latin America, where an estimated 16 to 18 million persons harbor the parasite chronically and approximately 45,000 die each year of the illness. T. cruzi is transmitted primarily by triatomine insects (i.e., kissing bugs); infection also can occur via blood transfusion, congenital transmission, organ transplantation, laboratory incident, and ingestion of triatomine-contaminated food or drink.
Due to migration movements, Chagas disease is now found in non endemic areas where the main transmission risk is due to transfusion, organ transplantation, or congenital transmission. European countries are unequally concerned by the disease depending on the rate of immigrants from endemic areas. European legislation requires deferral of donors with history of Chagas disease. However, the disease may remain asymptomatic for years and donors harboring the parasite represent a threat for transfusion safety and must be detected and permanently deferred.
We performed a survey aimed to collect data regarding the risk magnitude in different European countries and the policies implemented to prevent this risk.
Material and methods
A questionnaire was designed in order to collect data regarding the different policies applied in the European blood banks for the prevention of Chagas disease transmission. The questionnaire was sent to blood bank representatives of the following countries: Belgium, France, Finland, Germany, Italy, Netherlands, Norway, Portugal, Spain, Switzerland and United Kingdom. Questions concerned demographics of the population from endemic countries, prevalence of the disease, and the rate of Chagas transmitted cases if exist. Blood banks were also questioned about whether they have implemented one or more of the following prevention strategies: donor selection, donation testing, deferral policy and pathogen inactivation.

Results
The response rate to the questionnaire was 63 % (7/11). Most questionnaires were incomplete. The rate of migrant population from endemic areas ranged from 0.13 % in the UK to 4.1 % in Spain. Spain houses approximately 4 million immigrants, and 1.5 million of them were born in a country endemic for Chagas disease.

Screening at-risk donors for *Trypanosoma cruzi* antibodies is mandatory and applied only in Spain, France and UK. At-risk donors are people born in endemic countries, or born of a mother native to an endemic country, and / or travellers and residents. Donations are tested simultaneously by 2 ELISAs in France and some Spanish blood banks (Catalonia). In France and Catalonia, one of the ELISAs is based on crude antigens (parasite lysate) and the other on recombinant antigens (recELISA). In France, when at least one of the 2 ELISAs is repeat reactive, an Immunofluorescence assay is applied. In Catalonia, in case of initial reactive test donations are further tested with a particle agglutination test in combination with an IFA test and a PCR.

In the other countries, two categories of prevention strategies are applied. Some countries declare that no interview, no testing and no deferral policies are applied (Finland, Netherlands, and Switzerland). In other countries donors are temporarily deferred, after their return from an endemic area and then accepted for donation if they do not present with symptoms of chronic disease.

In France, the prevalence of *T cruzi* antibodies is 0.4 in 10,000 among at-risk population and 1 in 500,000 donations in the whole donor population (calculated for the ten-month period of mandatory testing, from May 1st 2007 to April 29, 2008). In the UK the seroprevalence calculated from 1999 to 2007, is 1 in 24300 donations. In Spain the seroprevalence reported in July 2007 is 0.9 % among at-risk donors and 1 in 10,000 donations for the whole donor population. Catalonia reported a seroprevalence of 0,64 % among at-risk donors. Among the questioned blood services only Spain reported cases of *T. cruzi* transmission by transfusion or organ transplantation. Three cases where reported from November 2005 to May 2007. Out of the 3 transmission cases, one was fatal.

Conclusion
Few countries in Europe have policies for prevention of transfusion-transmission of Chagas disease. Some countries consider that this risk is very low or does not exist and do not plan donor screening implementation. Only France, Spain and UK have implemented prevention strategies. The highest risk of Chagas disease transmission is in Spain where immigration from Latin America is the most important in Europe. Test selection and algorithms for result interpretation are crucial because of the number of indeterminate results that cause 1 % of discarded components in France.

The outcomes of the survey showed that there is a need for policy harmonisation in Europe countries, regarding donor selection, donor deferral and donation testing.
New approaches with pathogen reduction techniques seem to be the preferred strategies by most blood banks for the prevention of the transmission of T. cruzi by transfusion.

**Strategy of Blood Banks in the USA**

**Celso Bianco, Executive Vice President of America’s Blood Centers, USA**

The US has a very large population of immigrants from Mexico, Central and South America. These immigrants are gradually becoming part of the fabric of the American society. Consequently, they are an important fraction of both, the blood donor and the blood recipient population. Trypanosoma cruzi is endemic in Latin America and the population of infected individuals has been estimated at 7.7 million. There are about 17.9 million people in US born in Latin America.

There have been 7 cases of transmission of T. cruzi reported in North America in the last 20 years. (Dr. D Leybi – ARC)

- 1987 California via Mexican donor
- 1989 New York City via Bolivian donor
- 1989 Manitoba via Paraguayan donor
- 1993 Houston via unknown donor
- 1999 Miami via Chilean donor
- 2000 Manitoba via German/Paraguayan donor
- 2002 Rhode Island via Bolivian donor

Most of these cases were donors that came from South or Central America, and interestingly they were directed donors. Is not really well understood why only 7 cases in 20 years, are cases being missed, bad diagnosis, a problem of surveillance, and issues with parasitemia or changes in practice, among other.
Seroprevalence of T. cruzi among blood donors in the Americas

In addition, the parasite has been transmitted to several recipients by organ transplantation. Epidemiological studies published by Dr. David Leiby in Transfusion in 2002 showed an increased prevalence of donors positive for antibodies to T. cruzi in Los Angeles and in Miami between 1996 and 1999.

These results together with the increase in the population of Latin American immigrants raised concerns about the potential for transmission of the parasite to blood recipients and led the US Food and Drug Administration (FDA) to encourage the development of better blood donor screening assays than the ones that were been used in Latin America.

Blood banking in the USA have multiple collectors and providers for a total 15 million whole blood collections and 2 million platelet doses/year: 75 USA Members of America’s Blood Centers, ABC (~47%), American Red Cross, ARC (47%), Hospitals with collecting facilities (6%).

Regulations and Standards: Each ABC Member has its Food and Drug Administration (FDA) license; ARC has a single FDA license, hospitals in general are not licensed (work within the state), and an accredited by AABB, College of American Pathologists.

FDA Regulates Blood Banking
- Blood is a drug (pharmaceutical).
- Strict requirements for donor selection, testing, manufacture of components and distribution.
Requirements for the licensure of donor screening tests are much more extensive than those for diagnostic tests.

There is usually a gap of years between the time of licensure and the time for a recommendation for use or a mandate; blood centers in general implement the test before a formal FDA recommendation.

FDA indicated in 2002, the availability of an assay with high sensitivity and high specificity for antibodies to T. cruzi would lead to a recommendation for screening of the 15 million whole blood donations in the US. Ortho Clinical Diagnostics took the challenge and their ELISA for antibodies to T. cruzi was licensed in December, 2006. Several blood banking organizations, based on recommendations made by AABB (formerly the American Association of Blood Banks, started testing on Jan 29, 2007. FDA brought the question of universal screening (all donors on every donation) versus selective screening (only immigrants from Latin America or every first time donor or other approaches) to a meeting of its Blood Products Advisory Committee in April 2007. The committee recommended screening of all donors on all donations for an initial period of two years until sufficient data is accumulated for a future decision based on epidemiological data. The committee has not yet issued a formal guidance or recommendation for the screening of blood donors but it is estimated that between 75-80% of the whole blood and apheresis platelets collected in the US are being screened for antibodies to T. cruzi.

Testing for antibodies to T. cruzi among ABC Member Centers approx 8 million units/year: Yes - 59 centers (79%), 8 of these began testing in the last 6 months, No testing 6 centers (8%), Unknown – 10 centers (13%)

Over 9.6 million donors have been screened by ELISA; 0.12% are repeatedly reactive and about 30% of these specimens are positive on a RIPA confirmation assay (Dr. Susan Stramer, American Red Cross, personal communication).
The antibody prevalence is about 1:30,000 donors in the US.

Donors positive on RIPA for T. cruzi May 1, 2008:
- Over 11 million donors screened
- ~0.01% repeatedly reactive (~1:29,000)
- 1495 repeatedly reactive donations
- 472 RIPA positive; 1008 were RIPA non-reactive, three were RIPA indeterminate
- ~31% of repeatedly reactive are RIPA positive
- Higher prevalence in states with higher proportion of immigrants from Latin America such as Florida, California (1:3,700-1:8,000)

Country of Origin of Confirmed Donors

<table>
<thead>
<tr>
<th>Country</th>
<th>Confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARGENTINA</td>
<td>1</td>
</tr>
<tr>
<td>BOLIVIA</td>
<td>7</td>
</tr>
<tr>
<td>CHINA</td>
<td>1</td>
</tr>
<tr>
<td>EL SALVADOR</td>
<td>11</td>
</tr>
<tr>
<td>GERMANY</td>
<td>1</td>
</tr>
<tr>
<td>GUATEMALA</td>
<td>1</td>
</tr>
<tr>
<td>HONDURAS</td>
<td>2</td>
</tr>
<tr>
<td>MEXICO</td>
<td>26</td>
</tr>
<tr>
<td>UNITED STATES</td>
<td>16</td>
</tr>
<tr>
<td>UNKNOWN</td>
<td>408</td>
</tr>
</tbody>
</table>

AABB Biovigilance, May 1, 2008

Summary results of the geographical distribution and prevalence of antibody positive donors in the US are continuously updated on the AABB website at http://www.aabb.org/Content/Programs_and_Services/Data_Center/Chagas/chagas.htm

The American Red Cross has been tracing recipients of prior donations by donors with positive antibody results. Dr. Stramer indicated in a recent report that initial follow up of 55 recipients did not identify a case of actual transmission by transfusion. The number of recipients studied is still small but the initial conclusion is that the rate of transmission may be lower than that published in the 1950’s in Latin America.

United Blood Services (ABC Member) Implemented testing for T. cruzi
(Brian Custer, PhD, MPH)

UBS prospective donors are asked
1. Race and ethnicity
2. Country of birth
3. Three additional questions
   • Have you spent time that adds up to 3 months or more in Mexico, Central America or South America?
   • Has your mother spent time that adds up to 3 months or more in Mexico, Central America or South America?
   • Since your last donation have you traveled to Mexico, Central America or South America?
This testing shows an association of RIPA + and the country of origin with a higher prevalence from people coming, being born in Mexico.

<table>
<thead>
<tr>
<th>Country</th>
<th>All Donors Number (%)</th>
<th>RIPA+ Number (%)</th>
<th>RIPA+ Prevalence by Birth Country or Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>541,033 (83.0)</td>
<td>6 (20.7)</td>
<td>1:90,172</td>
</tr>
<tr>
<td>Mexico</td>
<td>12,690 (1.9)</td>
<td>7 (24.2)</td>
<td>1:1813</td>
</tr>
<tr>
<td>Central or South America</td>
<td>1,853 (0.3)</td>
<td>11 (37.9)</td>
<td>1:168</td>
</tr>
<tr>
<td>All other countries</td>
<td>13,410 (2.1)</td>
<td>2 (6.9)</td>
<td>1:6,705</td>
</tr>
<tr>
<td>Missing/Unknown</td>
<td>82,485 (12.7)</td>
<td>3 (10.3)</td>
<td>1:24,495</td>
</tr>
</tbody>
</table>

Due to the high cost of the ELISA test of 8 US per test, many different selective screening strategies for T. cruzi have been considered.

- Geographic location – high prevalence regions.
- Geographic exposure of donors.
  - Screen only donors who report a geographic risk.
  - Screen each first time donors and only repeat donors who report a geographic risk factor.
  - Screen each donor once followed by rescreening of donors who report a geographic travel risk factor – 1 time screening.
- Screen each donor on two donations followed by screening of repeat donors who report a geographic travel risk factor – 2 time screening.
- Universal ongoing screening
  - Hybrid approach: After a defined period of universal screening of every donation (1-2 years), screen all FT donors and repeat donors that report a geographic risk factor.
  - Specific component screening (platelets and/or whole blood).
  - Platelets only screening.
  - Platelets and whole blood screening.

The following is a cost-effectiveness analysis compared to screening strategy:

<table>
<thead>
<tr>
<th>Screening Strategy</th>
<th>Cost-Effectiveness (US$/QALY)</th>
<th>95% Results Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets only</td>
<td>207,000</td>
<td>93,000 – 582,000</td>
</tr>
<tr>
<td>First time donors</td>
<td>381,000</td>
<td>175,000 – 1,060,000</td>
</tr>
<tr>
<td>and repeats with</td>
<td></td>
<td>499,000 – 2,970,000</td>
</tr>
<tr>
<td>risk</td>
<td></td>
<td>781,000 – 4,639,000</td>
</tr>
<tr>
<td>1 time screening</td>
<td>1,080,000</td>
<td>842,000 – 4,997,000</td>
</tr>
<tr>
<td>Platelets and whole blood</td>
<td>1,654,000</td>
<td>1,005,000 – 5,950,000</td>
</tr>
<tr>
<td>2 time screening</td>
<td>1,783,000</td>
<td></td>
</tr>
<tr>
<td>Universal screening</td>
<td>2,123,000</td>
<td></td>
</tr>
</tbody>
</table>
Focus on Neglected Tropical Infectious Diseases: Chagas Disease a Public Health Threat in the Americas & Beyond
Report issued: September 30, 2008

Cost effectiveness in blood safety

<table>
<thead>
<tr>
<th>Intervention (comparison)</th>
<th>Cost effectiveness proximate to time of adoption ($/QALY)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV Antibody (no screening)</td>
<td>Cost saving to 3,600</td>
</tr>
<tr>
<td>HIV NAT (HIV AB)</td>
<td>1,966,000</td>
</tr>
<tr>
<td>HCV Antibody (no screening)</td>
<td>Cost saving</td>
</tr>
<tr>
<td>HCV NAT (HCV AB)</td>
<td>1,830,000</td>
</tr>
<tr>
<td>WNV (no screening)</td>
<td>520,000 – 897,000</td>
</tr>
<tr>
<td>HTLV (no screening)</td>
<td>420,000 – 63,000,000</td>
</tr>
<tr>
<td>SD plasma (ID marker screening)</td>
<td>2,200,000 – 9,700,000</td>
</tr>
<tr>
<td>T. cruzi universal (no screening)</td>
<td>2,123,000</td>
</tr>
</tbody>
</table>

Dr. Brian Custer, PhD, MPH, concluded the following implications after this study.
- Opportunity cost: Spending $4 – 8 ($5 – 12) per donation on T. cruzi testing is not a question of whether blood operators, hospitals, insurers and payers can afford the cost, but rather is a question of whether the cost is the best use of that money.
- Ongoing universal testing for T. cruzi is a poor use of resources from a value for money perspective.

As mentioned before, a number of US Blood Centers are waiting for a formal FDA recommendation before they initiate donors screening for T. cruzi. Others are only testing selectively.
The major reasons for a delay in test implementation stated by these centers have been (a) the limited number of transmissions of Chagas’ disease reported in the literature and (b) the lack of licensed confirmatory tests that would allow the reentry of blood donors. Hopefully, the extensive testing being performed in the US and as importantly, the lookback studies, will provide a scientific basis for the policy many decisions that need to be made regarding screening of blood donors for antibodies to T. cruzi in the United States.

Conclusions
- There has been great concern about the introduction of Trypanosoma cruzi, the agent of Chagas’ disease, in the USA by immigrants from endemic areas.
- The number of recognized cases of transmission by transfusion or transplants so far recognized is small.
- A new ELISA test for antibodies to T. cruzi (Ortho) was licensed in December 2006
  While the assay has not yet been mandated by the FDA, about 70% of collections are being screened.
- Most centers screen every donor on every donation.
- Some centers only screen donors at risk.
- The overall prevalence of confirmed positive donors is ~1:30,000, higher in states with many immigrants.
- 57 recipients of prior donations from positive donors tested negative for antibodies to T. cruzi suggesting that penetration is small.
- Ongoing universal testing for T. cruzi appears to be a poor use of resources from a value for money perspective.
- Selective screening is being considered as a more effective approach to donor screening, if indicated.
- Blood centers await final recommendations from FDA.

Special thanks to: David Leiby, PhD, Susan Stramer, PhD, Brian Custer, PhD, Sally Caglioti
Who generated most of the data presented here.

Laboratory Quality Control in Blood Banks

The procedures for Quality Control of Infectious Disease Screening include:
Quality Assurance
  – Quality Management System
Internal Quality Control
  – Internal control sera (ICS)
  – Kits evaluation (Performance panel)
    • Before to be used
    • Batch by batch
Quality Assessment
  – External Quality Control

Laboratories that carry out serological screening in blood banks must have a quality management system that allows the different phases (pre-screening, screening and post-screening) to be monitored.

Laboratory Quality Assurance Phases
Certain procedures are fundamental to ensure adequate quality during the screening phase, namely, prior evaluation of the diagnostic kits, batch-by-batch validation and the use of low-reactivity internal control sera (ICS) to monitor and clear the results of the tests carried out daily in the laboratory for release.

Reliable Serological Screening of Blood Donors
- Participation of well-trained and periodically up to date professionals
- Quality management system
- To use good quality diagnostic kits
- To adopt internal quality control procedures (IQC)
- To participate on programs for external quality evaluation (EQAS)

Participation in external quality assessment schemes (EQASs) allows the laboratory’s performance to be assessed from the pre-screening phase to the post-screening phase. Organizers of EQASs should promote continuous education programs with participating laboratories as a source of education and up-to-date information about serological screening.

It is also very important that the whole laboratory team have access to the documentation generated by the quality control process and that they participate actively in the analysis of the results.

The Most Important Items of the Internal Quality Control (ICS)
- Use of internal control sera (ICS), for daily validation of the tests performed.
- Ex: OD/CO: 2.5 - 4.0 or 0.3 - 0.7 for the competitive methods.
- Monthly determination of the variation coefficient for each method performed.
- Use of serum panels to evaluate the kits, before they are used.
- Use of serum panels to evaluate each new batch of kits before their use in laboratory routine.
An example of the use of the ICS to validate the assays performed in the laboratories

From the above graph a card is done with the results with the westgard rules, for instance as follows:

Evaluation of the diagnostic kits as a pre-requisite for commercialization

- Beneficial practice to avoid the commercialization of products with untruthful quality.
- As stipulated the period between evaluations is quite long (up to five years), the performance of different batches is not evaluated for each product, which might represent a great number of non-evaluated products.

Batch by Batch Changes of Kits Performance

- Different levels of reactivity, implementing the adoption of internal quality controls for daily validation of serum reactions which is difficult and expensive for the labs. This problem is continuously present in the Brazilian and Latin America laboratories.
- Verify when a change of batch results in significant losses of sensitivity and/or specificity.
- The application of internal laboratory performance panels has shown good results, to evaluate each new batch before use.
Internal Kits Evaluation before use and batch by batch control:

- All samples are analyzed for all tests used in the serological screening of blood donors + anti-HBs.
- Panels with sera samples positive and negative.
- Also for other tests when necessary (leishmania).
- Confirmatory tests are performed in positive samples.

Example of a T.cruzi performance Panel
Panel of sera samples 7 positive and 13 negative.

<table>
<thead>
<tr>
<th>Samples</th>
<th>ELISA</th>
<th>IHA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OD/CO</td>
<td>OD/CO</td>
</tr>
<tr>
<td>1</td>
<td>4.2</td>
<td>3.3</td>
</tr>
<tr>
<td>2</td>
<td>5.0</td>
<td>3.5</td>
</tr>
<tr>
<td>3</td>
<td>4.8</td>
<td>3.4</td>
</tr>
<tr>
<td>4</td>
<td>5.6</td>
<td>4.0</td>
</tr>
<tr>
<td>5</td>
<td>7.0</td>
<td>3.7</td>
</tr>
<tr>
<td>6</td>
<td>6.1</td>
<td>3.4</td>
</tr>
<tr>
<td>7</td>
<td>4.0</td>
<td>3.4</td>
</tr>
<tr>
<td>8</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>9</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>10</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>11</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>12</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>13</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>14</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>15</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>16</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>17</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>18</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>19</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>20</td>
<td>0.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

This method gives ok results and is not as expensive.

External Quality Assessment (EQA) or External Quality Control Programs (EQCPs)

EQA is a procedure to verify the quality of results generated by a laboratory, also called proficiency testing which are done with a periodicity of 2 or 3 times per year.
The programs intend to assess the performance of the participating laboratories and should be considered a challenge to check the efficacy of the Quality Assurance and the Internal Quality Control, in each laboratory.
Possible failures in the internal procedures of the laboratories may give rise to FPR and / or FNR in processing the Multipanel.

Multipanel: It is a panel with positive samples for all tests normally applied in the screening and some negative ones, this sort of serum panel is called Multipanel. In the specific case of the serological trial of blood bank, the main interest is to evaluate the performance of the laboratory responsible for the screening tests.

It is very important that the testing samples used in serological screening are qualitative tests (determines whether the substance being tested for is present or absent). That results obtained by the participating laboratory (PL) will be compared with a reference panel sent by de OC. The reference panel must be very well tested, to assure the certainty of the results. The characterization used for the multipanel is very important to evaluate them, and the use of a confirmatory test.
Blind Panels: For a single screening test, for instance: anti-\textit{T. cruzi}, samples $N = 5$ to 10 samples are used, 5-7 positive and 3-5 negative. Testing is done for least 6 different ELISA tests, two IHA tests and if possible, one complementary test.

The Development of the EQAS included:
- Participating Laboratories (PL) in EQAS receive by mail anonymous vials containing samples of serum.
- The samples should be tested by the methods routinely used in the laboratory and the results submitted to the Organizing Center (OC) for evaluation.
- Full anonymity is assured. The identity of individual laboratories is not revealed to other laboratories, or to third parties.
- When the results of each cycle of the EQAS have been evaluated, a final summary report is prepared and distributed to all participating laboratories.

Evaluation of the Laboratories’ Criteria
- A Correct Results
  - Without FPR or FNR
- B1 False Positive Results
  - $< 5\%$ of the total assays
- B2 False Positive Results
  - $< 5\%$ of the total assays
- C False Negative Results

EQCPs - Final Report Contents
- Number, type and geographic distribution of the Participant Laboratories.
- Characterization of the Multipanel.
- Strategies (type of methodologies) used by each Participant Laboratory.
- False Positive and False Negative Results
  - Number, \% and distribution by methodology, by disease and by trademark of the used kits.
- Relevant information.
Development of EQAS in Brazil and Latin America

Since 2008 a program was created so that private and public labs were able to participate in the EQAS programs.

Main problems detected during EQAS development

- Contamination of samples, creating FPR that might occur as a consequence of human mistakes or by failure of the equipment used.
- Errors in the transcription of the results (FPR and FNR).
- Lack of sensitivity and/or specificity of the kits used (FPR and/or FNR).
- Unsuitable procedures for ICQ (FPR and/or FNR).

EQAS PAHO/WHO – Latin America - False-Negative Results and False-Positive Results for Chagas Screening (2001 / 2004)

During that period, by these results we can observe a high percentage of FNR.
FPR and FNR observed in EQAS - Brasil (2004-2006)
In these results we can observe the importance of the procedures as mistakes can take to a very different results.

Analysis of the different strategies used for serological screening to anti-*T. cruzi*

- WHO 1991 : 2 tests
- WHO 2002 : 1 test
- Argentina 2004: 2 tests
- Brazil: until 2002: 2 tests (>70% ELISA + IHA)
- Brazil: since 2003: 1 test ELISA
- Costa Rica 2006: 2 tests (ELISA rec + Lys)
- Spain 2008: 2 tests (ELISA rec + Lys)
My recommendations for serological screening of T. cruzi are when:

- Using a Single Test: ELISA with high sensitivity
- Using Two Tests:
  - ELISA Tests (Ag lys + Ag rec), or (Ag lys + Ag Lys), or (Ag rec + Ag rec)
  - Not to use ELISA + IHA.

Strict Quality Control Procedures, including Batch by Batch Evaluation.

In general recommended are EIA Tests: ELISA, Clia. No recommended are: IHA, IIF, Non conventional assays as Rapid Tests.

Also is very important the definition of the minimum acceptable values of sensitivity and specificity for anti- T. cruzi serological tests that should be used.

Following the evaluation results of the kits more used in Latin America, we can observe that the sensitivity varies from 87 to 100% with more concentration towards 100%. In terms of specificity around 98 to 100%.

The minimal acceptable values of sensitivity are of:

**SENSITIVITY:** 99.8 – 100 %

And of specificity

**SPECIFICITY:** ≥ 99.5%

In the absence of a universally accepted confirmatory test, how can the samples that were reactive in the serological screening be confirmed?

- There is a urgent need for a supplemental assay for use in clinical laboratories and blood banks.
- Some tests that has been developed in the last years, are potentially suitable for use as a confirmatory test Unfortunatily none of them are available commercially.
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Criteria for screening in non-endemic of donor candidates from endemic areas

Using a Supplemental assay to confirm positive samples.

Definition of the minimum acceptable values of sensitivity and specificity for anti-\textit{T.cruzi} serological tests that should be used

Final Comments
- To strengthen internal quality control procedures:
  - Adequate application of ICS for daily validation of the reactions.
  - Prior and batch by batch evaluation, by panel performance application.
- Participation in the EQAS
  - By careful analysis of the information on final reports.
  - Disclosure of the results obtained in each program by the organizing centers to guide the regulation authorities.
4. Session IV: Research and Implementation on Congenital and Pediatric Chagas Disease

The Contribution of the IRD in the Fight Against Chagas

Laurent Brutus, “Mother and Child Health in the Tropics” Research Unit, IRD, France

In collaboration with Simone Frédérique Brenière, Jean Philippe Chippaux, Jean Pierre Dujardin, Frédéric Lardeux, François Noireau, Ali Ouaissi & Michel Tibayrenc, IRD, France

The regional initiatives to fight against Chagas’ disease have reduced dramatically the transmission of the parasite responsible for the disease, Trypanosoma cruzi. There is however some worry about persistence of vector transmission because of resistance to insecticides or incursion of wild populations of major vectors such as Triatoma infestans. In addition, transmission by blood transfusion or organ transplantation and from mother to child is now prevailing in some places, and requires the implementation of new strategies. These transmission patterns may also impact most industrialized countries (USA, Canada, Japon, EU), where migration of large numbers of citizens from Latin American countries infected by T. cruzi is increasing.

Entomological research of IRD teams focuses on three main aspects: 1) the introduction of wild populations of triatomines in human environments favoured by socio-economic and environmental changes (including weather), 2) the epidemiological role of sylvatic populations of T. infestans in the persistence of vector transmission, and 3) the acquisition of resistance to insecticides by vector populations.

Research in Parasitology of IRD teams is directed according to three axes: 1) the genetics and the evolution of populations of T. cruzi in order to determine their role in the transmission and morbidity, 2) the molecular and immunological characterization of T. cruzi with the aim of identifying virulence factors useful in the development of diagnostic tests and candidate vaccine antigens, and 3) the identification of genetic factors for human susceptibility to infection by T. cruzi.

The IRD team of Public Health concentrates on strategies for early detection and treatment of congenital Chagas’ disease: validation of screening and diagnostic tests, screening procedures in remote environments, clinical trial for the optimization of treatment for newborns. It has been suggested by IRD teams, and confirmed recently that Bolivia was the epicentre of the historic expansion of one of the main vector of Chagas’ disease (T. infestans) in the region. Bolivia remains the country where the indicators are most alarming (morbidity, incidence of new infections, reinfestation and resistance of the vectors, seroprevalence in blood banks and rate of congenital transmission, much higher than in other countries). Collaborative IRD researches tend to demonstrate that the Fight against Chagas’ disease might be more difficult to achieve in this country.

What can jeopardize vector control efforts?

The expected success of the vector control programs is based on the assumption that triatomine vectors are almost exclusive domestic populations, but:

-To reinforce the implementation of kit evaluation systems as a pre-requisite to commercialization in each country.
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-Wild and domestic populations do coexist and the former are more widely distributed than initially suspected in Southern Cone countries (principally *Triatoma infestans*).
- *T. infestans* has already undergone a domestication process in the past.
- Some wild triatomine species are displaying synanthropic trends such as domestication process

Some scientific questions that arise are:
- Do wild and domestic populations of *T. infestans* belong to the same species?
- Do *T. infestans* belong to the same species among its wide range of distribution?
- Do wild populations of *T. infestans* account for the risk of re-infestation after control interventions?
- Do emergent insecticide resistance compromise vector control efforts?
- Do emergent vectors constitute threats for new foci of transmission?

Some issues about *Triatoma infestans* in the Andean region
- Lack of speciation between wild and domestic *T. infestans* populations of Andean valleys in central Bolivia.
- Wild and domestic *Trypanosoma cruzi* cycles overlap in this region.
- Andean population of *T. infestans* is panmictic with spatial structuring (isolation by distance with low migration rate).
- *T. infestans* spread in association with recent human migrations.
- *T. infestans* apparently originated from a sylvatic rupicolous population, probably near Cochabamba and Sucre’s Andean valleys in Bolivia and, secondly adapted to human dwellings.

Population genetics of *Triatoma infestans*
2 allopatric groups of *T. infestans* with strong chromosomal and DNA content differences attributable to intraspecies variations.
Andean group (>1800 m)
- Large quantity of heterochromatin and high DNA content.
- Origin from a sylvatic population and adaptation to human dwellings.
- Geographic expansion to Andean regions of Bolivia and Peru.

Non-Andean group (<500 m)
- Heterochromatin and DNA loss by random genetic drift once from Andean group probably in the austral Chaco (founder event).
- Rapid and recent geographic expansion to the South of the Neotropical region (Argentina, Paraguay, Brazil).
- Greater domestic dependence and inability to return to sylvatic ecotopes = More susceptible to control interventions?

Re-infestation by *Triatoma infestans*
- Studies of re-infestation: In case of re-appearance of insects 1 to 2 years following insecticide applications and in absence of resistance to insecticide.
- Monitoring of re-infesting individuals from Cochabamba (Jamach’uma) and Santa Cruz (Vallegrande) valleys.
- Results in favour of a residual domestic population instead of a re-invasion of the houses from surrounding sylvatic foci.

Dispersal ability of *Triatoma infestans*
Active wild transmission site in Andean valleys of Bolivia
- 60% of wild *T. infestans* infected by *T. cruzi*.
- 50% wild mammals infected by *T. cruzi*.
- All those *T. cruzi* isolates belong to TcI lineage.
- Morphologically similar to domestic specimen, but with 1 generation/year, inhabit rocky habitats.
- Low dispersal ability of *T. infestans* attested by restricted gene flow between domestic and sylvatic populations using microsatellites markers.

*Triatoma infestans* in the Chaco region
Presence of a sylvatic arboreal population of *T. infestans* (dark morph) in the Bolivian Chaco. Morphologically similar to domestic *T. infestans*, except for their overall darker coloration. Closely related to members of the non-Andean group.
Inhabit hollow trees.
Only 2.5% positive for *T. cruzi* infection.
Origin of “dark morph” population?
From domestic members of non-Andean group (“Ability” to return to sylvatic ecotope?). From sylvatic members of Andean group with loss of DNA content by random genetic drift (Dispersion without previous domestication?).

Chaco Region Insecticide resistance
High resistance of *T. infestans* to pyrethroid insecticides and especially to deltamethrin (first mention in North Argentina, border with Bolivia).
Recently confirmed in Bolivian Chaco and in Cochabamba valleys.
Use of Carbosulfan (Carbamate) as an alternative in Bolivia with very poor results (low persistence).
Matter of concern for Southern Cone Initiative (INCOSUR) since June 2006 meeting in Brasilia.
Emergent vectors

- **Rhodnius stali** (candidate vector)
  - Normally sylvatic species
  - Domestication process in Alto Beni (La Paz) due to extensive deforestation, new human settlements and dwellings made of palm leaves
  - 7 to 9% of houses infected by *R. stali*
  - 6% of *R. stali* infected by *T. cruzi*
  - 3 to 4.5% of human infections

- **Triatoma sordida** (secondary vector)
  - 2 cryptic species:
    - **Group 1**: in Santa Cruz and Southern Cone countries, wide panmictic unit, more able to invade houses; 16 to 21% infected by *T. cruzi* (with clones belonging to lineage I, distinct from domestic *T. infestans* cycle); only 3% of human infections
    - **Group 2**: in Chaco, more sylvatic, ancestral species of the complex
  - Insecticidal control interventions more difficult against *T. sordida* because of sylvatic foci (house re-infestation) and wide panmictic unit (reappearance of domestic vectors due to immigrants from neighbouring localities)

How to improve disease management?
After decrease or interruption of vectorial and transfusional transmission of *Trypanosoma cruzi*:
- Persistence of a huge human reservoir
- Persistence of paramount alternate modes of transmission

Less attention given to human aspects of case detection and treatment

Some scientific questions that arise regarding this:
Better understanding of current epidemiological trends and genetic structure of *T. cruzi*
- Susceptibility to drugs
- Clinical expression: examples of congenital and childhood cases

Strategies for better treatment approaches
- Strategies to prevent congenital transmission
- Evaluation of chemotherapeutic efficacy
- *T. cruzi* virulence factors and new therapeutic targets
- Trypanocidal activities of plant extracts

Clonality in *Trypanosoma cruzi*
Widespread distribution of certain isozyme strains (from Bolivia to Chile and Brasil).
Majority of possible recombinant genotypes is lacking even in southern part of Bolivia where sympatric occurrence of different isozyme strains is common.
Strong linkage disequilibrium:
Suggest absence of frequent genetic recombinations and existence of a clonal population structure
- With genotypes being clones stable enough to be tracked for years.
- With natural clones being genetic entities to be studied separately for biomedical characteristics.

The stability of the multilocus genotypes conferred by clonality allows:
Epidemiological tracking and taxonomic studies (DTUs).
Downstream studies (impact of genetic diversity on biological properties such as virulence or persistence to drugs).
**Trypanosoma cruzi** genetic diversity
2 main phylogenetic lineages (upper DTUs).
6 Discrete Typing Units (lesser DTUs) as most reliable subdivisions of *T. cruzi* for epidemiological studies: 1 DTU for lineage I, and 5 DTUs for lineage II.

Biological properties and clonal evolution
Analysis of biological properties (virulence and pathogenicity in mice, infectivity in vectors) confirmed the correlation between phylogenetic distance among *T. cruzi* clonal genotypes and their biological properties
- Clones 20 and 39 more virulent in mice.
- Clone 20 more efficiently transmitted by vectors than clone 39.

However, no apparent difference of pathogenicity found between the 2 more frequent zymodemes isolated from humans.
Natural susceptibility to Benznidazole in *T. cruzi* expressed as IC50 level is not related with its genetic structure represented by the different DTUs.

Multilocus enzyme electrophoresis (MLEE) and random amplified polymorphic DNA (RAPD)

**Trypanosoma cruzi** major genotypes in Bolivia (clones 20 & 39)
- Presence of two major lineages (*T. cruzi* I & II) in triatomine bugs in different Bolivian localities (Cochabamba, La Paz).
- High frequency (46%) of mixed infections (clones 20 (TcI) and 39 (TcII)) in *T. infestans* in Bolivia.
- No significant association between *T. infestans* and any particular clone circulating in the area.
- Different frequency of clones among localities suggesting limited exchanges mainly governed by limited dispersion of triatomine bugs.
- High discrepancy in clones 20 and 39 distribution in vectors and in patients in one Bolivian community (only 18% of mixed infections in patients).
- What occurs in humans? Selection of a specific clone in humans (clone 39) explained by difference of infectivity or parasitaemia control by immune system:
  - Similar transmission behaviour of the two clones (proportion of mixed infections during acute phase similar to that in vectors).
  - Selection occur later in the infection (further drastic control of clone 20 parasitaemia by immune system) during chronic phase.
Epidemiological trends in childhood

Clinical presentation: High rate of abnormal ECG tracing among Chagasic patients.
- Already present among individuals younger than 13 years (early treatment strongly recommended).

Diagnosis: PCR 84% sensitive in chronic patients (higher results in children less than 13 years).
- Only 72% positive for anti-SAPA response (higher results in children 1-6 years old).
- Higher proportion of clone 39 than clone 20 except in younger patients harbouring more frequently mixed infections (1-6 years old).

Treatment: Absence of reliable criteria for evaluation of chemotherapeutic efficacy.
- 12 months after specific treatment of children (5-10 years old), only 6% of negative conversion by conventional serology and 32% with IgG anti-SAPA.

Follow-up: High variability of strain detection and identification before and after treatment
- Effect of benznidazole on activation of intracellular amastigotes, in which their differentiation is subsequently controlled by the immune system?

Parasites and vertical transmission

DNA polymorphism is not associated with the:
- Occurrence of congenital infection
- Development of severe clinical forms of congenital Chagas’ disease

High frequency of T. cruzi sublineage IId (clone 39) in blood of infected newborns indicative of general distribution of T. cruzi genotypes in Bolivian patients (absence of preferential association of one genotype with congenital infection).

Mothers display low parasitic DNA levels (qPCR), corresponding to parasitaemia < 10 parasites/mL in 90% of them.
Newborns display high DNA levels corresponding to parasitaemia > 1000 p/mL in more than 76% of them.

Cohort study of 128 pregnant vs 156 non pregnant chagasic women matched for age, parity and place of residence: RR=5 (1-17) for parasitaemia in pregnancy (9.4 vs 1.9%).
Cohort study of 148 chagasic women (28% positive by micromethod) during pregnancy: RR=15 (2-118) for congenital transmission when parasitaemic vs non parasitaemic (13.9 vs 0.9%).
Dose-effect relationship of intensity of parasitaemia in mother’s blood with the risk of vertical transmission.

<table>
<thead>
<tr>
<th>Mother’s parasitaemia (Parasites/mL)</th>
<th>Proportion of congenital transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>None detectable</td>
<td>0.9%</td>
</tr>
<tr>
<td>5 to 14</td>
<td>4.5%</td>
</tr>
<tr>
<td>15 to 24</td>
<td>12.5%</td>
</tr>
<tr>
<td>25 to 70</td>
<td>66.7%</td>
</tr>
</tbody>
</table>

Incidence of congenital transmission

All published studies on congenital transmission in Bolivia or abroad took place in large urban settings with well installed health facilities.
What’s up in poor remote rural settings with high home birth rate (40% in Bolivia)?
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<table>
<thead>
<tr>
<th>Place of study</th>
<th>Place of delivery</th>
<th>Seroprevalence in mothers</th>
<th>Proportion of vertical transmission</th>
<th>Incidence rate of congenital infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Santa Cruz (800000 inhab.)</td>
<td>Non-endemic Hospital birth</td>
<td>23.3%</td>
<td>3.4%</td>
<td>0.8%</td>
</tr>
<tr>
<td>Bermejo (25000 inhab.)</td>
<td>Non-endemic Hospital birth</td>
<td>33.9%</td>
<td>5.2%</td>
<td>1.5%</td>
</tr>
<tr>
<td>Yacuiba (70000 inhab.)</td>
<td>Endemic Hospital birth</td>
<td>42.2%</td>
<td>6.0%</td>
<td>2.6%</td>
</tr>
<tr>
<td>Carapari (10000 inhab.)</td>
<td>Endemic Hospital birth</td>
<td>60.1%</td>
<td>7.1%</td>
<td>4.3%</td>
</tr>
<tr>
<td></td>
<td>Endemic Home birth*</td>
<td>64.4%</td>
<td>9.3%</td>
<td>6.0%</td>
</tr>
</tbody>
</table>

* 40% of all births and congenital cases in this setting

Strategy to prevent congenital disease
-70% treatment compliance (60 days) in case of passive follow-up by health teams vs 90% treatment compliance during active follow-up by research teams.
-Need for better treatment scheme (twice daily vs single daily dose and 60 vs 30 days).

On going study (ending 2008)
Open randomized controlled superiority trial comparing Benznidazole treatment efficacy (negative seroconversion at month 9) and compliance (25% difference between arms) in two groups of congenitally infected newborns.
-60 newborns treated by 7.5 mg/Kg/d, single daily dose for 30 days
-60 newborns treated by 5 mg/Kg, twice daily for 60 days
Provision of automatic pill dispensers and active follow-up at home during and after treatment.

Evaluation of treatment efficacy
Recombinant protein from *T. cruzi* (rTc24: flagellar calcium-binding protein) useful:
For specific diagnosis of *T. cruzi* (Tc24-based PCR).
For monitoring cure of chronic human Chagas’ disease by distinguishing dissociated treated patients from treated but uncured patients:
-All sera from the treated but uncured patients (TNC) reacted with rTc24, showing absorbance values of greater than 0.1.
-Whereas 80% of sera from treated dissociated (TD) and cured (TC) patients showed absorbance values of less than 0.1, similar to non chagasic individuals (NCh).
-ELISA, Western Blotting, Tc24-based PCR.

*Trypanosoma cruzi* virulence factors
Identification of a parasite-released molecule Tc52 produced by the two forms of the parasite. With immunosuppressive activities in vitro:
- stimulation of IL-10 secretion and down regulation of IL-2 production and T cell proliferation.
In vivo, infection with single mutant parasite clones (targeted deletion of Tc52 protein encoding allele; Tc52+/−) in mice failed to induce:
- high parasitaemia during acute phase
- suppression of IL-2 and T cell proliferation
- inflammation in heart tissues during chronic phase
Active immunization with Tc52 stimulated both arms of the immune system and induced a significant level of protection in term of parasitaemia and mortality in mice.

Tc52 is among candidate molecules that may be used to design new therapeutic strategies (block of enzymatic activity of Tc52) or to develop vaccinal strategy against Chagas’ disease.

Natural products as trypanocidal
Plant-secondary metabolites isolated from medicinal plants from Bolivia and Paraguay demonstrated moderate to high activity in in vitro and in vivo bioassays against T. cruzi. 3 extracts exhibit trypanocidal activity in vivo in the mouse model of acute and chronic infection.

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**Chagas Pediatric and Congenital**

**Jaime Altchech**, Pediatrician, Clinical Researcher, Parasitology unit, Children’s Hospital of Buenos Aires, University of Buenos Aires, Argentina

*Trypanosoma cruzi* (*T. cruzi*) is primarily transmitted by vector-borne, blood transfusion or during pregnancy. In the last years, the control of both vectorial and blood transfusion transmission of this parasite has been consolidated. Consequently, congenital transmission has become more relevant as source of new cases.

Transplacental infection may occur in some or all gestations from a *T. cruzi* seropositive mother. The prevalence for *T. cruzi* in pregnant Latin American women varies from 5 to 40%, depending on the geographical area. The transmission rate ranges from 4 to 12 %. These discrepancies may be attributed to different diagnostic methods, parasite lineages and/or immunological status of infected mothers.

There is a consensus about diagnosis criteria of congenital infection:

- In newborn from mother with reactive serology for *T. cruzi*: present parasitemia by direct parasitological method (i.e. microhematocritic test);
- In infants older than 7 months: *T. cruzi*-specific antibodies detected by 2 tests, having discarding previous blood transfusion and vectorial route.

PCR can also be a convenient diagnosis test but the procedure needs to be standardized since false positive results can be observed in centers without large expertise or skilled professionals.

Regarding clinical manifestations, 60-90% of congenital cases are asymptomatic, with low rate of mortality. There is no specific clinical marker of congenital Chagas disease. Symptomatic patients show hepatomegaly and/or low birth weight related to prematurity. Myocarditis and meningoencephalitis are infrequent and mainly found in areas of high vectorial density and in infants with *T. cruzi/HIV* coinfection.

It has been largely documented that, independently of the transmission route, the administration of parasiticidal chemotherapy during the acute phase of infection is effective in ensuring a high cure rate, particularly in children younger than 15 years old. This is most relevant considering that installation of etiologic treatment during pregnancy is not
recommended. Unfortunately, there are only 2 drugs currently available for the treatment of Chagas disease, benznidazole (Bz) and nifurtimox (Nfx). Both drugs were developed over 30 years ago; however, their mechanisms of activity and pharmacokinetics in children still remain unexplored.

Serious side effects, such as allergic dermopathy, peripheral neuropathy, and granulocytopenia, have been reported in patients treated with benznidazole. However, children below the age of 12 years show good tolerance and remarkable adverse events are infrequent in this group of patients.

The treatment can be accomplished at ambulatory level but it should be supervised to allow rapid and appropriate intervention whenever any adverse reaction appears.

A relevant issue that still needs to be addressed is the cure definition of treated patients. So far, the universally accepted criterion is determined by negative conversion of conventional serology for \textit{T. cruzi}. However, the drawback is that, in those patients whose disease has been evolving since long, the evidence for this change may only arise many years later.

On the other hand, classic parasitological methods, in spite of their limited sensitivity, are useful to confirm therapy failure. PCR is currently being evaluated as post therapeutic marker. Yet, the procedure still requires go through the process of standardization for its application.

Recently, a WHO/PAHO meeting of the Scientific Working Group for Chagas disease of TDR (Buenos Aires, April 2005) defined as priorities among research topics in the treatment in \textit{T. cruzi}-infected children:

\begin{itemize}
  \item To develop new formulations of Nfx and Bz for children and pharmacokinetics studies for these drugs.
  \item To evaluate the parasite strains involved in congenital transmission.
  \item To assess the adherence of patients under treatment with conventional or new formulations of existing drugs.
  \item To advance in the development of new chemotherapeutic approaches for the treatment of pediatric Chagas’ disease, with higher efficacy and less side effects than existing drugs.
\end{itemize}

In conclusion, Chagas should be mainly considered as a pediatric disease. Therefore, infected children constitute the target population for chemotherapy. Particularly, in areas with active insect-mediated transmission, the vector control should be integrated with case detection and children treatment. This approach is highly cost–effective compared with insecticide spraying campaigns alone.

Because of migrations, congenital \textit{T. cruzi} infection is increasingly seen in urban areas and in traditionally non endemic regions all over the world. Accordingly, screening of pregnant women should be accomplished to identify infected mothers and to diagnose and treat infected newborns.


Hope for our Children: Chagas Disease Treatment Experience in Bolivia
Carlos Salinas, Silvia Nole, Gustavo Tapia. Plan International Inc. Bolivia, Edificio El Greco, Avenida Ballivian 2550, esquina calle 12, Calacoto, La Paz, Bolivia

BACKGROUND: Chagas disease (or American trypanosomiasis) is endemic in Bolivia. Nearly 1.5 million Bolivians (especially in rural areas) are currently infected with Trypanosoma cruzi, causing nearly 15% of deaths among persons aged 15-75 years. Due to this situation, Plan Bolivia and Pro-Habitat implemented successive Chagas prevention and control projects for more than ten years (1995 - 2008) in the departments of Sucre and Tarija. Both departments harbor the heaviest burden of Chagas disease in the country. “PROPLAN I” project (1995 – 1999) introduced home improvement interventions. “PROPLAN II project (2000 -2005) added a treatment component and was funded by the Government of Bolivia, DFID, the European Union, Community Fund (UK National Lottery Charity Board) and the Government of Netherlands. The on-going PROPLAN III continues Chagas control interventions and is funded by the Government of Bolivia and Plan. PROJECTS’.

OBJECTIVES AND STRATEGIES: The projects aimed to: (a) the reduction of the house infestation by the Triatoma vector, by promoting preventive behaviors’ among household members, by improving the physical environment (home improvement) and by providing indoor residual spraying, and (b) in communities achieving low infestation rates, the provision of treatment (with Benznidazole) to sero-positive children under 15 years of age. Communication-for-Behavioral-Impact, active community participation, involvement of
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children and women, appropriate experts’ advice, and involvement of governmental duty-bearers are key strategic elements.

![Chagas intervention model](image)

**PROJECT RESULTS:** A total of 60,000 inhabitants living in 15,608 houses of 6 municipalities of both departments were directly benefited in the first two projects and other 9000 houses are being improved through the third project. *Triatoma* infestation within the houses decreased from 80% to less than 3% in the outdoors and near 0% in the indoors. More than 80% of household members practiced recommended preventive behaviors, 3,331 (23%) of 14,000 tested children were found infected with Chagas, and 3,095 (93%) of those infected children started treatment with Benznidazole. Adherence to treatment was high, 96% of children who started have completed it and 86% had a significant decrease in their serologic titers. The project had a total cost of USD 16 million, of which 8 million (50%) were family contributions and USD 3 million (19%) came from the central and local governments.

<table>
<thead>
<tr>
<th>Projects</th>
<th>Number of Improved houses</th>
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<tbody>
<tr>
<td>PROPLAN I</td>
<td>3,639</td>
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<td>PROPLAN II</td>
<td>11069</td>
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<tr>
<td>PROPLAN III</td>
<td>9000</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>24,608</strong></td>
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Other Outcomes: (1) Participating families building new homes follow the project’s guidelines, (2) international NGOs are using the project’s experience to develop similar endeavors in other parts of the country, (3) central and local governments are budgeting project components within their regular allocations and (4) the central government is issuing policy norms for Chagas control which apply the best of the projects’ experience.

Experience of the MSF Chagas Programs: South & Central America
Nines Lima, Tropical Medicine Advisor MSF-OCBA, Barcelona, Spain

MSF History in Chagas Disease Projects

<table>
<thead>
<tr>
<th>Year</th>
<th>Yoro - Honduras</th>
<th>Tarja - Bolivia</th>
<th>Matapica - Nicaragua</th>
<th>Olopa - Guatemala</th>
<th>Sucre - Bolivia</th>
<th>Cochabamba - Bolivia</th>
<th>New Challenges</th>
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</tbody>
</table>

MSF Projects in Central America
- Yoro, Honduras.
- Olopa, Guatemala.

Objectives:
- To evaluate the safety of benznidazol in children <15 years of age with Chagas infection.
- To evaluate the efficacy of benznidazol.
Methodology:
- MSF-S collaborated with Chagas National Programs.
  The Areas of Intervention were selected according to High Seroprevalence and the presence of an active Vector Control Program (IRS and Entomological Evaluations).
  The selected areas were also required to have health structures, HR, equipment and immediate operational capacity for adequate diagnostic, treatment and follow up.
- Vector Control Activities were included in the projects (primarily in Yoro), IEC and Training for Health Staff with regards to Diagnostics and Treatment.

Methodology Diagnostics:
- Blood samples of digital puncture were collected in rural communities on filter paper.
  Screening with conventional ELISA was performed in the laboratories of Referal Hospitals.
  Diagnostic Confirmation was done with recombinant ELISA.
  Quality Control for all positives and 10% of the negative results (External QC in Tegucigalpa Regional laboratory)

Methodology Treatment:
- Counselling to parents/guardians of infected children: potential benefits of treatment, possible risk factors, adverse events and how to proceed if and when they occur.
  Informed Consent.
  Dosage: 5 - 7.5mg/Kg/day  2 or 3 times during 60 days (max. 300 mg/day).
  Weekly Follow Up, registering side effects: clinical presentation and degree of severity (mild, moderate or severe).

Methodology Treatment Efficacy:
- First serology evaluation at 18 months post-tx:
  - Processing simultaneously blood samples post-treatment and pre-treatment (preserved at –20ºC ) with recombinant ELISA.
  - Negative Results with recombinant ELISA were confirmed with conventional ELISA. If both samples were negative, the patient was notified of their cured status.
- Second serology evaluation was performed at 36 months post-treatment of patients with positive or indeterminate results in previous evaluations.
  - In case of a new positive or indeterminate result we measured the % difference between the optical density (OD) of both results.

$$TC = \frac{OD\hspace{10pt} post\hspace{10pt} treatment - OD\hspace{10pt} pre-treatment}{OD\hspace{10pt} pre-treatment} \times 100$$
Etiological treatment, adverse events and seroconversion in patients with infection of *Trypanosoma cruzi* in Honduras and Guatemala, Central America

Sílvia Morote¹, Josep Mª Escribà¹, Gema García², Paul Roddy¹, Pedro Albajar-Viñas³, Mª Ángeles Lima¹.

Background
Chagas disease, a highly prevalent parasitic illness in Latin America, infects approximately 13 million people worldwide and kills over 14,000 each year. In 2003 it was estimated that in Central America there were still 26 million inhabitants living in endemic areas with risk of being infected and 2 million infected people whom morbidity-mortality was unknown. In Guatemala and Honduras Médecins Sans Frontières implemented two projects assessing the safety and efficacy of benznidazol for the treatment of children under fifteen of age with an early chronic phase of *Trypanosoma cruzi* infection.

Methods
Serological detection of *Trypanosoma cruzi* infection was performed using the ELISA conventional assay. ELISA conventional results were confirmed using ELISA recombinant assay for all positive results and 10% of negative results.

After consent was given, patients were treated with benznidazol (Radanil®, Roche) at a dosage of 5 – 7.5 mg/Kg every 8 to 12 hrs during a 60 day period. Using a follow-up protocol adverse events were registered and clinical evaluations were given periodically when indicated.

Patients were followed-up at 18 months after the completion of treatment by extracting blood samples and processing them simultaneously with the pre-treatment blood samples which were preserved at –20°C. Disease classification from these blood samples were determined using the ELISA recombinant assay (ELISA Chagas Test Ag recombinantes Wiener Lab Argentina). Negative diagnostic results were confirmed with the ELISA conventional assay. When both assays were negative the patient was definitively classified as cured and no other follow-up appointments were scheduled.

The patients with positive or doubtful results at 18 months post-treatment were again followed up 18 months later (i.e. month 36 after post-treatment). Simultaneously, a blood sample in paper filter pre-treatment and the 36 months post-treatment, with ELISA recombinant, negative results were confirmed with negative results on ELISA conventional and a positive or doubtful result established the indication of calculation of the percentual variation between the spectrophotometer lecture and the optical density of both.

Results
**Honduras** 24,471 children were screened, of which 232 were confirmed positive (seroprevalence of 0.93%). 231 patients were treated with weekly follow up and post-treatment serological follow-up. Side effects were present in 50.2% of the patients, but most of these were mild and only three patients had to stop their treatment. The most frequent were gastrointestinal (53.4%), followed by cutaneous (25.9%) and neurological (20.7%) manifestations. 88.2% of the patients seroconverted at 18 months and 93.9% at 3 years after treatment.

**Guatemala** 8,927 children were screened, resulting in 124 positive cases (prevalence 1.5%). Side effects were reported in 50.8% of the patients and the most common were gastrointestinal (25.7%) and cutaneous (25.7%), followed by neurological (22.8%)


manifestations. In Guatemala the verification of the post-treatment seroconversion is currently ongoing but preliminary results shows that out of 47 patients followed up to 18 months 61.7% already seroconverted.

**Conclusions** The results of these two treatment experiences suggest that in these two Central American countries, both the diagnostic and treatment of *T. cruzi* infection are feasible, needed and ethically unquestionable.

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**5. Session V: Physiopathological, Clinical and Epidemiological Aspects of Chagas Disease**

**Chagas Heart Disease: Physiopathological Mechanisms Prognostic Factors and Risk Stratification**

*Anis Rassi Jr, MD, PhD, FACC, FACP, FAHA, Past Chairman of Guidelines of the Brazilian Society of Cardiology - Scientific Director, Anis Rassi Hospital Avenida José Alves 453 - Setor Oeste Goiânia (GO), Brazil*

Chagas’ disease represents the third largest parasitic disease burden globally, after malaria and schistosomiasis. It is estimated that 10 to 12 million people are infected, and about one third of these have cardiomyopathy, with over 21,000 annual deaths attributed directly to this etiology. Chronic Chagas’ cardiomyopathy (CCC) is the most common form of non-ischemic cardiomyopathy worldwide, and one of the leading causes of morbidity and death in Latin America.

Current knowledge suggests that the pathogenesis of CCC is dependent on a low-grade, persistent parasite presence, coupled with the participation of antiparasite and/or anti-self immune responses. Among other causes of chronic myocardial damage, autonomic derangements and microcirculatory disturbances constitute ancillary rather than fundamental mechanisms.
The chronic cardiac form of Chagas’ disease is characterized by a focal inflammatory process composed of lymphomononuclear cells that produce progressive destruction of cardiac fibers and marked reactive and reparative fibrosis affecting multiple areas of the myocardium.

The parasympathetic cardiac nerves and the conduction system are preferentially involved, producing intraventricular and atioventricular blocks, sinus node dysfunction, and ventricular arrhythmias. The right bundle and the left anterior fascicle are most frequently affected. The focal myocardial fibrosis provides the anatomic substrate for ventricular and/or atrial arrhythmias, predisposes to cardiac dilation and failure, and leads to formation of narrow necked left ventricular (LV) apical aneurysms, a hallmark of CCC. Thrombi are often present in the left ventricular aneurysm and in the right atrial appendage. This may explain the common occurrence of thromboembolic phenomena in the systemic and pulmonary circulation. Annual mortality for outpatients has been estimated to be about 4%. Sudden death accounts for 55-65% of the deaths, heart failure for 25-30% and thromboembolic phenomena for the remaining 10-15%.
The Contribution of Autoimmunity to Chagas’ Cardiomyopathy
(Marin-Neto JA, Rassi A Jr, Maciel BC, Simões MV, Schmidt A. Chagas Heart Disease. Evidence Based Cardiology - 3rd Edition (In press))

- Demonstration of predominant mononuclear cells in the inflammatory infiltrates suggesting a delayed hypersensitivity reaction.
- Identification of heart- *T cruzi* cell cross-reactive T-cell antigens with reproduction of pathobiological changes by passive transfer of immune cells in murine models.
- Attenuation of the inflammatory process as a consequence of tolerance induction to myocardial antigens.
- Induction of myocardial aggression after immunization with cardiac myosin.
- Isolation of cardiac myosin autoreactive T cells in molecular mimicry with *T cruzi* B13 protein from affected tissue.
- In vitro immunization with B13 protein eliciting T cell clones cross-reactive with cardiac myosin.
- Immunization with *T cruzi* ribosomal antigens inducing crossreactive antibodies and heart conduction abnormalities.
- Similar cross-reactive autoantibodies present in sera from patients with CCC disease inducing arrhythmia in explanted hearts.

Although autoimmune mechanisms have been demonstrated, their relative contribution to producing myocardial damage is still debated. Autoimmunity may be an epiphenomena of the parasite related myocardial inflammation.

Neurogenic Hypothesis of Chagas Cardiomyopathy
This theory has several pitfalls from clinical trials and pre-clinical trials studies that do not correlate between denervation and type of death or pathological features in the Chagasic heart, among other factors.

**Autonomic Dysfunction and Prognosis in Chagas Heart Disease**

<table>
<thead>
<tr>
<th>Heart Rate Variability</th>
<th>Alive (n=107)</th>
<th>Death (n=64)</th>
<th>Sudden death (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDNN &lt;100ms</td>
<td>19%</td>
<td>41%*</td>
<td>31%*</td>
</tr>
<tr>
<td>rMSSD &lt;25ms</td>
<td>38%</td>
<td>36%</td>
<td>33%</td>
</tr>
<tr>
<td>PNN50 &lt;1%</td>
<td>13%</td>
<td>13%</td>
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</tr>
</tbody>
</table>

*p < 0.05 vs alive

**Myocardial Perfusion Abnormalities in Chagas’ Heart Disease**
Dysfunction in Chagas’ disease perfusion
Proposed Model for Disease Development in *T. cruzi* Infection

The severity of disease is determined at least in part by:
- tissue parasite load
- quality of the anti-parasite immune response


Proposed model for lesion progression in patients with Chagas disease

Modified from Tarleton RL. Trends Parasitol 2001;17:447-51
Chagas’ Heart Disease Independent Predictors of Mortality

- NYHA functional class III/IV
- Cardiomegaly (chest X-ray)
- Impaired LV function (echo)
- NSVT (Holter)

Manifestations associated with poor prognosis include New York Heart Association (NYHA) class III or IV, cardiomegaly on chest radiography, ventricular tachycardia or complex ventricular arrhythmias, increased left ventricular systolic diameter, and segmental or global LV wall motion abnormalities.

Patients with symptoms or electrocardiographic (ECG) changes consistent with CCC should undergo a comprehensive cardiac evaluation, including 24-hour Holter monitoring to detect arrhythmias; exercise testing to identify exercise-induced arrhythmias and assess functional capacity and chronotropic response; and 2-dimensional echocardiography to assess biventricular function, wall motion, and structure.

Patients who present with symptoms of heart failure (NYHA class III/IV) or have LV dysfunction on echocardiogram associated with episodes of nonsustained ventricular tachycardia (NSVT) on Holter monitoring are at the highest risk of death and should be regarded as candidates for aggressive therapeutic management. It is not uncommon for chagasic patients with ECG and marked LV segmental abnormalities to be asymptomatic hard workers. When heart failure symptoms occur (NYHA class III/IV), invariably all patients...
manifest associated cardiomegaly on the chest radiography, global systolic dysfunction on echocardiogram, and NSVT on Holter monitoring.

Conversely, patients with abnormal ECG but in NYHA class I/II with neither LV dysfunction on echocardiography nor NSVT on Holter are at low risk of death. These patients should be followed up annually or biannually. Between these 2 extremes are patients with either LV dysfunction or NSVT. In such patients, who are at intermediate risk, the optimal treatment strategies should be individualized.

The Epidemiology of Chagas Disease in Latin America in 2008 and the Future Risks for its Control
Joao Carlos Pinto Dias, MD, PhD Senior Resercher of the Oswaldo Cruz Foundation, Brazil

Human Chagas Disease (HCD) is largely spread in all Latin American Continent, where 19 countries are considered endemic and about 30 million of individuals are still under the risk of contamination. Twelve million are estimated to be infected by Trypanosoma cruzi and about thirty per cent of them are under risk of severe cardiopathy. Official estimation (PAHO) considers 12,500 annual deaths due to HCD in 2006. All these epidemiological figures are progressively decreasing, chiefly since 1980, when important demographic and social changes occurred along the region and effective control programmes where implemented in several countries.

Chagas Disease: estimated figures for Latin American Region in 2007
(Source: Dr. R. Salvatella, PAHO, 2007)
-Total Population: 531,432,850
-Number of infected individuals: 15,632,000
-Incidence of vector transmission: 41,200 (yearly)
-Congenital transmission: 14,385 cases/year
Focus on Neglected Tropical Infectious Diseases:
Chagas Disease a Public Health Threat in the Americas & Beyond
Report issued: September 30, 2008

-Seropositive women in fertile age: 1,809,507
-Population under risk in endemic areas: 28,595,000
-Cardiopathies: 1,772,365
-General prevalence in blood banks: 1.28%

Contextual considerations for CD epidemiology & management in LA
- Most of the chagasic people are very poor and depend of public health system (PHS).
- In all Latin America PHS is being decentralized and requires strategic clinical and laboratory
reference centres for CD management.
- The production and distribution of active drugs against T. cruzi remain complicated.
- Most of chronic and acute cases are oligosymptomatic.

Main contextual factors have been the increased migration, the intensive anthropic action, the
urbanization of rural populations, and the expansion of agriculture frontiers in endemic
countries. Regarding Public Health, undoubtedly the principal factors are the increased of
vector control programs, and of blood donors selection along the whole continent.

Evolution of Chagas Disease in Latin America
Change of the epidemiologic parameters because of transmission reduction between 1990 and
2000. (Source: TDR/WHO, PAHO, WHO)
Some particular aspects at the social and political side are:
- Unstable social policies: fragile health authorities (ministers staying about one year in their post).
- Difficulties in the transition from vertical to horizontal control programmes.
- Progressive loosing of interest in HCD from the scientific side.
- Markets not interested in developing drugs for HCD as it did not represent profit.
- Low political interest on the disease.

By the political angle, the major achievement was the beginning of intergovernmental initiatives, launched in Southern Cone since 1991. The transmission of HCD was really minimized in most endemic countries, but residual foci still exist in non controlled and/or poorest and more isolated areas, in parallel with a heritage of almost thirteen million of already infected individuals. For the near future, the major challenges will be the maintenance of control and surveillance activities; together with the adequate medical and social care for the infected people. The risk of new cases will remain on congenital and oral transmission, as well as on residual vector transmission.

All of these situations tend to decline if vector control and surveillance are maintained. By the biologic and ecological side, the risk of transmission restarting will depend of secondary and sylvatic vector species, chiefly in isolated rural areas and or primary forests. The risk of the development of insecticide resistance has been demonstrated in some isolated situations, being a possibility for the late future. Nevertheless, the mayor risk for HCD in Latin America remains on the side of political will. Current there is lack of disease visibility, of health priorities and the progressive weakening of technical, scientific and administrative expertise; all this factors must be considered at present to overcome the challenges of HCD control.

Aspects regarding the visibility and priority of HCD
- The chronic evolution of disease.
- The proportion of sub clinical acute and chronic cases.
- The problem of medical access for poor people.
- The low return profits for political and financial inversion in rural poor areas.
- The still existent difficulties of medical staff for HCD diagnosis and management.
- The competition of HCD with other acute diseases (malaria, dengue fever) and common health demands.

Major risks and problems at present in Latin America
The reached success in the decreasing of the disease presentation and taking into consideration the political and administrative inconsistency in the Region, some risks must be appointed:
- De-structure of the programmes and surveillance.
- Progressive loosing of skill Human Resource.
- Lack of following of epidemiological information.
- Absence or weakness of the educative component.
- Several difficulties for the counter-reference of cases.
- Absence of a pharmacological “basic basquet” for chagasic people.
- The pragmatic misunderstanding of isolated and marginal populations.
- Loosing of research priority.
- Loosing of consistence and priority in the University Curricula.

Vector control: main challenges
- To clarify the factors that determines reinestation by *T. infestans, T. brasiensis, T dimidiata* and other vectors.
- To reach effective vector control in the peridomiciliary environment.
- To address the threats imposed by sylvatic vectors?
- To improve the integration of vector control, environmental management and housing improvement based on locally available resources.
- To detect low density populations of triatomines.
- To enhance community participation in vector control.
- To address and prevent resistance to insecticides.

Oral Transmission: a new challenge
- Outbreaks and occurrence since 1968 (RS, PA, PB, México, SC, BA, CE, Amazon, Venezuela.)
- The case is the key point: diagnosis and criteria.
- Cases generally depend on neighboring triatomines, but other possibilities exist.
- Research and attention: diagnosis confirmation, case treatment, exclusion-inclusion of Hypothesis, contact examinations and focus elimination.

Scoring some risks for the near future
Epidemiological:
- Recrudescence of classical vectors (low)
- Increasing of housing colonization by wild vectors (low)
- Appearance of new endemic areas of vector transmission (low)
- Recrudescence of transmission in blood banks (very low)
- Increasing of congenital transmission (very low)
- Increasing of oral transmission (unpredictable)

Institutional:
- Incompetence of decentralized structures (high)
Decreasing of priority in PAHO and WHO (possible)
Loosing of consistence & effectiveness of the current Initiatives (possible)

Scientific:
- Lack of priority and financial help (high)
- Decreasing of scientific interest (middle/high)

Political: Major decreasing of priority (high)

Some general conclusions and advice for Countries in advanced control stage
- *T. infestans* elimination and the control of HCD in Brazil, Chile, Uruguay etc. depends on political responsibility and continuous surveillance.
- Technical control and reference staff’s at the central level are required to support the implementation and continuity of decentralized control activities.
- Similarly, technical laboratory references are necessary to help municipalities and possible new clinical and epidemiologic situations.
- The Public Health System must be open and linked with other correlate instances such as Education, habitation and social security.
- Operative research will be always necessary.
- These Countries have responsibility as a reference to other endemic Countries.
The Epidemiology of Chagas disease has changed along the years. Classically described as an endemic Latin America disease related to poor rural areas, nowadays we can find Chagas disease in urban areas, far away from the rural endemic areas. Migration flows are at the root of this phenomenon.

During the last year, Spain has received an increasing flow of migrants from Latin America. Around 1.5 million people from this area live in Spain. Data from 2001, show that Spain has received 49% of Latin-American people migrating to Europe, followed by Italy (13%), United Kingdom (12%) and Germany (10%).
Latin American migration by country in Spain (National Institute of Statistics, Spain 2007)

Total population in Spain: 45,200,737  
Total immigrant’s population: 4,307,464 (9.52 %)  
Total Latin American immigrant population: 1,558,203 (36 % of immigrants; 3.4 % of the total population).

<table>
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<tr>
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<td>BRASIL</td>
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<td>VENEZUELA</td>
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<td>OTRAS</td>
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</tbody>
</table>
European Cases of Chagas Disease

- **Italy**: 22 cases
  - 1 acute case (traveller)
  - 1 reactivation
  - 2 chronic cardiac forms
  - 1 chronic digestive form

- **France**: 18 cases (Paris), 7 chronic cardiac forms, 1 acute case.

- **Germany / UK / Portugal**: No cases reported, (or anecdotic).

- **Sweden**: 2-4 cases/year reported.

- **Spain**: >1,000 cases

- **Switzerland**: 28 cases
Regarding the cases in Switzerland: A closer look


-2002-2007: 28 cases with positive serology or with borderline serology and positive animal inoculation.
  - 2 congenital cases (Bolivian)
  - 1 post heart transplant reactivation (Brazilian): fatal
  - 4 cardiomyopathies, 3 pacemaker (Bolivian)

  Most cases in indeterminate phase

-Latin-american migrants, mostly undocumented women, living in Western Switzerland (Geneva).

-No affected travelers.

-Serosurvey in 72 latin-american undocumented pregnant women.
  - 7/72 positive (9.7%)
  - Bolivian 5/30 (16.7%)


First cases of Vertical Transmission described in Spain (out of studies):


Few epidemiological studies have been undertaken in Europe concerning Chagas disease. In Spain, studies at the blood banks show that 0.9% of Latin-American blood donors are infected with T.cruzi. On the other hand, in two studies in pregnant women, the global percentage of those infected by T.cruzi reaches 4.8%. Isolated cases of vertical transmission have been published in Europe.

Conclusions:
In Europe, the challenges are: 1) to avoid/control T.cruzi transmission through the control and screening in blood banks and in pregnant women; 2) to start a protocol for the diagnostics and management of infected people; 3) to give adequate education to health professionals in order to keep in mind the diagnosis of Chagas disease.

References:

6. Session VI: Research Priorities and Novel Chemotherapeutic R&D Findings

The Knowledge, Gaps and Research Priorities in Chagas Disease with Emphasis on Finding Solutions to Address the Needs of the Vulnerable Populations

**Janis K. Lazdins**, Coordinator, Drug development and evaluation for helminths and other neglected tropical diseases. Special Programme for Research and Training in Tropical Diseases, World Health Organization, Geneva Switzerland

With the enhanced access of available drugs for the treatment of Chagas disease (e.g.: Nifurtimox donation by Bayer), the availability of epidemiological data, definition of global case distribution, the international interest in supporting disease control programs and unfortunately the lack of any prospects for innovative treatments available in the short term, there is an opportunity to promote expanded Chagas disease treatment as part of national disease control policies. However, key research questions must be addressed if rational and safe use of drugs is to be promoted.

Among the key gaps that should be addressed through research the following can be highlighted:

1.- The validity of treating acute new cases is unquestionable; however, there is need to define the threshold of prevalence of vectorial transmission (and patient reinfection rates) that may justify implementation of treatment strategies with currently available drugs.

2.- Evidence to support the need (or not) of differential use of Nifurtimox vs. Benznidazole depending on geographical areas (anecdotal reports claim that both drugs are not equally effective in different geographical areas). This could be approached through randomized control efficacy studies in targeted geographical areas.

3.- Evaluation of safety of drugs used under field conditions where availability of trained personnel to detect adverse effects early during the treatment is limited or not existent. For this in the first instance there would be need to conduct research to establish the tools and methods to develop a pharmacovigilance that is compatible with local conditions (build on a
model that TDR is developing for miltefosine use in VL in the Indian sub-continent elimination program).

4.- Clinical research to validate the dosages and regimens for benznidazole or nifurtimox that not only would be most adequate to achieve the clinical benefits intended through expansion of treatment actions but also reassure the control programs that the risk of emergence of resistance is minimal. Needles to emphasize the consequences if one or both drugs would be lost to resistance.

Risks associated with expanded access to treatment

Safety
Drug resistance
Sustainability
Patient exclusion

Regarding Safety, treatments can not always be conducted under the best medical supervision especially in resource poor countries. By the nature of the drugs that are used there is risk that late detection of drug reactions can increase the number and seriousness of the adverse events some times resulting in lethality.

If treatments are implemented in areas where vectorial transmission can not be fully excluded, multiple rounds of treatment will be requested by the patients and this will probably increase the chance of serious adverse events due to sensitization.

Research to provide evidence and support to guide patient treatment strategies to reduce safety risks:

To manage drug adverse reactions there is need to conduct research to develop appropriate pharmacovigilance tools to:

- establish the true risk / benefit of the currently available drugs
- design strategies to detect SAEs and provide early treatment
- strengthen Health Systems

The experience on developing tools for pharmacovigilance for the use of miltefosine for vicerel leishmaniasis could be a model for Chagas disease control programs

Regarding drug resistance, risks account for:

- Uncertainty on dosages and regimens.
- Treatment compliance can not always be assured.
- Nifurtimox is used more and more in areas where previously poor response to this drug was reported or in new areas.
- Benznidazole and nifurtimox can induce cross resistance.

Research to provide evidence and support to guide patient treatment strategies to reduce the risk of drug resistance:

- There is urgent need to develop molecular tools to monitor T. cruzi genetic and functional changes, especially in areas where treatment is expanded.
- There is urgent need to examine the pharmacokinetic and pharmacodynamic properties of nifurtimox and benznidazole to establish if the dosages and regimens currently used are appropriate to fully eliminate the parasites especially in geographical areas where these drugs have not been used previously.
Regarding sustainability, risks account for:
Country & donor commitment to sustain gains will depend on demonstration of impact of treatment and demonstration that it can be sustained at the level of national health systems programs especially when other health problems compete for resources.

Today cures can not be readily and rapidly certified. Therefore, it is difficult to measure the benefit and impact of treatment strategies (either at patient or community level).

Research to provide evidence and support to guide patient treatment strategies to reduce the risk of lack of sustainability:
- There is urgent need to develop highly sensitive parasite detection tools that can establish parasitological cures
  - Validation and standardization of PCR technologies for clinical use
  - Promote novel antigen detection methods
  - Research on biomarkers
- There is urgent need to promote health systems research to integrate Chagas disease control with other health interventions.

Regarding patients treatment exclusions, risks account for:
- Infected individuals in the indeterminate phase or with early clinical manifestations are excluded from treatment strategies w/o evidence to do so
  - This group of infected individuals is numerically the largest and would benefit the most, it also represent the highest risk for blood transfusion and congenital transmission.
- As long as we do not have the appropriate tools to address children some of them will continue to be excluded
  - Need to study drug pharmacology in children
  - Need to develop formulations
- Probably the most excluded group is the patient with clinical manifestations
  - Difficulty in access to medical attention and drugs to treat clinical manifestations.

Research to provide evidence and support to guide patient treatment strategies to reduce the risk of treatment exclusion:
- Clinical studies to determine treatment efficacy and safety in patients with clinical manifestations.
  - BENEFIT (Benznidazole)
  - BENEFIT bis with nifurtimox?
- Drug efficacy and safety studies (in different geographical areas) in the indeterminate stage of infection.
- Research to determine disease progression determinants (host/parasite) to selectively treat those at risk.
- Pharmacological research to address needs in children.
- Drug discovery and development research strategies that will not only provide new, safe and effective drugs but also drugs AFFORDABLE to ALL.
- If POSACONAZOLE would be validated as a new treatment entity it will be a challenge to guarantee access to all
  - Current treatment cost: 4,000 US Dollars per patient
  - Limited production capacity
5.- Currently we lack adequate tools to define patient cure (and impact of treatment campaigns) within a time scale where disease control programs can evaluate efficacy of interventions or redirect treatment strategies. The development of such tools (e.g.: use of PCR based assays) should be seen as high priority.
6.- If diagnosis and treatment of Chagas disease is to be considered in the frame of an integrated disease management approach (e.g: malaria, TB, Dengue, leishmaniasis, etc) pharmacological studies to guide program managers on the best approach to use of diverse drugs will be required to avoid undesirable drug interactions.
7.- The biggest uncertainty for such programs would be the uncertainty associated with treatment of individuals in the indeterminate phase of the disease. There is need to generate evidence demonstrating that treatment in these individuals will result in prevention of clinical disease or in those with signs or symptoms that anti parasitic treatment will result in clinical benefit. The BENEFIT study is addressing this, however, this study is conducted with benznidazole, there would be need to replicate this with nifurtimox. Another consideration is the frequency and severity of drug adverse reactions are different depending on the way treatment is managed.
8.-The needs for management of infections in special populations (congenital, pregnancy, immunosuppressed patients -AIDS or transplants) raise many questions concerning the adequacy and use of available diagnostic and treatment tools.
9.- Last but not least relevant operational research (standardized case management protocols) will be required to create disease (cardiac/digestive) treatment strategies that would ensure access to adequate health care to these patients.

Chagas dream will only be possible if research is integral part of disease control
We must move away from the Classical Model with boundaries between Research & Disease Control.
-Research priorities are left to the intuition of researchers or funders.
-Research Funding availability is very limmited or missdirected.
-Disease control managers continue to engage in policies that are no longer sustainable by evidence (e.g.: continuation of use of chloroquine in the presence of resistance.
-Disease control strategies usually are slow to incorporate innovation.

We must move to a model of Seamless interaction:

-Research goes beyond “hypothesis testing” moving into implementation.
-Research priorities and agendas are defined by opportunities in science and by disease control needs.
-Research outputs are measured by their impact.
-Advocacy and Funding allocation for research and control is done on a rational basis with a health as a goal.
-Disease control actively seeks new knowledge and participates in the definition of R&D agendas and work plans.

The need to see science and research as an "art in defense of life"

-WHO is recognizing and taking clear position in relation to research for health in the context of its mandate.

-PAHO addressed this need during the 42nd meeting of the Committee on Health Research (13-15 April 2008, Rio de Janeiro, Brazil) and established the basis for a framework for PAHO research policy to promote health, innovation and product access as key and integral element of its mandate from the countries in the region.

New Chemotherapeutic Approaches for Chagas Disease
Isabela Ribeiro and Rob Don, Drugs for Neglected Diseases Initiative, DNDi, Regional Office, Latin America, Rio de Janeiro, Brazil

Chagas’ disease ranks among the world’s most neglected diseases. In Latin America, 21 countries are endemic with an estimated 95 million people at risk of contracting the disease. Recent estimates (PAHO 2006) indicate 7.54 million infected people and 55,185 new cases per year. Chagas’ disease is becoming an important health issue also in the United States and Europe due to immigration from disease-endemic countries. Congenital transmission has also gained increasing importance – congenital T. cruzi infection will be a significant public health issue for many years to come.

Nifurtimox (Nfx) and Benznidazole (Bz) are the only two registered compounds for T. cruzi infection. Both are far from being ideal: treatment regimens are long, poorly tolerated - particularly in adults- and have limited anti-parasitic activity in adult chronic patients.

With the limitations of the current therapeutic arsenal, research strategies should incorporate short, medium and long term goals. In the short term, the main prospects for impact in the chemotherapy of Chagas’ disease include the reformulation of existing compounds, therapeutic switching and evaluation of combination therapies.

Issues with current available treatments
Recommendations for use
- Lack of comparative data on efficacy and safety
- Therapeutic regimens empirically derived, with very few randomised clinical trials and pharmacokinetic data
- Regimens in children largely extrapolated from adult data

Lack of easy-to-use, affordable, adapted paediatric formulations.
Long treatment courses.
Low efficacy rates, particularly in adult chronic patients.
Concerns about safety, particularly in adult patients.

Strategy to deal with limitations of current therapeutic arsenal and available data
Short term
- Reformulation of existing compounds
  - Paediatric benznidazole
- Therapeutic switching: evaluation of new azoles
Medium term
- Combination therapy with currently registered Chagas products
- Therapeutic switching: evaluation of back-up azoles and other C14 demethyase inhibitors, squalene synthetase inhibitors,…

Long term
- Lead optimization for new chemical entities

Reformulation of existing compounds would address the fact that the two currently available treatments, Bz and Nfx are formulated as adult strength tablets. Despite their proven efficacy and better tolerability in children, there are no adequate formulations for pediatric use. Tablet fractionation is needed for most of the treated children, with the related potential for improper dosages, safety concerns particularly in the very young and malnourished and possible decrease of efficacy with cover modification, the addition of diluents and stability concerns. An affordable, age-adapted, easy to comply with, formulation is clearly required.

Evaluation of Combination Treatment
Different types of combination treatments depending on the main objectives of the treatment.
Objectives:
- Improvement of efficacy.
- Delay of development of resistance to the individual components of the combination
  - With low levels of resistance, low prevalence and deficiencies in laboratory testing: impact of resistance to antiparasitic agents is insidious.
  - Unless clinical drug trials are conducted, resistance and its impact often go unrecognized
- Improvement of safety profile.
- Reduction of dose and duration of treatment regimens.
  - Side effects of Bz and Nfx are both dose and time-dependent

Principe: « Simultaneous use of two or more drugs with independent modes of action and thus unrelated biochemical targets in the parasite »

Rationale: The rationale for combining antiparasitic agents with different modes of action:
- Often more effective
- Mutual protection is thought to prevent or delay the emergence of resistance.

- To realize the two advantages, the partner drugs in a combination must be independently effective.
- Possible disadvantages and challenges with combination treatments:
  - Potential for increased risk of adverse effects
  - Increased complexity
  - Increased cost.

Side effects to Bz and Nfx are both time- and exposure-dependent. As such, it may be possible to reduce the toxicities by reducing the dose of the available compounds and combination with a second anti – T. cruzi drug. Priorities for the definition of optimal combination treatments are the review and assessment in animal models of candidate partner drugs among compounds with demonstrated anti-trypanosomal activity in clinical and non-clinical studies and prioritization of hits as potential partners for clinical studies.
Combination of registered compounds (Benznidazole/Nifurtimox) with drugs with demonstrated activity in Chagas’ disease.

Potential Partner compounds:
Steroid biosynthesis inhibitors A
Amiodarone C Steroids C
Lovastatin C Terbinafine C
Allopurinol B Itraconazole C
Miltefosine A- Thiotic Acid C
Bisphosphonates C Cyclosporin C
Pentamidine C Phenothiazines B
Arginine C

Animals studies are being perform to evaluate certain combination candidates with the two main therapeutic compounds.

Animal Studies + Combination candidates :
Benznidazole + Nifurtimox +

Itraconazole
Ravuconazole
Posonoconazole
TAK 187
Miltefosine

Animal studies aim the:
Determination of suboptimal doses of monotherapy in an acute murine model of Chagas.
Combination of suboptimal doses to assess additive or synergistic effects.
For additive or improved combinations, assay in 21-day murine model in more than one trypanosoma strain.
Exploration of different regimens:
  • Reduced doses of both partner compounds
  • Short initial course of Bz, followed by partner compound
  • Pulse treatment with Bz

An additional approach, therapeutic switching, offers substantial advantages in terms of research and development time, costs and risks. A potential target for evaluation is ergosterol biosynthesis, a pathway effectively targeted for anti-fungal therapy and sharing considerable similarity with the trypanosome pathway. A number of the antifungal triazoles, including the new generation compounds (posaconazole and ravuconazole) show considerable promise as anti-trypanosomal agents in monotherapy or combination. Other promising targets in the same pathway would include HMG CoA reductase, squalene synthase and epoxidase inhibition. Research on these and other drug targets should be continuously reviewed with reference to their potential for therapeutic switching.
Proline Racemases: Potential Targets for the Development of a Therapy Against Chagas Disease


We had previously identified a parasite B-cell mitogen, the first eukaryotic proline racemase (TcPRAC), essential to T. cruzi viability, virulence and fate. Saturation of TcPRAC catalytic site with specific inhibitors induces conformational changes of the protein precluding its interaction with B-cell ligands. Immunoprotection against parasite infection is possible by ‘vaccination’ of mice with TcPRAC sub-mitogenic doses or TcPRAC-DNA. A signature for PRACs allowed the identification of functional PRACs in other pathogens. Ongoing experiments revealed that the solubility of the PRAC inhibitor can be improved by medical chemistry. Through structural and molecular dynamic analysis of PRAC with/without its inhibitors new insights were obtained on conformational opportunities for enzyme stabilization and ligand binding; yet, calculations of the protonation state of residues of the binding site were performed and appropriate pharmacophoric/docking models were derived for further virtual screening of compound libraries.

Collaborations: L. Masgrau, Institut Pasteur, Unité de Bio-informatique Structurale; P. Alzari, Unité de Biochimie Structurale and M. Afshar, Ariana Pharma SA, Paris, France; W. Degrave and N. Soeiro, IOC, FIOCRUZ, Rio de Janeiro, Brazil.

The European Diagnostic Manufactures Association

Paul Contestable, Ortho-Clinical Diagnostics, Rochester New York, USA

Addressing the needs of patients, bloodbanks and clinicians with respect to the diagnosis of infections of Trypanosoma cruzi is the challenge, which the IVD industry has to address when developing assays for Chagas’ disease detection. This challenge is compounded by the nature of the T. cruzi, not only because of the way it infects and presents itself within its host but also due to its particular distribution and strain differing characteristics. What these needs imply for the IVD industry, as well as an overview of the different solutions which are available to IVD manufacturers and the way in which these are being implemented will be addressed in this presentation.

Definitive diagnosis requires the detection of the parasite within its human host. Direct detection of the parasite in non-acute Chagas’ disease is insensitive due to often low levels of circulating parasite in chronic disease.

T. cruzi antibody detection methods can be more sensitive than direct parasite detection methods in asymptomatic chronic disease; however specificity is variable method to method.
Antibody Detection Challenge
Antibody response is variable due to \textit{T. cruzi} strain variability and host response variability. An antibody detection method must be able to detect this variability.

### Chagas Positive Samples

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Blood Donor Screening criteria
- High throughput is a must.
- High sensitivity (no tolerance for false negative results).
- High specificity (low rate of false positives is clearly an advantage as less donors are lost and less retesting is needed).

Blood Donor Screening with ELISA tests
ELISA methods are well established and allow for high throughput.
- Various approaches to the ELISA method have been taken by manufacturers.
  - Antigen source
    - Parasitic lysate
    - Recombinant proteins
  - Detection method
    - Direct capture/indirect detection
    - Direct capture and detection
  - Substrate
    - Colorimetric
    - Chemiluminescent
  - Processing
    - Manual / POC
    - Automated

How is the ELISA tests performance
- Today ELISA assays obtain over 99\% sensitivity.
- Specificity is being continually improved with assays having over 99.9\% specificity—important with high volume of screening.
- The performance level is comparable to that of well established blood screening analytes (HIV, HCV, etc.).
But there is more to testing
- The greatest assay in the world is of no use unless it is deployed and used where needed.
- This requires greater situational awareness on Chagas’ disease, and action, throughout areas where the disease is not endemic.

With immigration numbers from Chagas endemic regions increasing in non endemic countries, there is an ever increasing risk of transmission through blood transfusion, organ donation and child birth in these countries that needs to be addressed. Although various approaches have been taken to improve the safety of blood and organ supply in several of these countries, there are currently no requirements for testing in countries outside Latin America.

Availability of Chagas Testing in Non Endemic Countries requires
Policy changes on assay use (e.g. bloodbank practices) combined with supporting policies (e.g. reimbursement of Chagas assays) are needed and will lead to greater deployment of testing and better protection of the blood supply and of patients.

A glance about the EDMA
- European Diagnostics Manufacturers Association
- 35 Company members – 21 National Associations across Europe
- Dedicated to serving the IVD industry gathering the knowledge of over 300 technical regulatory and communications experts.
Press release 24/04/2008

Focus on Neglected Tropical Infectious Diseases: Chagas Disease a Public Health Threat in the Americas & Beyond
May 5 – 7, 2008

Lyon, April 24, 2008— In partnership with the World Health Organization, Fondation Mérieux organizes a symposium in relation to Chagas disease at “Les Pénies” Conference Center in Veyrier du Lac, France. During the three day symposium experts on the subject will unveil the current status of the disease and its potential public health risk beyond its known geographical boundaries.

Since first discovered by Brazilian doctor Carlos Chagas in 1909, Chagas disease gradually emerged as a significant public health problem in the Latin American populations.

Chagas also known as American trypanosomiasis is caused by parasite Trypanosoma cruzi mainly transmitted to humans through the bite and fecal contamination from Triatominae vectors, but also via congenital, via blood transfusions, and other forms. An important percentage of the cases, about 30%, follow a silent chronic presentation that could last for decades finally showing fatal irreversible symptoms principally related to heart and gastrointestinal pathologies.

Due to political, social, and economical (poverty) related reasons, the populations of “Campesinos” began to migrate to the periphery of the cities in Latin America bringing with them Chagas and urbanizing the epidemiological path of the disease.

The disease is endemic in Mexico, and all countries of Central American and South America; today due to migration patterns, and to the fact that Latin America are important blood exporters, cases of Chagas disease have been found in North America and Europe becoming a potential emerging public health problem in these new geographical territories.

Special attention must be given to determine the extent of Chagas impact in out of known endemic zones; as well as, to tackle in all aspects the burden that Chagas disease represents in Latin America.

The event will count with the participation of foremost international experts on the subject, and of international organizations that will present their work and programs through sessions as follows.

- GNChE, Triatominae Vector and Information Systems
- T. cruzi, Immune Response & Diagnosis in Chagas Disease
- Latin America, USA 1 Europe: Blood Banks
- Research and implementation on Congenital and Paediatric Chagas Disease
- Physiopathological, Clinical and Epidemiological Aspects of Chagas Disease
- Research Priorities and Novel Chemotherapeutic R&D Findings
The symposium intends to consolidate knowledge on the disease novel findings, to foster knowledge and feedback sharing, and to review the current status of the disease in parallel with the ongoing program strategies to respond to the entire scope of Chagas disease control.

The symposium, consistent with the foundation’s core mission, contributes to the dissemination of scientific information worldwide and to the epidemiological surveillance of infectious diseases.

**About Fondation Mérieux**

Fondation Mérieux was created in 1967 by Doctor Charles Mérieux and was granted charity status in 1976. Presided by Alain Mérieux, the Foundation’s mission is to fight infectious diseases affecting developing countries. The Foundation works to develop and make available new and affordable approaches based on biotechnologies, in the field of prevention, diagnostics and therapeutics.

To achieve its goal, Fondation Mérieux plays a catalyst role in Research and Development by mobilizing a network of excellence that gathers the foremost international experts working in the scientific world today. The Foundation fosters the dissemination of scientific information and innovation through international seminars and conferences, like the Santiago symposium. The Foundation also provides high-level, practical scientific training for health practitioners in the developing world. Finally, Fondation Mérieux works directly in the field by strengthening and building local health infrastructures to enable long-term sustainable development. It is present in Africa, Asia and in Haiti.
Monday May 5, 2008

17:30 – 19:15
Participants Reception
Welcome Address
Christophe LONGUET - Medical Director, Fondation Mémoire
Keynote Presentation: Chagas Case Study performed in Salta, Argentina
Speaker: Hector FREILIJ

Dinner

Tuesday May 6, 2008

8-10 h
Session I: GNChE, Triatominae Vector and Information Systems
Chairman: Mario Zaidenberg

Speaker: Jean JANNIN
Phylogeny and Evolution of Triatominae
Speaker: Chris SCHOFIELD
Strategies for Vector Control and its Sustainability
Speaker: Ricardo GURTNER
Geographic Analysis as a Support Tool for Triatominae Control Programmes
Speaker: David GORLA

Coffee Break

10.30-12.30 h
Session II: T. cruzi, Immune Response & Diagnosis in Chagas Disease
Chairman: Glauco Paranhos Baccala & Felipe Guhl

T. cruzi Genome Project
Speaker: Nagib EL SAYED
Phylogenetics in T. cruzi
Speaker: Michel TIBAYRENC
Immune Response: Protective or Pathogenic?
Speaker: Manuel FRENSNO
New Serological Diagnostic Tools
Speaker: Alejandro LUQUETTI
Novel Molecular Diagnostic Tools
Speaker: Philippe BUSCHER

Lunch
Focus on Neglected Tropical Infectious Diseases: Chagas Disease a Public Health Threat in the Americas & Beyond
Report issued: September 30, 2008

14 – 16:00 h
Session III: Latin America, USA & Europe: Blood Banks
Chairman: David A. Leiby & Carlos Ponce

- Strategy of Blood Banks in Latin America: The Experience in Sao Paulo
  Speaker: Ester Cerdeira SABINO
- Strategy of Blood Banks in Europe
  Speaker: Azzedine ASSAL
- Strategy of Blood Banks in the USA
  Speaker: Celso BIANCO
- Laboratory Quality Control in Blood Banks
  Speaker: Amadeo SAEZ-ALQUEZAR

Coffee Break

16.30 – 18.30 h
Session IV: Research and Implementation on Congenital and Paediatric Chagas Disease.
Chairman: Faustino Torrico & Graciela Russomando

- Contribution of the IRD in the Fight against Chagas: Research on the Congenital, Paediatric & other fields
  Speaker: Laurent BRUTUS
- Chagas Paediatric & Congenital
  Speaker: Jaime ALTCEH
- Experience of the International Plan in Bolivia.
  Speaker: Carlos SALINAS
- Experience of the MSF Chagas Programs: Central & South America
  Speaker: Nines LIMA

Dinner

Wednesday May 7, 2008

8 – 10 h:
Session V: Physiopathological, Clinical and Epidemiological Aspects of Chagas Disease
Chairman: Robert Don & Yves Jackson

- Chagas Heart Disease: Physiopathological Mechanisms, Prognostic Factors & Risk Stratification
  Speaker: Anis RASSI Jr
- Epidemiology of Chagas in Latin America: Future Risks
  Speaker: João Carlos PINTO DIAS
- Epidemiology of Chagas in Europe
  Speaker: Joaquim GASCON
- Chagas Disease in the USA: Epidemiology & Challenges
  Speaker: James MAGUIRE
Coffee Break

10.30 – 12.30 h

Session VI: Research Priorities and Novel Chemotherapeutic R&D Findings

Chairman: Julio Urbina

   Speaker: Janis K. LAZDINS-HELDS

New Chemotherapeutic Approaches for Chagas Disease - DNDi
   Speaker: Isabela RIBEIRO

Proline Racemases: Potential Targets for the Development of a Therapy against *T. cruzi* Infection and Disease
   Speaker: Paola MINOPRIO

Chagas Disease: The European Diagnostic Manufacturers Association - EDMA experience
   Speaker: Paul CONTESTABLE

Lunch

14:00 – 16 h

Workshops

Main Moderator: Pedro Albajar Vinas

Workshop - Creation of 5 groups as per Chagas Disease main Subjects:

Group 1: Epidemiological Surveillance & Information Systems
   Moderators: David Gorla & Mario Zaidenberg

Group 2: Prevention of Transfusional & Organ Transplantation Transmission
   Moderators: Amadeo Sáez-Alquezar & Azzedine Assal

Group 3: Diagnostic Test(s) for Screening and Diagnosis of *T. cruzi* infection
   Moderators: David A. Leiby, Carlos Ponce & Felipe Guhl

Group 4: Prevention & Control of Congenital Transmission and Case Management of Congenital and Non-congenital infections
   Moderators: Hector Freilij, Joao Carlos Pinto Dias & Yves Jackson

Group 5: Research and Novel Chemotherapeutic Findings
   Moderators: Julio Urbina, Bernard Pecoul & Shing Chang

15:10 – 16h Groups Reconstitution: Feedback & Conclusions
   (Time allocated 10 min per Group)

16 h Closing Remarks
   Speaker: Hector FREILIJ

End of Symposium