PAHO/HDM/CD/512-2008

Update

of

American Trypanosomiasis

and

Leishmaniasis Control and Research:

Final Report

(Rio de Janeiro, Brazil, 6–7 November 2007)

Organizers: Pan American Health Organization / World Health Organization (PAHO/WHO)
Sociedade de Pediatria do Estado de Rio de Janeiro (SOPERJ)

Table of Contents

Report of the Scientific Meeting

"Update of American Trypanosomiasis and Leishmaniasis Control and Research"

Introduction

The meeting was held in Rio de Janeiro, Brazil on 6 and 7 November 2007, organized by the Communicable Disease Reseach Program, Comunicable Disease Unit, Health Surveillance & Disease Management Area of the Pan American Health Organization / World Health Organization (PAHO/WHO), the The UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, and the Pediatrics Society of Rio de Janeiro State, Brazil.

The objective of the meeting was to set up an information platform about control and research gaps of American Trypanosomiasis and Leishmaniasis in order to establish a road map for the academia and control programs.

Lecturers

1) Antonio Carlos Silveira, Nucleo de Medicina Tropical de la Universidad de Brasilia (colaborator). Panamerican Health Organization / World aHealth Organization (PAHO/WHO) (eventual consultant) atcrs@uol.com.br

2) Marcelo Aguilar, Representante para el Ecuador del Organismo Andino de Salud-Convenio Hipólito Unanue Coordinador Nacional Proyecto Control de la Malaria en las Zonas Fronterizas de la Región Andina. Un Enfoque Comunitario-PAMAFRO. maguilar@conhu.org.pe ; maguilarv3@hotmail.com

3) François Noireau, UR 016, Institut de Recherche pour le Développement (IRD), Montpellier, France, and Centro Universitario de Medicina Tropical, Facultad de Medicina, Universidad Mayor de San Simón, Cochabamba, Bolivia. Francois.Noireau@ird.fr

4) Carlota Monroy, Laboratory of Applied Entomology and Parasitology –LENAP- Faculty of Pharmacy, San Carlos University, Guatemala. C.A. cmonroy@usac.edu.gt

5) Nicolás Jaramillo, Instituto de Biología, Universidad de Antioquia, Medellín, Colombi.a nicolas.jaramillo@siu.udea.edu.co

6) Elsa Segura, CONICET (National Council of Research and Technology) of Argentina, at the Instituto Nacional de Parasitología "Dr. Mario Fatala Chabén," ANLIS, Ministerio de Salud, Argentina. elsasegura@fibertel.com.ar

7) Antonieta Rojas de Arias, Vector Control National Consultant, Pan-American Health Organization / World Health Organization (PAHO/WHO), Asunción, Paraguay. arias1@telesurf.com.py

8) Elci Villegas, Universidad de los Andes, Núcleo Universitario "Rafael Rangel", Instituto Experimental "José Witremundo Torrealba", Trujillo, Venezuela. elciv@ula.ve

9) João Carlos Pinto Dias, Oswaldo Cruz Foundation, René Rachou Research Center, Belo Horizonte, Brazil. jcpdias@cpqrr.fiocruz.br

10) José Rodrigues Coura, Laboratório de Doenças Parasitárias, Instituto Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brasil. coura@ioc.fiocruz.br

11) Carlos Morillo, Arrhythmia Service, Cardiology Division, McMaster University - Population Health Research Institute, Hamilton, Ontario, Canada. morillo@hhsc.ca, morillc@univmail.cis.mcmaster.ca

12) Roberto Badaró, Federal University of Bahia, Brazil, University of California San Diego, USA. badaro@ufba.br

13) Oscar Salomón, CeNDIE-ANLIS, Ministry of Health / CONICET, Argentina. danielsalomon@hotmail.com

14) Rolando Oddone, Instituto de Investigaciones en Ciencias de la Salud, Universidad Nacional de Asunción, Asunción, Paraguay. roloddone@yahoo.com

15) Iván Darío Vélez, Programa de Estudio y Control de Enfermedades Tropicales (PECET), Universidad de Antioquia. Medellin-Colombia. id_velez@yahoo.com, idvelez@udea.edu.co

16) Eric Rosenthal, Department of Internal Medicine, Archet Hospital, University of Nice Sophia Antipolis, France. rosenthal.e@chu-nice.fr

17) Roberto Docampo, Center for Tropical and Emerging Global Disease and Department of Cellular Biology, 350A Paul D. Coverdell Center, University of Georgia, Athens, GA, USA. rdocampo@uga.edu

18) Joana Martins de Sena, Secretaria de Vigilância em Saúde, Ministério da Saúde, Brasília-DF, Brazil. joana.sena@saude.gov.br

19) Dr. Myriam Lorca, M.T. MSc., PhD, Post Ph.D, Associated Professor Parasitology, PAHO/WHO Expert in Chagas disease, Faculty of Medicine, University of Chile, Santiago, Chile. clorca@med.uchile.cl (Dr. Lorca could not attend the meeting due to

health problems but her manuscript was included because the content was considered important.)

Narrators

22. Lileia Diotaiuti (American Trypanosomiasis), Oswaldo Cruz Foundation, Centro de Pesquisas Renée Rachou, Belo Horizonte, Brazil. diotaiuti@cpqrr.fiocruz.br

23. Oscar Salomón (Leishmaniasis). danielsalomon@hotmail.com

Participants

24) Jose Jurberg, Instituto Oswaldo Cruz (FIOCRUZ), Río de Janeiro, Brazil. jjurberg@ioc.fiocruz.br

25) Angela Junqueira, Instituto Oswaldo Cruz (FIOCRUZ), Río de Janeiro, Brazil. junqueir.rlk@terra.com.br

26) Teresa Cristina Monte Goncalves, Instituto Oswaldo Cruz (FIOCRUZ), Río de Janeiro, Brazil. tcmonte@ioc.fiocruz.br

27) Cléber Galvao, Instituto Oswaldo Cruz (FIOCRUZ), Río de Janeiro, Brazil. clebergalvao@hotmail.com

28) Gabriel Grimaldi, Instituto Oswaldo Cruz (FIOCRUZ), Río de Janeiro, Brazil. grimaldi@ioc.fiocruz.b

29) Marisel Maldonado, Instituto de Investigaciones en Ciencias de la Salud, Universidad Nacional de Asunción, Asunción, Paraguay. mariselm@gmail.com

30) Isabel Sánchez, Panamerican Health Organization (PAHO), Asunción, Paraguay.

31) Ana Laura Carvajal, Instituto Oswaldo Cruz (FIOCRUZ), Río de Janeiro, Brazil.

32) Catarina Macedo Lopes, Instituto Oswaldo Cruz (FIOCRUZ), Río de Janeiro, Brazil.

American Trypanosomiasis

Control of Vector Transmission Of Chagas Disease, Its Limits and the Research of Situations or Areas with Persistent Infestation by Introduced Species

Antônio Carlos Silveira
Nucleo de Medicina Tropical de la Universidad de Brasilia (colaborator)
Panamerican Health Organization / World Health Organization (PAHO/WHO) (eventual consultant)
atcrs@uol.com.br

There are important epidemiological and technological restrictions for the control of Chagas disease transmission starting with the fact that the disease is primitively an enzootia which avoids its eradication.

Other limiting factors, related to its natural history are:

i) multiple transmission mechanisms;
ii) a large number of wild and domestic reservoirs, that makes the exhaustion of infection sources almost Impossible;
iii) scarce or none clinical appearance of most acute cases, a condition that hinders the epidemiological surveillance;
iv) great diversity of vectors with different behavior, requiring distinct approaches or methodologies for their control; and,
v) primarily economic and social determinants expressed more immediately in precarious life conditions.

On the other hand, the available control tools are limited taking into account that there are not:

i) a vaccine that allows the immunization of the population under risk;
ii) drugs that could be used at large scale and side-effects free.

Thus, the only control possibility of the primary transmission mechanism (vectorial) is the reduction or suppression of the contact opportunities between man and the infected vector. From that point on, it could be considered that the disease vulnerability to control is low or minimum.

Attributes proper of the disease triatomine vectors make that, on the contrary, there was a high vulnerability of the domiciliary Chagas transmission to control in spite of all the limitations above indicated. They are vectors characterized by their small mobility, slow population restocking and the fact that all stages are always present in the same ecotope (14, 17).

It is true that neither all the diverse numerous vector nor the potentially vector species respond equally to control actions. An initial and fundamental distinction refers to the different degree of domicile adaptation of the diverse species. This determines that the possible control goals are different. *Introduced* species—i. e. completely domiciled in a determined area—are eliminable at the beginning while for *native* species— present in their wild foci, apart from invading and colonizing the domicile— it can only be attempted to stop them from colonizing intradomiciles, suppressing or exhausting those already established inside dwellings (17).

The control of vector transmission can be made by physical, mechanical and chemical means by the systematized use of residual action insecticides for some continuous time in the infested houses.

The *physical control* might be understood as a promotion measure or as a specific protection measure depending on its scope and the purposes of the implemented actions.

The housing improvement is frequently made by the substitution or almost complete rebuilding of the house often without including the peridomicile. Specificities of the disease transmission or peculiar risk circumstances represented by vector habits and behavior are not considered; i. e. it is not a rigorous *specific protection* measure though it can contribute to control in variable degrees. In that case, it has more a *promotional* character, not of health conditions, but of the own life conditions of the benefited populations. Its scope goes beyond the control of a particular disease and even the strictly sanitary issue itself. Evidently, it is always desirable but it can not be completely efficient for Chagas disease control.

The physical control has to be understood as a specific control measure when it is adequate to determined local particularities or junctures involved in the risk of domiciliary infestation. “Localized improvements” in the house can be mentioned such as the substitution of roofs for the control of specimens of *Rhodnius* genre or the placement of floors in areas where species using mimicry mechanisms with the floor (*Triatoma dimidiata*) prevail or even the substitution of wood or cane fences in barnyards (*Triatoma brasiliensis* and others). Other example is the simple management of peridomicile with clearing and spatial reordering of annexes placing them further from the house.

It is important to distinguish the approach type to be provided by physical control when the transmission pattern is the usual one—that presupposes domiciliary colonization as a condition for a reiterative contact between man and the vector (which is, in turn, condition for the existence of a permanent transmission risk)—from that occurring without colonization through recurrent or episodic visitis of adult vector specimens to the house. In this last situation, the physical control might be the only possible intervention, creating mechanical barriers to the entry of triatomines or by the management of the extradomiciliary environment in the most immediate house environment.

Though room improvement can be considered the preferential strategy because it is a lasting or "definitive" change in the determinants of domiciliary transmission of chagasic endemics, the universalization as a measurement especially aimed at the control of Chagas disease finds restrictions that hinder a large-scale application. The first and most important obstacle is economic considering the extension of the risk area and consequently the volume of resources required. Likewise, there is the issue of the land property that frequently is not in the hands of the directly benefited population.

In spite of these limiting factors there are very precise indications for physical control apart from what has already been said about "visiting vectors". In areas where autochthonous vectors prevail, present in high density wild foci and with great invasive capacity, the housing improvement, including the peridomicile management, is always recommendable if necessary resources are available. Though chemical control is considered to be enough to eliminate strictly domiciliary vectors, persistent reinfestation foci, predominantly domiciliary, are observed repeatedly demanding specific physical control wide measures covering different intervention types.

It is interesting to notice that sometimes it is proposed to qualify physical control measures (use of agents such as temperature, sun exposure, photo-period or diverse radiations) of lethal intensity for vectors as *mechanical control*. However, these techniques are rarely applied in public health as the manipulation has to be made in closed environments (15).

Concerning *chemical control*, proven efficient tools for triatomine control in domiciliary environment are available since half of the last century. The advent of a new chlorinated insecticide, hexaclhorocyclohexane (HCH or Gamexanne), highly lethal for triatomines in laboratory assays at first (2) then confirmed to be toxic enough to achieve an important reduction of vector density in treated houses in successive field assays made in Brazil, Argentina, Venezuela, Uruguay y Chile (4,11,19). Other insecticides of the same chlorinated group like Dieldrin and from other chemical groups like carbamates and phosphates were later assayed and sometimes used.

At first, the most important factors for the product elections were residual action, an indispensable property, easy manipulation and application and evidently high toxicity for triatomines and the lowest possible for man and domestic animals.

The toxicity issue of this products generation, especially chlorinated ones, was determinant for the reluctance to use them for longer periods at the beginning. On the other hand, the results of the first "campaigns" of vector chemical control with limited scope and discontinuous, contributed to certain discredit about the efficacy of this type of interventions. The results always seemed to be insufficient or transitory.

The development of synthesis pyrethroids and their introduction in control programs since 1980 (8,13) was decisive for the improvement of control actions. Their lower toxicity for man and potent toxicity allied to insect repelling properties represented an important quality gain.

The efficacy of responses and stability of the control level achieved mainly depend on two basic principles of the chemical control of Chagas disease vectors: continuity in time and spatial contiguity. The domiciliary chemical treatment has to be continuous, regular and for the necessary time depending on the finding of vectors through permanent actions of entomological surveillance. On the other hand, the intervened areas have to be contiguous and progressively increasing.

If these requirements have been fulfilled, the interruption of the domiciliary vector transmission has to be expected excepting peculiar situations and exceptional circumstances that make infestation persistent.

The periodicity and extension of interventions are determined by the expected final objectives, the vector species present and the different degree of infestation risk in the area. What is accepted as a guideline (9,10,20) is the performance of at least two integral spraying cycles, selected by infested locality, previously identified by entomological search and taken as baseline for future impact evaluations. In the subsequent cycles, several alternatives are being adopted:

iii) the selective treatment per infested domiciliary unit (DU), known by searches made after two initial treatment cycles that also act as a first verification of results;

iv) the infested DUs and the neighboring ones;

v) the DUs positive for vectors and those included in an area with a determined extension in meters (which is being defined pretty variably and arbitrarily); or even,

vi) the infested dwellings and those more vulnerable to infestation/reinfestation in the locality.

The selectivity, that confers rationality and could provide a significant cost reduction of spraying operations, is partially limited by the low sensitivity of the available techniques and methods for vector detection, particularly when the density is low or little expressed as it happens in already intervened areas with successive interventions cycles. There is not enough reliability in the findings that allow the restriction of treatment of the proven infested unit in any situation. This is undeniable for those cases where the elimination of domiciliated vectors is expected.

In these circumstances, the spraying scope has to be established based in variables such as infestation rates (verified in the post-spraying evaluation), physical characteristics of dwellings and their spatial distribution. It could be interesting to incorporate other indicators or aspects considered relevant for the specific situation in discussion.

Though the technical accumulation for vector control is considered enough, the previously mentioned limits have to be taken into account. Likewise, it is important to mention and make a conceptual distinction between these *limits* and situations with *unsatisfactory responses to control* and *control failures*.

The *limits* are determined by the facts that enzootic vector transmission will continue occurring and that autochthonous vectors in an area could invade or colonize a room establishing or re-establishing the domiciliary transmission cycle. Though the domiciliation of these vectors does not happen, the accidental transmission to man may rarely occur. Besides, control methods are not completely elaborated or enough tested and validated in some particular cases. This happens with the control of transmission caused by the repeated visiting of wild vectors to dwellings (seen with *Rhodnius pallescens* in Panama) which is being proposed by the installation of mechanical barriers and house protective sprayings.

The *unsatisfactory responses* to control affect situations where the results do not correspond to the expected in function of evidence resulting from previous experiences where the efficacy or sufficiency of the techniques used was proved. There are many variables to be considered among the possible causes of these events:

vii) fast insecticide degradation due to excessive alkalinity of the solution or substrate, excessive sun exposure or rain;

viii) environmental conditions exceptionally favorable to vector persistence that could be caused by the high complexity of the domiciliary and peridomiciliary environments or precariousness of the room physical conditions;

ix) resistance of local vector populations to the insecticide used.

The *control failures* will be specific cases of unexpected responses involving operational mistakes such as an insufficient concentration of the insecticide active ingredient, inadequate dilution or preparation of the solution and irregularities, lapses or omissions in applications.

These diverse unfavorable conditions for control are obviously not excluding. An exemplary case is the "Gran Chaco" region where several contingencies are added. There, in a large territory covering important parts of Argentina, Bolivia and Paraguay, the initial expectations of eliminating *Triatoma infestans* (as part of the "Southern Cone Initiative") were not fulfilled (5).

Since the beginning of the INCOSUR/Chagas Initiative (1991), *T. infestans* wild foci have been identified in several areas of the Bolivian Chaco (3, 6, 7) and also in Argentina more recently; i.e. *limits* for the projected control of the species elimination have been known (16,18). Also, resistance of vector populations has been shown in Tarija, Cochabamba (Bolivia) and Salta, La Rioja (Argentina) (1,12) that added to the anarchic organization and profusion of annexes in the peridomicile—often without a clear distinction of the intradomicile—determined control *unsatisfactory response.* Considering the weak supervision of field activities, discontinuity of spraying actions and spraying performance by the community and collaborators it can be suspected that *control failures* have occurred.

Alternatives techniques and methodologies have to be generated and assayed in areas with persistent reinfestation by introduced species like in the Chaco and other eventual areas. The identification of causes or agents of this type of events has to be systematically and thoroughly made as a condition to create new control means or forms

The following algorithm could orient the research of control failures and identification of environmental conditions possibly involved in cases where an unsatisfactory response to chemical control of Chagas disease vectors occurred (Figure 1). To simplify and facilitate its use, the adoption of "check lists" is proposed to be used in field application (Figures 2 and 3).

A peculiar methodologic or operational "*failure"* is the lack of continuity of the actions (or its limited scope) almost always determined by insufficient resources where causes have different nature. In this case, they depend on political decisions and health policies, i.e. they depend on the priority confered to the disease surveillance and control in one hand and in the other hand on the structural and functional viability for the full development of actions.

The conditions determining that a health public problem is *recognized and treated* as a priority are mainly its magnitude, trascendence and vulnerability to control, i.e. the power of control instrument existent (19).

Besides, social demand is absolutely determinant in the definition of priorities. In the case of control of transmissible diseases, the existence of demand is influenced by the populational groups affected or low risk, clinical visibility, damage perception by the population and evolution and transmission form or celerity. Thus, diseases compromising politically and socially influent groups, clinically more evident, occurring in acute form and transmited epidemically in urban zones usually receive priority treatment. Chagas disease, affecting predominantly rural and marginal populations, presenting long chronic course and slightly evident in the acute phase, is not among those diseases in spite of its magnitude, trascendence and control vulnerability. From all this, it results an insufficient or irregular provision of resources and consequently the *discontinuity of actions in time*.

On the other hand, the operative decentralization of control programs which benefetis are undoubtefull in their conception — *extension of the operative basis*; greater *opportunity and property of actions* approximating the technical decision of events; and, *"permanence" of activities*, because of the possibility of carryinng them put from local health services—is evidencing that the transfer of political decision to municipal or regional governmental levels frequently implies a *spatial discontinuity of actions* (20).

The great difficulty to overcome is the lack of recognition of local authorities—subjected to requests perceived by the population as urgent or more urgent— of the priority represented by the control of diseases with magnitude exceeding municipal or regional level. From this contingency, it results the lack of cohesion of the actions performed in disrupted form and without the necessary constancy.

Evidently it is not being proposed a return to the old order, where the programs of the so called "great endemics" (Chagas disease among them) were historically marked for a vertical structure and developed as long term "sanitary campaigns" characterized by:

i) *transitoriness*, according to clearly established goals to be accomplished in a determined time;

ii) *high specificity*, with isolated, independent and exclusive actions;

iii) *guidelines rigidity* and consequently a technical/technological undifferentiated treatment; and

iv) *mobilization of a large volume of resources* in short time.

However, it is indispensable to search for strategic, methodological and legal alternatives that guarantee the advances achieved in the control and implementation of actions in areas where there is still an active transmission of Chagas disease.

Figure 1: Research of “Failures” or Unsatisfactory Responses to Control

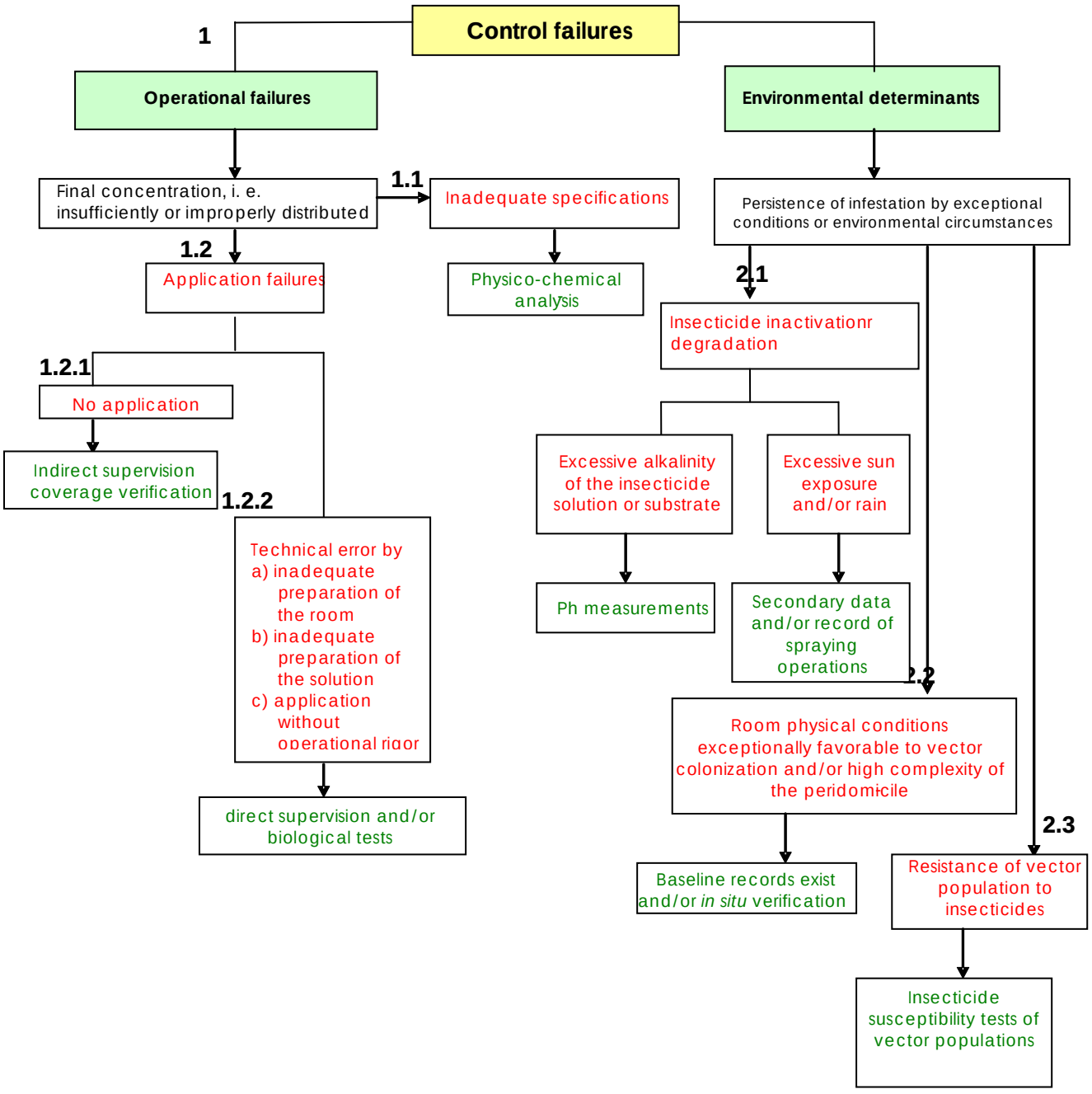


Figure 2: "Check list" for the Research of Unsatisfactory Responses to Chemical Control of Chagas Disease Vectors

1. Operational Failures

Municipality _____

Locality _____

Possible Failure	**Research**		**Result**		
			Yes	*No*	*Inconclusive (or unknown*)*
Inadequate insecticide specifications	Was (or is) the insecticide within the required technical specification?	Physico-chemical tests			
	Was (or is) the insecticide within the registered validity period?				
Improper application	Did (or does) the application follow the technical guidelines for the use of residual action insecticides?	Biological tests Direct supervision**			
No application	Is the administrative datum real? (was the application performance really verified?)	Indirect supervision: coverage verification			

* Tests or activities not made

** According to model of activities follow-up

Figure 3: "Check list" for Research of Unsatisfactory Responses to Chemical Control of Chagas Disease Vectors

2. Environmental determinants (persistence of infestation by exceptional environmental or eco-epidemiological conditions)

Municipality _____

Locality _____

Determinants		**Research**	**Result**		
			Yes	*No*	*Inconclusive (or unknown*)*
Insecticide inactivation or degradation	Excessive alkalinity of the insecticide solution or substrate	pH tests			
	Excessive sun exposure or rain	Secondary data (meteorological)			
Houses and/or peridomicile physical structures exceptionally favorable to the permanency of infestation or reinfestation		In situ observation			
Existence of vector wild foci with permanent reinfestation risk		Search of wild foci in the area			
Resistance of vector populations to insecticides		Susceptibility/resistance tests			

* Tests or activities not made

References

1. Audino PG, Vassena C, Barrios S, Zerba E, Picollo MI. 2004. Role of Enhanced Detoxication in a Deltamethrin-resistant Population of *Triatoma infestans* (Hemiptera, Reduviidae) from Argentina. *Mem Inst Oswaldo Cruzc99*(3): 335-339.

2. Busvine JR, Barnes S. 1947. Observations on mortality among insects exposed to dry insecticidal films. *Bulletin of Entomological Research 38*: 80-81.

3. Cortez MR, Emperaire L, Piccinali RV, Gürtler RE, Torrico F, Jansen AM, Noireau F. 2007. Sylvatic *Triatoma infestans* (Reduviidae, Triatominae) in the Andean valleys of Bolivia. *Acta Trop. 102(1):*47-54.

4. Dias E, Pellegrino J. 1948. Alguns ensaios com o "Gamexanne" no combate aos transmissores da doença de Chagas. *Brasil Médico 62:* 185-190.

5. Gürtler RE. 2007. Eco-epidemiología regional de la transmisión vectorial: enfermedad de Chagas en el Gran Chaco. *In*: La enfermedad de Chagas a la puerta de los 100 años del conocimiento de una endemia americana ancestral. Buenos Aires: OPS (OPS/CD/426-06), Mundo Sano, P. 137-55.

6. Noireau F, Flores R, Gutierrez T, Dujardin JP. 1999. Detection of wild dark morphs of *Triatoma infestans* in the Bolivian Chaco. *Mem. Inst. Oswaldo Cruz 92:* 583-84.

7. Noireau F, Cortez MR, Monteiro FA, Jansen AM, Torrico F. 2005. Can wild *Triatoma infestans* foci in Bolivian jeopardize Chagas disease control efforts? *Trends Parasitology 21*: 7-10.

8. Oliveira Filho AM, Melo MTV, Santos CE, Lustosa ELB. 1997. The flushing-out activity of pyrethroid formulations on triatomines. *In:* V Congresso Latinoamericano de Medicina Tropical. La Habana, Cuba, 1997. Anais, p. 87.

9. Organización Panamericana de la Salud. 2003. Informe de la Reunión Internacional para el establecimiento de criterios de certificación de la eliminación de Rhodnius prolixus. Ed. OPS/DPC/CD/245/03, Guatemala.

10. Organización Panamericana de la Salud. 2003. Consulta OPS en Criterios, Indicadores y Parámetros de Eliminación de *Triatoma infestans.* Santiago de Chile, 23 y 24 de octubre de 2003. Mimeo. 7 p.

11. Osimani J, Verissimo S, Bayce Carbonell P. 1950. La profilaxis de la enfermedad de Chagas en el Uruguay por medio del gamexano. Experiencias realizadas y plan de lucha contra el *T.infestans*. *Bol.Of.Sanit.Panamer,29(11):*1125-1134.

12. Picollo MI, Vassena C, Santo Orihuela P, Barrios S, Zaidenberg M, Zerba E. 2005. High resistance to pyrethroid insecticides associated with ineffective field treatments in *Triatoma infestans* (Hemiptera: Reduviidae) from Northern Argentina. *J Med Entomol. 42(4)*:637-42.

13. Pinchin R, Oliveira Filho AM, Pereira. ACB. 1980. The flushing-out activity of pyrethrum and synthetic pirethoids on *Panstrongylus megistus*, a vector of Chagas' disease. *Trans. R. Soc. Trop. Med. Hyg. 74* (6): 801-803.

14. Schofield CJ. 1979. The behaviour Triatominae (Hemiptera, Reduviidae): a review. *Bull. Ent. Res. 69:* 363-379.

15. Silva PC, Braga IA, Calderón G. 1997. Métodos de Controle de Insetos (Pragas agrícolas/vetores de doenças), 24 p

16. Silveira AC. 1993. Indicadores operacionais para um programa de eliminação do *Triatoma infestans*. *In: Resumos – Taller: Definición de Indicadores para la Certificación de la Eliminación del T. infestans*. *Rev. Soc. Bras. Med. Trop. 26 (supl. III):* 51-54.

17. Silveira AC. 1999. Profilaxia. *In: Trypanosoma cruzi e Doença de Chagas.* 2.ed. Rio de Janeiro, Guanabara-KOOGAN, p. 75-87.

18. Silveira AC. 2002. El control de la enfermedad de Chagas en los países del Cono Sur de América. Historia de una iniciativa internacional 1991/2001. Uberaba: Sociedade Brasileira de Medicina Tropical, 316 p.

19. Romaña C, Abalos JW. 1948. Acción del "Gamexanne" sobre los triatomídeos. Control domiciliario. *Anales del Instituto de Medicina Regional. Tucuman 2:* 95-106.

20. World Health Organization. Control of Chagas Disease. (WHO Technical Report Series 905). 2002.

Critical Aspects of *T. cruzi* Infection and Chagas Disease in the Ecuadorian *Amazonía*: Considerations for Other Amazon Areas with Probable Endemic Transmission

H. Marcelo Aguilar V.

Representative in Ecuador of the Organismo Andino de Salud- Convenio Hipólito Unanue Nacional Coordinator of the Malaria Control Project in the Frontier Zones of the Andean Region, A Community Approach-PAMAFRO

maguilar@conhu.org.pe

Introduction

Before 1990, there were no reports indicating the existence of *Trypanosoma cruzi* human infection cases in the Ecuadorian *Amazonía* in spite of the existent general health services and the Malaria Control Service (SNEM in Spanish) that systematically examined blood samples for malaria diagnosis and could have accidentally discovered *T. cruzi* infections (Abad-Franch & Aguilar 2003).

In the Ecuadorian *Amazonía*, the first acute cases of Chagas disease were reported in 1991 and since then other cases habitually occur. The presence of vector insects in sylvatic zones and environments closed to the dwellings is also reported (Amunárriz, 1991). *Rhodnius pictipes*, *Rhodnius robustus* and *Panstrongylus geniculatus* have been incriminated as vectors of *T. cruzi* transmission (Abad-Franch F et al., 2001). In 1997, Chico et al. showed *T. cruzi* transmission in indigenous populations, natives of the Ecuadorian *Amazonía*; in the provinces of Napo, Orellana and Sucumbíos where 1,011 people from 18 communities were examined, 85% were Quechua natives and the remaining were people of mixed race living in the localities studied and 6.08% positive cases were reported using ELISA with a recombinant antigen (Chico et al., 1997).

Grijalva et al. (2003) carried out a wide study in a significant sample of the *Amazonía* in 2003 and reported a global seropositivity of 2.91%. The study included the provinces of Sucumbíos, Orellana, Napo and Pastaza implying that about 14,105 people would be infected by *T. cruzi* in these provinces.

Currently, the epidemiological surveillance system, implemented by the staff of SNEM trained for the identification of *T. cruzi,* systematically reports Chagas acute cases in the Province of Sucumbíos and Orellana.

Seroepidemiology in the *Amazonía*

The seroepidemiological study performed by Grijalva et al. (2003) in the Amazonian provinces of Napo, Sucumbíos, Pastaza and Orellana reported a global *T. cruzi* prevalence of 2.91% in 6.839 samples from 158 communities. About 31% of the communities were seropositive and the prevalence ranged from 23.8% to 0.34%.

T. cruzi infected people were found in all age groups. The prevalence increased progressively with age until reaching the maximum in the 60-70 years old group and then decreased; 53.3% of the seropositive cases were under 15 years old implying an additional impact of the disease as most of the Amazonian population is young.

The increase of seropositivity prevalence with age revealed a profile of endemic and continuous transmission similar to the one occurring with domiciled vectors and without control intervention. The reduction of prevalence in higher ages is explained by the specific mortality of the disease. This epidemiological behavior characterizes a continuous transmission and shows an effect of accumulative risk proportional to age, showing the endemic character of the transmission (Aguilar et al., 2005).

The study "Dynamics of *T. cruzi* transmission in the Ecuadorian *Amazonía*" (TDR/OMS A20500) performed in 2003 showed major differences in the seroepidemidemiology and the association to different Amazonian landscapes and ways of life of the populations.

Risk Factors

In a global study of the *Amazonía*, seropositive people were mostly natives, internal migrant from localities of the same province or other Amazonian provinces. Migrants from other Chagas endemic zones like Manabí or Loja did not have any significance (only 7 seropositive cases).

Houses built with closed walls (cement, bricks, wood or blocks) were protective against the infection in comparison with houses having walls of bamboo cane or other vegetal materials ($p < 0.05$). Closed roof

of cement, tiles and corrugated sheets were also protective in comparison to open or permeable roof to the entrance of flying insects associated to a high risk of *T. cruzi* infection. The insecticide spraying of the house was also protective while stories of triatomine bites were associated to seropositivity. Others factors such as identification of insects by the population, number of inhabitants per house and stories of cardiac problems did not have any statistical association (Grijalva et al., 2003).

Triatominae and Palm Trees

The triatomines reported in the *Amazonía* included nine species. *R. pictipes, R. robustus*, and *P. geniculatus* are incriminated in *T.* cruzi transmission; these insects invade the houses without colonizing them. Others like *Eratyrus mucronatus, Cavernicola pilosa* and *P. lignarius* are strictly sylvatic. Occasionally specimens of *P. herreri, T. carrioni* and *T. venosa* have been collected (Abad Franch et al., 2001).

Francisco Palomeque (2003) studied 101 palm trees using live bait traps (Noireau et al., 2002) plus manual captures in several localities of the provinces of Orellana and Sucumbíos in the north of the Ecuadorian *Amazonía*. He found a global infestation of 48.5% in palm trees registering a 80% (n=15) positivity in *Oenocarpus bataua*, 56.5% (n=46) in *Attalea butyracea*, 53% (n=15) in *Phytelephas tenuicaulis*, 50% (n=2) in *Elaeis guineensis*, 25% (n=8) in *Astrocaryum sp*. Specimens of *Aphandra natalia* (n=5), *Cocos nucifera* (n=4) and *Mauritia flexuosa* (n=6) were not infested.

The landscape differences showed different associations with palm trees infestation. The highest percentage of infested palm trees were in forest strata with high human intervention and forest degradation like agricultural farms, grass fields and crops (+61%, average of captured insects 8.1± 16.9) followed by urbanized areas with higher density of dwellings and access to electricity (+ 43%, average of captured insects 4.7± 8.5) and finally zones of secondary forests (+34%, average of captured insects 2.4±6) (7). A higher number of systematic observations are required in relation to human domicile to establish the risk for human populations.

The exploratory study carried out in the *Amazonía* (TDR A, 2005) showed interesting data. The prevalence of human *T. cruzi* infection in rural areas (3.40%) was higher than in urban areas (1.64%) and transmission occurred by invasion of triatomines that do not colonize domiciles. The rural population was composed by native, small farmers, hunters and fishers living in wooden houses very permeable to insect invasion.

The higher prevalence of *T. cruzi* was associated to landscapes made up of secondary forest, plantations and forest mosaic while the lower urban prevalence was associated to grass and bushes coverages. The Amazonian landscapes composed by secondary forest, plantations and primary forest had higher quantities of palm trees surrounding human rooms. The palm trees (urban 33% and rural 42%) had *Rhodnius* colonies.

The native houses had worse general conditions, were built with wood and very open in their walls and had higher percentage of triatomine invasion (urban 0.75% rural 10.57%). The high percentage of hens in urban houses has to be explored as a possible protective factor.

The higher prevalence of *T. cruzi* was associated to the recognition of *Rhodnius* and its identification in the houses. These elements acted as predictor risk factors.

The knowledge about Chagas disease of the two populations studied was insignificant and this orients to the need of developing preventive actions as strong compounds of community education.

The occasional or accidental *T. cruzi* transmission to man in the *Amazonía* is well known. In the *Amazonía*, *T. cruzi* naturally circulates in an enzootic cycle implying a large quantity of mammal reservoirs and triatomines. In these conditions, transmission occurs when the man enters into the natural cycle of the parasitosis or when environmental changes altered the vector habitats and insects invade human environment. *T. cruzi* transmission through food or drinks contaminated with triatomine intestinal content has been well documented and epidemic outbreaks of acute Chagas by common source are known (Coura, 1990). However, the observation of *T. cruzi* transmission by wild vectors with endemic profile in the Ecuadorian *Amazonía* is unknown in the scientific literature.

The diverse situations of Chagas disease transmission seem to be related to serious alterations of the Amazonian territory originally covered by a humid tropical forest. The oil exploitation, agricultural colonization and urbanization have resulted in dramatic ecological and demographic changes in the region (Aguilar et al., 2005). The enzootic cycle of *T. cruzi* is well known in the *Amazonía* and human participation in the cycle is sporadic (Coura et al., 2002) and still with limited endemism as it might occur in some populations with ancestral ways of life (Briceño, 2006). However, our observations that sylvatic foci or from secondary forest show important potential transmission to humans contrasting completely with the transmission modality involving domiciliary vectors known in the coast and propose a technical or scientific challenge to develop effective control strategies.

T. cruzi transmission from natural foci has been known since the times of Carlos Chagas and it has been considered a zoonosis with natural foci and rare and accidental human transmission (Coura et al., 2002; Pinto Dias, Prata & Schofield, 2002). However, a careful review of the literature shows evidence of transmission with endemic profile in several Amazonian countries according to the observations of Guhl (2002) in Guianía, Colombia related to *Rhodnius*, the invasion and systematic transmission phenomena documented by the staff of Cristina Aznar in French Guyana, the presence of high serological rates and chagasic cardiopathy in humans and dogs exposed to the bites of triatomines that invade dwellings. The research studies of Professor Coura in Río Negro, Amazon, Brazil showed continuous transmission and severe forms of the disease which are only some evidences added to our observations in Ecuadorian *Amazonía* that Chagas disease is a more serious and complex problem than it was known until now. This situation requires systematic and consistent knowledge to face prevention and control based in scientific evidences.

Modes of *T. cruzi* Transmission Identified in the *Amazonía*

1. Occasional transmission to man by wild vectors in the natural cycle (forestry activities, tourists)
2. Continuous transmission by wild vectors in extraction activities. (Extraction of "piaçava" and other palm trees that are habitats of triatomines)
3. Domiciliary and peridomiciliary transmission by vectors with incipient domiciliation.
4. Oral transmission by cultural practices: a) Ingestion of *T. cruzi* reservoirs blood as medicine, b) Ingestion of raw or semi-raw meat (smoked) of *T. cruzi* reservoirs (natives and settlers)
5. Oral transmission by common source (familiar or community outbreaks)
6. Continuous domiciliary transmission by invasion of wild triatomines (Endemic profile)

The recognition of Chagas disease as an emerging problem happened in several countries and the scientific community and control organisms mobilized in the search of shared and coordinated actions. As a concrete result of this purpose, a first international technical meeting was held in 2002 in Palmarí-Brazil where some guidelines for research, surveillance and evaluation of control possibilities were established. During this meeting, the conformation of the Intergovernmental Initiative of Surveillance and Prevention of Chagas Disease in *Amazonía* (AMCHA in Spanish) was proposed and PAHO/WHO was designated as Technical Secretariat.

Meetings were held in Manaos (Brasil) in 2004, in Cayens (French Guyana) in 2005 and there was a joint meeting between the Andean and Amazonian Initiatives in 2006 in Quito. In these meetings a consensus was reached about: a) A surveillance international network/system adapted to the Amazonian sub-region; b) Surveillance and prevention measures of Chagas disease in *Amazonía*; c) Proposals of diagnosis and clinical studies of Chagas disease in *Amazonía*; d) Research in relation to the improvement of epidemiology, diagnosis and treatment of Chagas disease in *Amazonía*.

It has been agreed that the implementation strategy of AMCHA Initiative has to have an increasing character from a progressive characterization and demonstration of risks. This research proposal is inscribed within the technical and scientific recommendations of experts and tends to develop critical knowledge that orient preventive and control actions in a particularly complex epidemiological situation. An especially important point is the identification of transmission dynamics in hot spots from a holistic vision (Abad Franch, 2006) that rationally integrates the multidisciplinarity and different analysis scales, allowing the establishment of certain predictors elements in identifiable spatial patterns and helps in the identification of critical zones in a wide region with endemic structure for *T. cruzi* transmission.

We approached Chagas diseases from a holistic and multidisciplinary vision in several research studies (Aguilar, 1988, 2005) and we are convinced that this approach allows the identification of critical elements capable of modifying the structure of an endemic area and altering the transmission risks of a vector borne disease. The eco-health approach (Lebel, 2005; Bazzani, Noroña y Sancrez, w/d) proposed by IDRC with elements of multidiscipline, equity and community participation constitutes a stimulating vision collecting elements of sociology, environmental sciences and human geography as a contribution to epidemiology and public health. We consider that this line collects principles from an old and renewed work line oriented now to the knowledge of different scales of determination towards the solution of problems.

Some Questions that Have to Be Answered as an Operative Base for Control

- ✓ What are the forms of spatial occupation and environmental transformation more frequently associated to the transmission of *T. cruzi* to human population?
- ✓ Which are the landscapes structures associated to peridomiciles that determine a higher *T. cruzi* transmission?

- ✓ Which are the organization forms (familiar and social networks), behaviors of health protection against diseases, gender roles and practices of domicile and peridomicile management?
- ✓ Which are the behaviors and practices towards triatomines?
- ✓ Which are the risk factors associated to *T. cruzi* transmission in domicile and peridomicile? Which is the dynamics of *T. cruzi* transmission in the different sub-spaces: natives, settlers and periurban inhabitants?
- ✓ How do the zones of old and recent occupation behave?
- ✓ Which is the relation between the ingestion of wild meat and food that could contain *T. cruzi* and population seroprevalence?

These questions have been made in the Project "Risk of *T. cruzi* transmission in the Ecuadorian *Amazonía* (EA)" Nº 103696-011IDRC that will soon start under our direction. We think that the questions made are pertinent to other endemic situations of *T. cruzi* transmission in the *Amazonía* and that is necessary to establish institutional networks and teams working with harmonized methodology and complementary logic that in a reasonable time allow the settlement of technical bases for the prevention and control of Chagas disease in the *Amazonía*.

References

Abad-Franch F& Aguilar VHM 2003 *Control de la Enfermedad de Chagas en el Ecuador OPS/OMS* (Publicación auspiciada por Ayuda Popular Noruega, Catholic Relieve Services, COOPI, Médicos Sin Fronteras y Oxfam) Quito, Ecuador. Online http://www.opsecu.org/publicaciones/OPS.doc.

Abad-Franch F, Paucar CA, Carpio CC, Cuba Cuba CA, Aguilar VHM, Miles MA 2001. Biography of *Triatominae (Hemiptera: Reduviidae*) in Ecuador: implications for the design of control strategies. *Mem Inst Oswaldo Cruz* 96 (5): 611-620 July.

Abad- Franch F 2006 Capítulo 7: *Complejidad ecológica y enfermedad de Chagas en la* Amazonía*.* http://www.idrc.ca/en/ev.

Aguilar,VHM 1988. *Epidemiologia da doença de Chagas: Aspectos históricos, sociais e morbidade em duas áreas endêmicas de Minas Gerais. Tese de Mestrado.* Instituto Oswaldo Cruz. Rio de Janeiro, Brasil, 180 pp.

Aguilar, VHM; Coura, JR & Sabroza, PC 1988. Doenca de Chagas em duas áreas endêmicas com organização espacial diferente. *Rev. Soc. Bras. Med Trop* 20 (II).

Aguilar VHM, Abad-Franch F, Racines VJ, Paucar CA 1999. Epidemiology of Chagas disease in Ecuador. A brief review. *Mem Inst Oswaldo* Cruz 94 (Suppl. 1): 387-393.

Aguilar VHM, Grijalva M, Palomeque F, Castro E y Abad Franch F 2005 Enfermedad de Chagas en la *Amazonía* Ecuatoriana. *4to Foro Nacional de Investigación en Salud: Memoria*, Pags: 31-44

Aguilar VHM 2007. T cruzi *dynamics of transmission in the Ecuadorian* Amazonía *Region*. TDR Project A20500. Scientific Report TDR/WHO.

AMCHA 2004. *Reunión Internacional sobre Vigilancia y Prevención de la Enfermedad de Chagas en la Amazonía (Manaus, Estado de Amazonas, Brasil, 19–22 septiembre): Implementación de la Iniciativa Intergubernamental de Vigilancia y Prevención de la Enfermedad de Chagas en la Amazonía.* http://www.paho.org/Spanish/AD/DPC/CD/dch-amcha-2004.htm

AMCHA 2005 Memorias de la 2ª Reunión de la Iniciativa Intergubernamental de Vigilancia y Prevención de la Enfermedad de Chagas en la Amazonía. OPS/OMS-IDRC-CDIA-EC-UFR Medecine/Univ. Antilles Guyane/CH Andrée Rosemon - Is. Vs. Departement International et Tropical - DS DS Guyane-LHUPM-EA 3593- CIRE, Antilles Guyane, IRD-MSF-ECLAT Cayena. Guayana Francesa, 2–4 de noviembre. http://www.idrc.ca/cairo/ev-103576-201-1-DO_TOPIC.html

IPA-AMCHA Conclusiones y Recomendaciones de la Reunión Anual Conjunta. (Quito, Ecuador, 18–20 septiembre 2006) 0PS/HDM/CD/CHA/421/06 http://www.ops-oms.org/Spanish/AD/DPC/CD/dch-amcha-ipa-2006.pdf

Amunárriz M 1991. Enfermedad de Chagas. Primer foco amazónico. In: *Amunárriz M, Estudios sobre patologías tropicales en la* Amazonía *ecuatoriana*. Ed. CICAME, Pompeya, Napo, Ecuador, p. 27-37.

Aznar C., Liégeard P., Mariette C., Lafon S., Levin M., Hontebeyrie M. 1997 A simple *Trypanosoma cruzi* enzyme-linked immunoassay for control of human infection in non-endemic areas. *FEMS Immunol.& Med. Microbiol*. 18: 31-37.

Bazanni R, Noroña L & Sánchez A (s/d) *An Ecosystem Approach to Human Health: building a transdisciplinary and participatory research framework for the prevention of communicable diseases.* http://www.globalforumhealth.org/forum8/forum8-drom/OralPresentations/Sanchez%20Bain%20%20F8-165.doc

Briceño LR 2006 *Una perspectiva sociológica de la enfermedad de Chagas en la* Amazonía. http://www.idrc.ca/en/ev

Chico HM, Sandoval C, Guevara EA, Calvopiña HM, Cooper PJ, Reed SG, Guderian RH 1997. Chagas disease in Ecuador: evidence for disease transmission in an indigenous population in the Amazon region. *Mem Inst Oswaldo Cruz* 92: 317-320

Coura JR 1990. The risk of endemic Chagas disease in the Brazilian Amazon. *Rev Soc Bras Med Trop* 23: 67-70

Coura JR, Junqueira ACV, Fernandes O, Valente SAS, Miles MA 2002 Emerging Chagas disease in Amazonian Brasil. *Trends in Parasitology* 18: 171-176.

Grijalva MJ, Escalante L, Paredes RA, Costales JA, Padilla A, Rowland EC, Aguilar HM & Jose Racines. 2003. Seroprevalence and Risk Factors for Trypanosoma cruzi Infection in the Amazon Region of Ecuador. *Am. J. Med.Hyg*. 69(4): 380-385

Guhl F 2002 Distribución en la amazonía Colombiana y su papel en la transmisión de *Trypanosoma cruzi*. En: *Proceedings ECLAT-AMCHA. International Workshop on Chagas Diseases Surveillance in the Amazon Region.* Universidad de los Andes CIMPAT pag:24-30

Lebel J 2005 Salud. *Un enfoque ecosistémico*. 89 pags. Alfaomega Bogotá, Colombia

Noireau F, Abad-Franch F, Valente SAS, Dias-Lima A, Lopes CM, Cunha V, Valente VC, Palomeque FS, Carvalho-Pinto CJ, Sherlock I, Aguilar M, Steindel M, Grisard EC & Jurberg J (2002). Trapping *Triatominae* in sylvatic habitats. *Memórias do Instituto Oswaldo Cruz* 97, 61-63.

Palomeque FS 2003 *Preferencia de hábitat de* Triatominae (Hemiptera Reduviidae) *en palmeras* (Arecaceae) *en la amazonia ecuatoriana.* Tesis de Licenciatura en Ciencias Biológicas. Pontificia Universidad Católica del Ecuador, Quito Ecuador, 107 pp.

Pinto Dias JC, Prata A, Schofield CJ 2002. Doença de Chagas na Amazonia: esboço da situação atual a perspectivas de prevenção. *Revista da Sociedade Brasileira de Medicina Tropical* 35 (6): 669-678, Nov-Dec

Wild *Triatoma infestans*: Scientific Curiosity or Potential Threat?

François Noireau

UR 016, Institut de Recherche pour le Développement (IRD), Montpellier, France

Centro Universitario de Medicina Tropical, Facultad de Medicina, Universidad Mayor de San Simón

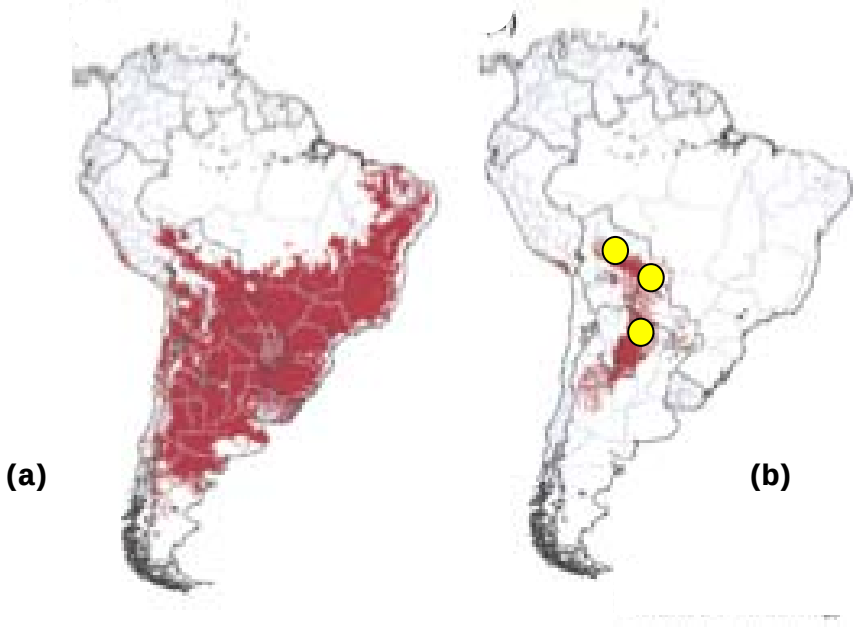
Cochabamba, Bolivia; francois.noireau@ird.fr

Triatoma infestans (Reduviidae, Triatominae) is the main vector of *Trypanosoma cruzi (Kinetoplastida: Trypanosomatidae)*, the causative agent of Chagas disease, in the Southern Cone countries. Therefore, it is the target of control programs within this region. The expected success for the elimination of *T. infestans* was particularly based on the assumption that it is an almost exclusive domestic vector (Schmunis et al. 1996). Whereas the maximum predicted distribution of *T. infestans*, reached during the 1970s, covered the 12 most populated states of Brazil in addition to vast areas of Bolivia, southern Peru, Chile, north of Argentina, Paraguay and Uruguay, an estimate shows that the current distribution of the vector has been reduced by over 80% (Schofield et al. 2006). Currently, *T. infestans* only persists in Andean valleys of Bolivia and the Gran Chaco region (Fig. 1). Interestingly, wild populations of *T. infestans* are precisely widespread throughout these regions. The following question deserves to be asked: does a relationship exist between the persistence of areas still infested with the vector and the occurrence of wild *T. infestans*? This paper provides an overview of knowledge on *T. infestans* wild foci.

Figure 1: Apparent distribution of *Triatoma infestans*

(a) Maximum predicted distribution reached during the 1970s according to Gorla (2002).

(b) Current distribution according to Schofield et al. (2006). Yellow dot = record of wild *T. infestans*.



Distribution of Wild Foci

Wild populations of *T. infestans* have a wide distribution in Bolivia, throughout the Andean valleys and the Chaco (Noireau et al. 2005). Up to date, wild *T. infestans* populations were found in three Andean departments (Cochabamba, La Paz and Potosi) and in the Chaco region (Department of Santa Cruz). Outside of Bolivia, wild *T. infestans* were recently detected in Santiago del Estero and Chaco Provinces, Argentina (Ceballos and Gürtler, unpublished data) and in the Metropolitan region of Chile (Bacigalupo et al. 2006). A wider distribution of the wild *T. infestans* throughout the Andean valleys and the Chaco is suggested by the fact that recent surveys have often been successful in detecting silvatic populations. Consequently, with regard to *T. infestans*, the notion of wild focus has not to be restricted to small areas but defined by large biogeographic regions as the Chaco and the mesothermic Andean valleys. Another important notion is the existence of wild foci in peri-urban environment as demonstrated in Cochabamba. In this large Andean city (> 500.000 inhabitants, census 2001), the soaring urbanization in the southern zone disregards the occurrence of wild *T. infestans* in the immediate outskirts of new settlements (Fig. 2).

Figure 2: New Settlement Located on the Outskirts of Cochabamba, 2,600 m asl. *Foreground:* Rocky Outcrops Providing Refuge for Wild *T. infestans*.

The Different Morphs of Wild *T. infestans* and Some Traits of Their Bioecology (Table 1)

The Andean wild *T. infestans* occur amongst rocky outcrops, which, regardless of their size, provide good refuges for them. High altitude populations (> 2,500 m asl) display a morphological and chromatic pattern similar to the domestic bugs whereas some populations found in the inter-Andean Chaco (< 2,000 m asl) exhibit a greater size and clearly different marks on the connexivum (Cortez et al. 2007). In contrast, the wild *T. infestans* from the Chaco (350 m asl) are arboreal. They are found in hollow trees, where the abundance of immature forms shows they constitute certainly a very favorable ecotope, and exhibit an overall darker coloration (dark morph) that distinguishes them from the other forms of *T. infestans*, either domestic or silvatic (Noireau et al. 1997). Particularly interesting is the considerable behavioral and chromatic plasticity displayed by the wild *T. infestans*. According to the biogeographic region, the vectors occur amongst rock-piles or in arboreal habitat whereas almost all other triatomine species do not display such feature. Similarly, we observe obvious chromatic differences between the rupicolous specimens collected in the Andes and the arboreal *T. infestans* (dark morph population but also *Triatoma melanosoma* Martinez, Olmedo & Carcavallo 1987) from the lowlands.

Table 1:

The Different Morphs of Wild *T. infestans* and Some Traits of Their Bioecology

			
Name	**Common Morph**	**"Mataral" Morph**	**Dark Morph**
Distinguishing morphochromatic traits*	None	Large size (> 30 mm) Great yellow markings on the connexivum	Overall dark coloration Small yellow markings on the connexivum
Area of endemism	Mesothermic Andean valleys (> 2.500 m asl)	Inter-Andean Chaco (< 2,000 m asl)	Lowlands of the Chaco (< 400 m asl)
Habitat	Rocky outcrops	Rocky outcrops	Hollow trees
Trypanosoma cruzi infestion rate	> 60%	N.R.	2.5%

* Distinguishing traits between the wild morph and the common domestic one; N.R.: Not recorded.

The Andean wild *T. infestans* is assumed to be the most ancient form (Dujardin et al. 1998; Panzera et al. 2004; Bargues et al. 2006). Nevertheless, the detection of wild *T. infestans* in the Boreal Chaco challenged this opinion and suggested an ancestral population native to this ecogeographic region (Carcavallo et al. 2000). The detection of a new morph of *T. infestans* in the inter-Andean Chaco (i.e. intermediate between the high Andean valleys and the Chaco) adds to the complexity of the question. Regardless of the ancestral form of *T. infestans* (Andean or Chaco), the other wild populations could be a consequence of a geographic expansion of the primitive wild population or derivatives from domestic insects recolonizing wild habitats.

The knowledge of the silvatic ecology and the role of wild *T. infestans* in the transmission of *T. cruzi* is still scarce in spite of the number and diversity of foci recorded The rupicolous wild insects

collected in the Andes are often heavily infected by *T. cruzi* (infection rate > 60%). Small wild rodents (genera *Phyllotis*, *Bolomys* and *Akodon*) and marsupials (*Thylamys elegans*) maintain this active *T. cruzi* transmission cycle. In spite of both *T. cruzi* genotypes (TCI and TCII) being prevalent in the valley of Cochabamba, only TCI is locally transmitted by wild *T. infestans* (Cortez et al. 2006). The infection rate observed in wild Andean populations is higher than that detected in the dark morph population from the Chaco (2.5%; Noireau et al. 2000). Such a low infection rate would be in favor of an ornithophilic tendency. A yearly pattern of population stage-structure, characterized by only one emergence peak of young nymphs and corresponding to the production of one generation a year, was found in the Andean wild *T. infestans* (Cortez et al. 2006). The age structure pattern of dark morph population is still unknown. Although the domestic *T. infestans* produce two generations a year in the warm climate of the Chaco (Gorla & Schofield, 1989), fluctuations in host availability in wild habitat might put the dark morph at a disadvantage and lead to an increased developmental time.

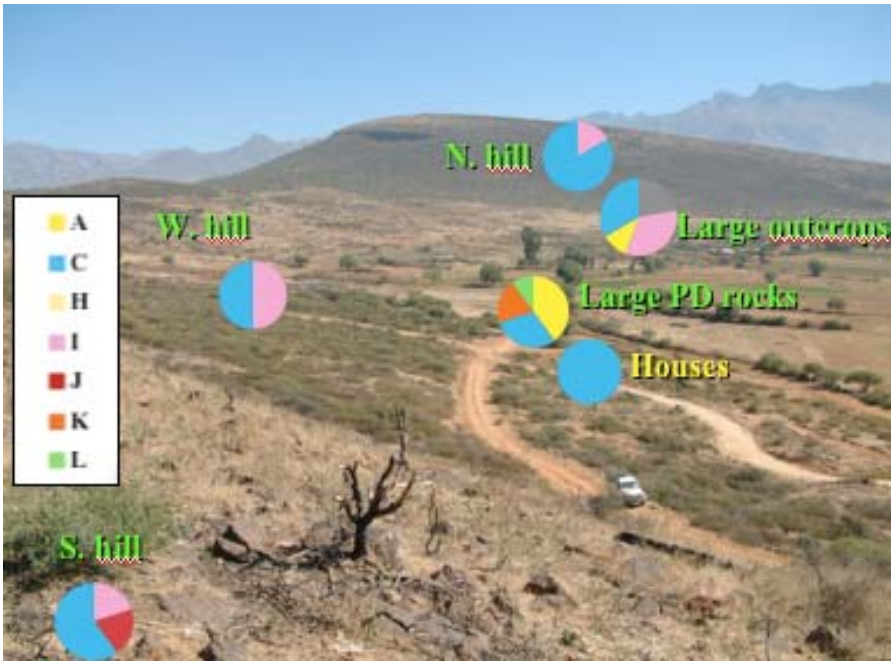
Genetic Diversity and Dispersal Ability for Wild *T. infestans* at High Altitude (2,750 m asl)

The sequencing of a mitochondrial cytochrome b gene fragment was performed in wild *T. infestans* sampled from a small area (< 1 km^2) located in the Cochabamba valley, at 2,700 m asl (Kengne et al. unpublished results). Wild *T. infestans* displayed a high genetic variation. Seven haplotypes were detected among the 38 insects analyzed and their distribution by collecting site is shown in Fig. 3. The silvatic haplotype C was the only detected in five domestic *T. infestans* from the same area, and the haplotypes A and C were also found in domestic bugs from the region of Sucre (Giordano et al. 2005). The detection of seven haplotypes in wild triatomines from a very small area supports the evolutionary theory that predicts a high genetic variation in the ancestral silvatic populations.

With regard to the threat represented by wild populations of *T. infestans*, the key question concerns the possibility for them to recolonize insecticide treated villages and thus jeopardize control efforts. In other words, it is essential to assess the dispersal of wild *T. infestans* and the gene flow between silvatic and domestic populations. In a study of dispersal performed in the Cochabamba valley at 2,700 m asl, the detection of restricted gene flow between close but distinct silvatic sites (rocky outcrops) supports the hypothesis that the vector does not disperse by flying at high altitude (Richer et al. 2007). Some studies dedicated to *T. infestans* flying ability under more favorable conditions (lowlands of the Chaco) pointed out that this species shows a flight potential on a village-wide scale and in silvatic environment (Schofield et al. 1992; Noireau et al. 2000; Vazquez-Prokopec et al. 2006). The results of Richer et al. (2007) rather suggest that, at 2,700 m asl in the Andes, the wild *T. infestans* gradually disperses over small distance by walking within a "patch" that might be characterized as a continuous

land cover, with all necessary resources for the persistence of triatomine population (Gustafson & Gardner 1996). On the other hand, when the land cover is disrupted by man made activities (building of dwelling and peridomestic structures, land or livestock farming…), the triatomine bugs bump into an unsuitable environment and cannot spread to separate patch by walking. The results of this study proceeding from highlands cannot be extended to other silvatic foci of *T. infestans* because of their ecological variety. Consequently, it is essential to determine the role that wild *T. infestans* populations may play as potential source of reinfestation in the different ecosystems where they occur.

Figure 3: Distribution of Cyt b haplotypes for *Triatoma infestans* in Five Silvatic Sites of Collection and Three Houses (Area of Cotapachi, Cochabamba Valley, 2,700 m asl)



No. of insects analyzed:

Southern hill:	5
Western hill:	8
Northern hill:	6
Large out-crops:	9
Large PD rocks:	10
Houses:	5

Acknowledgements

Special thanks to Mirko Rojas Cortez from the National Program of Chagas (PNCH), Bolivia. The study received financial support from IRD (France), CAPES and CNPq (Brazil), and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

References

Bacigalupo B.A., Segura M.J.A., Garcia C.A., Hidalgo C.J., Galuppo G.S. & Cattan, P.E. (2006). Primer hallazgo de vectores de la enfermedad de Chagas asociados a matorrales silvestres en la Región Metropolitana, Chile. *Revista Médica de Chile* 134: 1230-1236.

Bargues M.D., Klisiowicz D.R., Panzera F., Noireau F., Marcilla A., Perez R., Cortez M.R., O'Connor J.E., Gonzáles-Candelas F., Galvão C., Jurberg J., Carcavallo R.U., Dujardin J.P. & Mas-Coma S. (2006). Origin and phylogeography of the Chagas disease main vector *Triatoma infestans* based on nuclear rDNA sequences and genome size. *Infection, Genetics and Evolution* 6: 46-62.

Carcavallo R.U., Jurberg J., Lent H., Noireau F. & Galvão C. (2000). Phylogeny of the *Triatominae (Hemiptera: Reduviidae).* Proposals for taxonomic arrangements. *Entomologia y Vectores*, 7: 1-99.

Cortez M.R., Emperaire L., Piccinali R.V., Gürtler R.E., Torrico F., Jansen A.M. & Noireau, F. (2007). Sylvatic *Triatoma infestans (Reduviidae, Triatominae)* in the Andean valleys of Bolivia. *Acta Tropica* 102: 47-54.

Cortez M.R., Pinho A.P., Cuervo P., Alfaro F., Solano M., Xavier S.C.C., D'Andrea P.S., Fernandes O., Torrico F., Noireau F. & Jansen A.M. (2006). *Trypanosoma cruzi* (Kinetoplastida Trypanosomatidae): Ecology of the transmission cycle in the wild environment of the Andean valley of Cochabamba, Bolivia. *Experimental Parasitology* 114: 305-313.

Dujardin J.P., Schofield C.J. & Tibayrenc M. (1998). Population structure of Andean *Triatoma infestans*: allozyme frequencies and their epidemiological relevance. *Medical and Veterinary Entomology*, **12**, 20-29. Giordano R., Pizarro Cortez J.C., Paulk S. & Stevens, L. (2005). Genetic diversity of *Triatoma infestans (Hemipetra: Reduviidae)* in Chuquisaca, Bolivia based on the mitochondrial cytochrome b gene. *Memórias do Instituto Oswaldo Cruz* 100: 753-760.

Gorla D.E. (2002). Variables ambientales registradas por sensores remotos como indicadores de la distribución geográfica de *Triatoma infestans (Heteroptera: Reduviidae). Ecologia Austral* 12: 117-127.

Gorla D.E. & Schofield C.J. (1989). Population dynamics of *Triatoma infestans* under natural climatic conditions in the Argentine Chaco. *Medical and Veterinary Entomology* 3: 179-194.

Gustafson E.J. & Gardner R.H. (1996). The effect of landscape heterogeneity on the probability of patch colonization. *Ecology* 77: 94-107.

Noireau F., Cortez, M.R., Monteiro F.A., Jansen A.M. & Torrico, F. (2005). Can wild *Triatoma infestans* foci in Bolívia jeopardize Chagas disease control efforts? *Trends in Parasitology* 21**:** 7-10.

Noireau F., Flores R., Gutierrez T., Abad-Franch F., Flores E. & Vargas F. (2000). Natural ecotopes of *Triatoma infestans* dark morph and other wild triatomines in the Bolivian Chaco. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 94: 23-27.

Noireau F., Flores R., Gutierrez T. & Dujardin J.P. (1997). Detection of wild dark morphs of *Triatoma infestans* in the Bolivian Chaco. *Memórias do Instituto Oswaldo Cruz* 92: 583-584.

Panzera F., Dujardin J.P., Nicolini P., Caraccio M.N., Rose V., Tellez T., Bermudez H., Bargues M.D., Mas Coma S., O'Connor J.E. & Perez R. (2004). Genomic changes of Chagas disease vector, South America. *Emerging Infection Diseases* 10**:** 438-446.

Richer W., Kengne P., Cortez M.R., Perrineau M.M., Cohuet A., Fontenille D. & Noireau F. (2007). Active dispersal by wild *Triatoma infestans* in the Bolivian Andes. *Tropical Medicine & International Health* 12: 759-764.

Schmunis G.A., Zicker F., & Moncayo A. (1996). Interruption of Chagas disease transmission through vector elimination. *Lancet* 348: 1171.

Schofield C.J., Jannin J. & Salvatella R. (2006). The future of Chagas disease control. *Trends in Parasitology* 22: 583-588.

Schofield C.J., Lehane M.J., McEwan P., Catalá S.S. & Gorla D.E. (1992). Dispersive flight by *Triatoma infestans* under natural climatic conditions in Argentina. *Medical and Veterinary Entomology* 6: 313-317.

Vazquez-Prokopec G.M., Ceballos L.A., Marcet P.L., Cecere M.C., Cardinal M.V., Kitron U. & Gürtler R.E. (2006). Seasonal variations in active dispersal of natural populations of *Triatoma infestans* in rural northwestern Argentina. *Medical and Veterinary Entomology* 20: 273-279.

Ecosystem Approach Intervention for Long-Term Control of *Triatoma Dimidiata* in Guatemala

Monroy Carlota, Rodas Antonieta, Pineda Sandy, Castro Xotchilt, Ayala Virgilio, Moguel Barbara, Bustamente Dulce

Laboratory of Applied Entomology and Parasitology (LENAP), Faculty of Pharmacy

San Carlos University, Guatemala, Central America

Introduction

Experiences in Guatemala highlighted how integrated approach encompassing perspectives from epidemiology and community development can lead to sustainable, effective control of Chagas disease native vector by involving community members throughout the research and intervention processes.

The main strategy for the control of Chagas disease vectors in Central America has been the insecticide spraying; strong efforts have been done in Guatemala to diminish the populations of *Triatoma dimidiata*, but the insect reappears inside the houses few months after several rounds of insecticide applications. Those reinfestation problems suggest that new strategies must be developed in order to reduce insect populations. IDRC funded the following research

Methodology and Main Results

Four villages with persistent (post spraying) *T. dimidiata* infestation were studied; in two of them, an ecosystemic approach was implemented and wall improvements and house sanitation was carried out by house owners, with the support of the transdisciplinary team (Ecosystemic intervention). In the other two villages, a control approach based on insecticide application was followed (Traditional intervention).

Seventeen variables were evaluated as possible risk factors for the intradomiciliary infestation with *Triatoma dimidiata* in 644 houses in Jutiapa, Guatemala. Chi square analysis detected significant associations between vector presence and 8 variables related to house sanitary and construction conditions. Log-linear models supported that regardless of house age the odds of vector presence were 4.3 and 10 times lower in houses of good socioeconomic status than in poor and very poor houses, respectively. Log-linear models also supported higher odds of vector presence when walls lacked of plastering (3.85 times).

Selected risk factors, such as deteriorated wall conditions, were addressed by the community and a transdisciplinary research team to minimize the cracks or ridges in the mud walls. Engineers, Architects, Biologists and Anthropologists meet with community leaders to assess the best way to minimize the risk. The main risk factor for domestic infestations was the type of house; we divided the houses in types A, B, and C. Type C houses consist of adobe walls with deteriorated or no plaster at all, dirty floor, animal inside the houses and disordered or unclean house conditions. The type C houses were the ones with higher risk, while type A houses (all wall plaster in very good conditions, cement floor, clean environment and no animals inside) showed the lower risk.

Local materials pointed out by the community were tested under laboratory conditions to determine the best mixtures and proportions of local material to produce endurable wall plasters; a new wall plastering mix was formulated. The anthropologist determined that plaster application is a task traditionally crafted by women, using their bare hands, and it is considered a cosmetic aspect of the house. Our high opinion of their tradition led us to find a kind of plaster that does not require tools to be applied. It also had to match the people preferred colors and should consist in a simple mixture of their traditional material with sand.

After a short training, all family members in the ecosystem approach villages (La Brea, El Tule) were encouraged to perform the plasters using the new mixture proposed. The middle dry season corresponds to the Easter celebration, the agricultural work is over and the families have more free time to mix and apply the plasters.

Entomological evaluations were performed before and after insecticide application (four villages) and house improvements (Ecosystem approach). Both control interventions achieved a reduction in the infestation by *T. dimidiata*, but only the ecosystemic approach achieved high levels of house improvement (better sanitation and wall plastering) that could prevent in the long term, house reinfestation by *T. dimidiata*.

We focused the work on type C houses; 95 % of the poorest houses in the village El Tule made the wall improvements and 79% in village La Brea. Before the improvements, the vectors were found mainly inside the houses. After the wall plaster was applied, the vectors moved out into the chicken coops, dog houses and stone piles in the peridomestic environment in the ecosystem approach villages.

Hygiene improvements were also seen in the ecosystem approach villages, since the process required taking all the family belongings out of the house. They were selective to what they would bring

back and avoided to relocate things that would not look nice, and took better care on the way they arranged their “new painted house”.

The Ecosystemic and Traditional control strategies applied in this study were both successful in lowering *T. dimidiata* infestations. A few months after the spraying, all villages had less infested houses and a smaller number of domestic bugs were collected after the control interventions. The difference between the two strategies was in the significant improvement in the hygienic, construction conditions and in the location of the bugs collected after the intervention. Bugs were collected in both treatments after the interventions, the ecosystemic with an increase (293 %) of insects collected in few (two) peridomestic ecotopes, while the traditional approach decreased the amount of bugs collected (43%) in the peridomestic environments.

Hygiene and construction conditions of rural houses that make them suitable for triatomine infestation are directly linked to socioeconomic status and cultural practices. As some of this factors changed, the risk of human infection with the parasite of Chagas disease decreased. The challenge now is to maintain the peridomestic vector with the minimum human-vector contact, zooprofilaxic practices may be implemented with *Triatoma dimidiata*.

References

Dorn PL, Melgar S, Rouzier V, Gutierrez A, Combe C, Rosales R, Rodas A, Kott S, Salvia D, Monroy MC, 2003. The Chagas Vector, *Triatoma dimidiata* (Hemiptera:Reduviidae), is Panmictic within and Among Adjacent Villages in Guatemala*. J Med Entomol* 40: 436-440.

Dumonteil E, Ruiz-Piña H, Rodríguez-Félix E,Barrera, Perea M, Ramirez-Sierra, MJ, Rabinovich I.E, Menn F, 2004. Reinfestation of houses by *Triatoma dimidiata* after intradomicile insecticide application in the Yucatan peninsula, Mexico. *Mem Inst Oswaldo Cruz* 99: 253-256.

Hashimoto K, Cordon-Rosales C, Trampe A, Kawabata M, 2006. Impact of Single and Multiple Residual Sprayings of Pyrethroid Insecticides against *Triatoma dimidiata (Reduviiade; Triatominae),* the Principal Vector of Chagas Disease in Jutiapa, Guatemala. *Am J Trop Med Hyg* 75: 226-230.

Monroy C, Mejia M, Rodas A, Rosales R, Horio H, Tabaru Y, 1998. Comparison of indoor searches with whole house demolition collections of the vectors of Chagas disease and their indoor distribution. *Med Entomol Zool* 49: 195-200.

Monroy C, Rodas A, Mejia M, Tabaru Y, 1998. Wall plastering and paints as methods to control vectors of Chagas disease in Guatemala. *Med Entomol Zool* 49: 187-193.

Monroy C, Rodas A, Mejía M, Rosales R, Tabaru Y, 2003. Epidemiology of Chagas Disease in Guatemala: Infection Rate of *Triatoma dimidiata, Triatoma nitida*, and *Rhodnius prolixus* (Hemiptera, Reduviidae) with *Trypanosoma cruzi* and *Trypanosoma rangeli* (Kinetoplastida, Trypanosomatidae). *Mem Inst Oswaldo Cruz* 98: 305-310.

Nakagawa J, Cordon-Rosales C, Juarez J, Itzep C, Nonami T, 2003. Impact of Residual Spraying on *Rhodnius prolixus* and *Triatoma dimidiata* in the Department of Zacapa in Guatemala. *Mem Inst Oswaldo Cruz* 98: 277-281.

Nakagawa J, Hashimoto K, Cordon-Rosales C, Juares JA, Trampe A, Marroquin L, 2003. The impact of vector control on *Triatoma dimidiata* in the Guatemalan department of Jutiapa. *Ann Trop Med Parasitol* 97: 289-298.

Pinto Dias, JC.2001. *La comunidad y el control de la enfermedad de Chagas. Integración, Rol, Supervisión y evaluación de su participación. En OPS, Grupo de trabajo OPS para consulta en planificación operativa, estrategia y evaluación de etapas avanzadas de control antivectorial de la enfermedad de Chagas.* Montevideo. OPS/HCP/HCT/194/01.

Schofield CJ, 1985. Population dynamics and control of *Triatoma infestans*. *Ann Soc Belge Med Trop* 65: 149-164.

Rizzo NR, Arana BA, Diaz A, Cordon-Rosales C, Klein RE, Powell MR, 2003. Seroprevalence of *Trypanosoma cruzi* infection among school-age children in the endemic area of Guatemala. *Am J Trop Med Hyg* 68: 678-682.

Zeledon R, Guardia VM, Zuñiga A, Swartzwelder JC, 1970. Biology and ethology of *Triatoma dimidiata* (Latreille, 1811). II. Life span of adults and fecundity and fertility of females*. J Med Entomol* 7: 462-469.

Zeledon R, Rojas JC, 2006. Environmental management for the control of *Triatoma dimidiata* (Latreille, 1811), *(Hemiptera: Reduviidae)* in Costa Rica: a pilot project. *Mem Inst Oswaido Cruz* 101: 379-386.

Geometric Morphometrics Applied to the Study of *Triatominae*

Nicolás Jaramillo-O.
Instituto de Biología, Universidad de Antioquia, Medellín, Colombia
nicolas.jaramillo@siu.udea.edu.co

Morphological Variability

One of the main objectives in Biology is to understand the origin and nature of the variation of biological forms, and the causes of changes in the variation patterns. Quantitative analysis of phenotypic variation and its relationship with environmental, genetic and random (or from unknown origin) variation helps enormously to achieve such objective.

The first property of nature that stands out is the morphological variability of the organisms. Biological and non-biological factors underlie such variability, interacting in a complex way to model individuals, populations and species. Biological factors are products of micro- and macro-evolutionary processes. Individual size and shape are products not only of maternal effects and quality of life during ontogeny but of gene combinations inherited from parents. Moreover, populations and species have their own size and shape attributes, coded in their alleles, and product of their evolutionary histories. For example, consequent with their divergent trajectories, *Rhodnius prolixus* is a species of smaller size than *R. pallescens* and they exhibit different shapes.

Probably size and shape changes are significantly correlated; however, both traits are modulated by different genetic elements (Monteiro et al., 2002) which interact in a complex manner with environment. To quantify and analyze the morphological variation that results from such interactions is the goal of the geometric morphometrics. An additional objective is the study of co-variation with other variables (genetic, climatic, geographic, etc.). It is important to remark that the separate study of the portion of variation modeled by biological factors from that resulting from measurement artifacts is a great possibility of geometric morphometrics.

Geometric Morphometrics

Traditional morphometrics use distances between landmarks that are then translated into figures in paper sheets. But in the paper those figurers lose their relation with the biological forms they come from. They are processed in similar way than economic data, consumer surveys, psychological databases, etc.; thus, the direction of changes in the morphological differences is lost and either there is not any possibility of reconstructing shape.

On the contrary, geometric morphometrics keeps, throughout the different analytical algorithms, the relative position of landmarks. The morphometrics processing allows the elimination of the non-biological variation making possible to study the shape, visually examine its architecture, and analyze its co-variation with other variables.

Usefulness of morphometric analysis: it is easy to track the importance of morphometric analysis in the scientific literature. We will find that it has been used: (1) to detect and quantitatively describe the morphological variation, (2) to study the origin and nature of morphometric variation, (3) to study the genetic and environmental elements that can change morphometric patterns, (4) to support taxonomy, especially for populations hardly identified, (5) to detect evolutionary patterns, and (6) to support phylogenetic studies.

Besides these uses, morphometrics has been used in medical entomology as a low cost tool for entomological surveillance, helping to monitor and evaluate vector reinfestation after spraying. Also, it has been used to know the morphological variants present in the populations and weight their association to risk factors. Morphometrics helps to evaluate the spatial and temporal fluctuations of the morphological variants, and gives information about migratory process (gene flow). It is fundamental to support vector taxonomy in order to apply, in a focused and efficient way, control programs. Finally, it complements the information given by molecular biology, physiology and other disciplines.

Triatominae Studies Based in Morphometrics

In order to illustrate the possibilities of geometric morphometric analysis, some examples of the works performed at the Laboratory of Chagas of the Universidad de Antioquia, Colombia, are presented. The co-authors are: Dr. Maria Dora Feliciangeli and Dr. María Jesús Sanchez (Centro de Investigaciones Biomédicas, Sección de Entomología Médica, Universidad de Carabobo, Núcleo Aragua, Venezuela), Dr.

Omar Triana, MSc Harling Caro-Riaño and MSc. Sair Arboleda (Grupo de Chagas, Instituto de Biología, Universidad de Antioquia, Colombia), Dr. Cléber Galvão, Dr. Dayse de Silva Rocha and Dr. Jose Jurberg (Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Departamento de Entomologia, Instituto Oswaldo Cruz / FIOCRUZ, Rio de Janeiro, Brasil), Dr. Ana Laura Carbajal (Setor de Morfologia, Ultraestrutura e Bioquímica de Artrópodes e Parasitos, Laboratório de Transmissores da Leishmaniose, Instituto Oswaldo Cruz / FIOCRUZ, Rio de Janeiro, Brasil), Dr. Lileia Diotaiuti (Laboratório de Triatomíneos e Epidemiologia da Doença de Chagas, Centro de Pesquisas René Rachou-Fiocruz, Belo Horizonte, Brazil), Dr. François Noireau and Dr. Jean Pierre Dujardin (IRD, France).

At the north of the Amazon River basin, *Rhodnius prolixus* is the main vector of *Trypanosoma cruzi*. In the savannah region of Venezuela (where this species probably originated) and Colombia, domestic and sylvatic populations inhabit (López et al., 2007; Feliciangeli et al., 2007). In other areas of its distribution, *R. prolixus* seems to have only domestic habits, and its presence is possibly explained by human passive transport (Schofield and Dujardin, 1999). Because gene flow between domestic and sylvatic populations probably affects the response to control programs, we studied 297 sympatric insects of domestic, peri-domestic (coops) and sylvatic (*Attalea butyracea* palms) environments; all of them from Barinas state, Venezuela and collected before insecticides control. We used geometric morphometrics, processing wings by the generalized Procrustes analysis (Rohlf and Marcus, 1993). Size and shape variables obtained from this analysis were compared between populations by univariate and multivariate statistics. The results did not show significant differences in size, except for sylvatic males which are significantly smaller (Fig 1A, $F = 4.408$, $df = 2\&145$, $p < 0.05$). Shape did not show any differences between populations (Fig. 1B, $p > 0.05$).

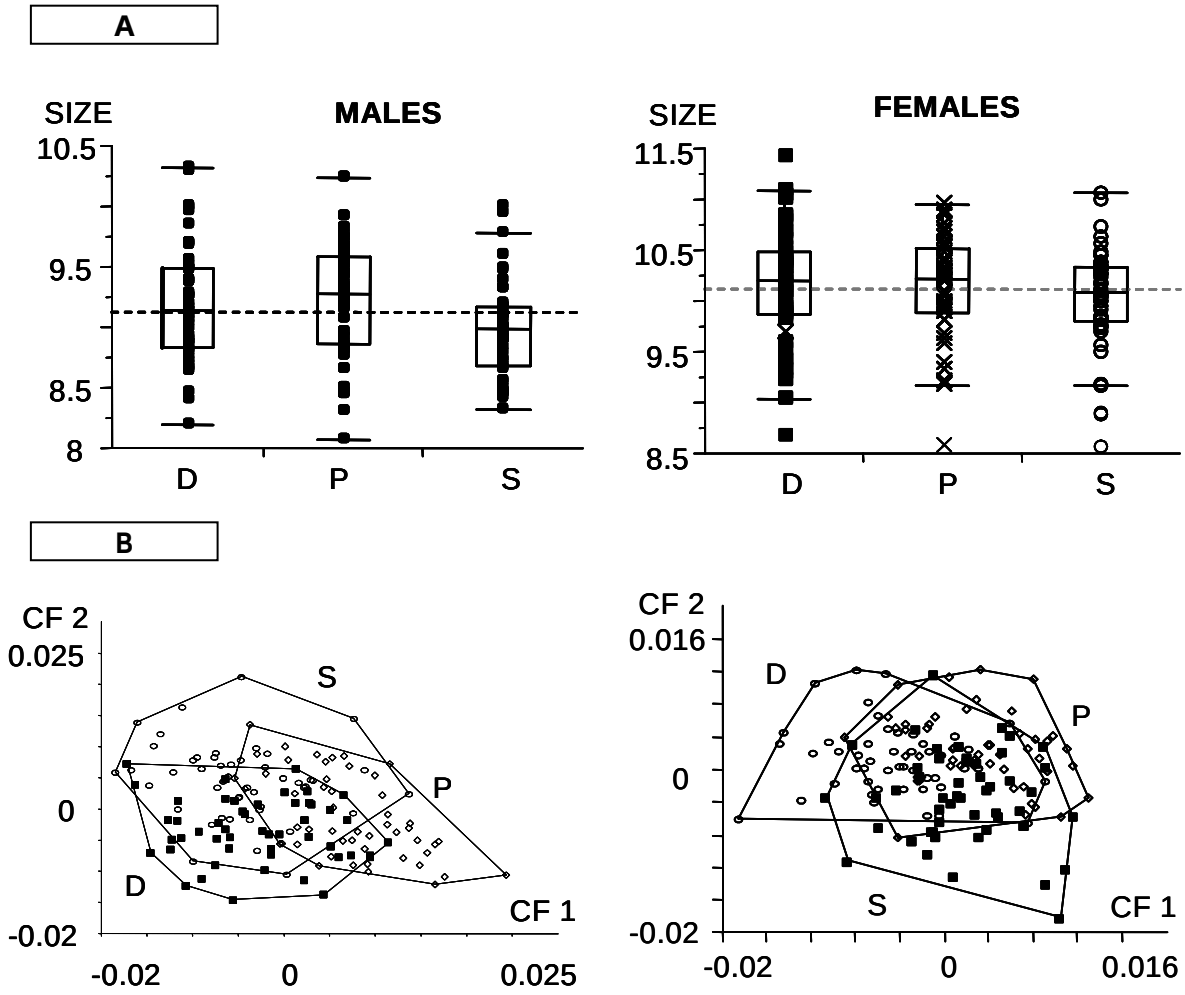
These results are similar to those presented by Feliciangeli et al. (2007) except by the notorious reduction in size variance of sylvatic specimens in relation to domestic ones, observed by these authors, which makes them to think about a unidirectional flow from sylvatic to domestic environment. On the contrary, our results show similar variance between both environments in females ($t = 1.127$, $df = 98$, $p = 0.26$) and different variance in males ($t = 2.37$, $df = 96$, $p = 0.020$ after Bonferroni correction). These results suggest that population dynamics between sylvatic and domestic environments is more complex than previously seen. This initial analysis suggest a bidirectional flow for females and unidirectional for males, and a significant phenotypic plasticity for acclimation to different habits. On the other hand, it was observed a significant small size in sylvatic males (Fig. 1, $p<0.05$) in relation to domestic and peridomestic ones, whereas females did not show such size reduction. This means more sexual size dimorphism in sylvatic environment, which is a marker trait of sylvatic populations of Triatomiane (Dujardin et al., 1999). With respect to shape, the lack of differences between the three habitats is a strong

suggestion that the three *R. prolixus* populations are a unique evolutionary unity with an important gene flow between them. In conclusion, size variation and shape uniformity tell us about the significant phenotypic plasticity of *R. prolixus*, which allows the acclimation of the individuals to different environments; but also the important gene flow in a complex way between sylvatic and domestic populations. This is signaling the importance of developing additional strategies to spraying such as the implementation of mechanical barriers that prevent the access of vector to human dwellings.

Figure 1: Variation in Size and Shape of Domestic (D), Peri-Domestic (P) and Sylvatic (S) *Rhodnius prolixus* from Barinas, Venezuela

A. The quantile box plots summarize the size distribution of males and females. The median is shown as a line between the 25 and 75 percentiles; a line above and below each box represent 10 and 90 quantiles respectively, and individuals distribution is shown as points along the box plots.

B. The points represent the individuals shape projected on the first and second canonical factors, which were derived from a canonical variate analysis. To facilitate visualization, lines connect the most external individuals of each population.



Most works have found *R. prolixus* as a depauperate species with significant genetic and phenotypic homogeneity inter-populations, a fact that has been used to argue its easy control by chemical methods (Dujardin et al., 1998). Such homogeneity and its adaptation to human dwellings, a relatively uniform and stable niche, allow the assumption of a high morphological similarity between domestic populations. However, the large geographical distances between populations, the differential exposure to insecticides, the different levels of gene introgression from sylvatic populations and the probably phenotypic plasticity could cause significant morphological variation. Consequently, it is important to search for differences between domestic populations because they could be a signal of variation in the responses to control.

In that sense, we use geometric morphometrics to study 241 domestic *R. prolixus* collected in 12 localities of three different ecological regions, each one separated by more than 400 km. The first region is located at a north slope of the Sierra Nevada de Santa Marta (SNSM), a humid tropical forest life zone, department of Guajira, Colombia. The other two regions are located in a continuous ecosystem of savannah: the Llanos in the Casanare department (CAS), Colombia, and in Barinas state, Venezuela (BAR). But the Llanos is not a homogeneous ecosystem because the relative humidity decreases gradually from west to east, making CAS climate that of a humid tropical forest, and BAR a dry tropical forest.

Geometric morphometric analyses showed SNSM individuals of significant larger size, while those of the other two populations did not show size differences between them (Fig. 2). It was not expected, because introduced populations without contact with their sylvatic co-specific populations show a notorious reduction in size (Dujardin et al., 1998, 1999; Jaramillo et al., 2002). It is possible that a similar effect to Bergman's rule accounts for these results, which show an inverse relationship between size and environmental temperature. The AVHRR remote sensor on board of NOAA satellites show lower average land surface temperature in SNSM (30.6 °C) than in BAR (36.3 °C) and CAS (36.8 °C), suggesting an important capacity of size change in response to environmental variation.

On the other hand, shape was different for each population, being BAR and CAS the most different and SNSM the intermediate. Multivariate regression analyses showed a significant relationship between size and shape, signaling an effect of growth on shape. Moreover, size and shape relationship was the same between BAR and SNSM and between CAS and SNSM (i.e. the pairs of populations grew in the same way); but it was not between BAR and CAS. These results are showing complex patterns of population dynamics and adaptation (or acclimation) to local environments. Moreover, it is probably that

migration (past or present), throughout intermediate populations, influences SNSM from BAR and from CAS. On the other hand, no contact between BAR and CAS is suggested, in spite of both populations being from a continuous ecological region, but with different life zones. In conclusion, domestic *R. prolixus* has important capacity of morphometric change that should be evaluated in more detail because of its possible correlation with variable responses to chemical control.

Keeping in mind the significant morphological plasticity of Triatominae, Dujardin et al. (2007) have been developing an interesting hypothesis: variance of domestic populations and species are more extensive than variance of sylvatic ones. To test it, we are executing several experimental designs, starting with genetically homogeneous laboratory lines of domestic and sylvatic Triatominae species. Controlled treatments of feeding on mammals or on birds and of growth in different levels of crowded blocks and feeding rhythm are performed to compare domestic and sylvatic species. Preliminary results (not shown) from *R. pallescens*, a predominantly sylvatic species, showed that higher population densities and prolonged feeding rhythm were significantly associated to smaller wing sizes, while no changes were observed in head sizes. Meanwhile, only higher population densities (no feeding rhythm) were associated to differences of variation in heads and wings shape being wings more susceptible to variation in shape than heads. The principal changes in wings shape involved the region between the coastal and cubital veins. In the heads, the changes were principally observed in the post-ocular region. Similar experiments are being conducted in *R. prolixus* and other *Rhodnius* spp. Finally, size and shape variances will be compared between species by means of sophisticated statistical analyses (e.g. 2-block partial least square analysis) in order to contribute to the understanding of domestication process in Triatominae.

Data from the Fundação Nacional de Saúde of 2002 shows *Triatoma rubrovaria* as the species more frequently collected after elimination of *T. infestans* in the Rio Grande do Sul state. *T. rubrovaria* adults are often found in peridomiciles suggesting an active dispersion behavior from their sylvatic habitats. Active dispersion in Triatominae is carried out by flying but some *T. rubrovaria* specimens show abilities of flying and others do not. What are the behavioral, structural and genetic differences between flying and non-flying *T. rubrovaria?* Answers have great epidemiological importance, but currently there is not a good understanding about this issue. As a first approach to this problem, we look for morphometric differences in the wings of flying and non-flying *T. rubrovaria.*

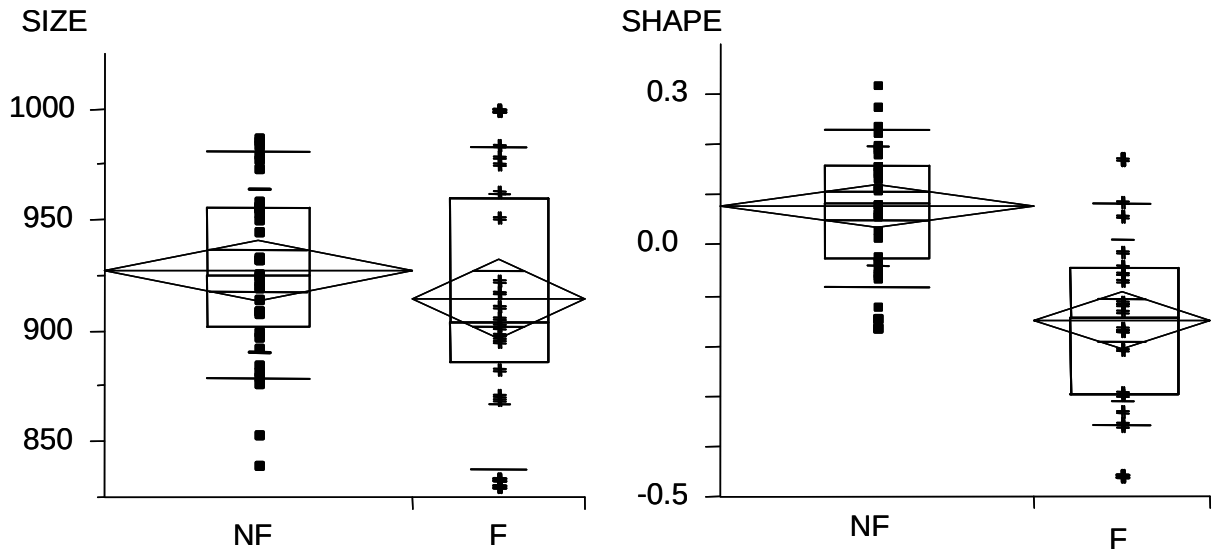
Twenty wings belonging to flying insects and 36 to non-flying were examined by geometric morphometric analyses. The size of the wings did not show significant differences between flying and non-flying insects ($p > 0.05$); but the wings shape showed significant differences among them (t-Test = 6.055, df = 54, $R^2 = 0.404$, $p < 0.0001$; Fig. 2). Differences in the mean shape of flying and non-flying

insects could not be explained by growth, because a linear regression of shape variables on size variable was not significant (p = 0.9063). Sixteen out of 20 flying insects and 29 out of 36 non-flying insects were correctly re-classified by discriminant analysis based on individuals shape. The Kappa statistics that measure the degree of agreement was equal to 0.586 indicating a moderate reclassification. Thin plate-spline technique showed differences between flying and non-flying insects as local deformations of traverse vein that intersects median and radial veins in the equatorial part of the wing. In relation to a consensus configuration, non-flying insects showed an expansion of that region while there is a reduction of the same region in flying insects. No differences in size but in the mean wing shape between flying and non-flying insects suggest a significant genetic background for this trait.

Figure 2:

Comparisons between the Wings of Flying (F) and Non-Flying (NF) *Triatoma rubrovaria*

The quantile boxes show the median as a line across the middle and the quartiles (10th, 25th and 75th, 90th percentiles) as its ends. The diamonds are signaling the mean of the sample and the 95% confidence interval about the mean.



T. arthurneivai and *T. wygodzinskyi* are two sylvatic Triatominae species from Brazil; they are potential vectors of Chagas disease but little is known about their ecology and biology. Santos et al. (2007) have evidenced that several authors mistakenly identified *T. arthurneivai* from several places; thus, publishing wrong information. In this work, we used geometric morphometrics in order to differentiate both species and detect wrong identifications that occurred in the past. We used insects from the field, collected in Minas Gerais and Sao Paulo states, and insects from museums of both states.

Results showed similar size of *T. arthurneivai* from Serra do Cipó, Minas Gerais, and individuals previously classified as *T. arthurneivai* from Ituparanga, Sao Paulo. These two populations were different in size from *T. wygodzinskyi* from Vargem Grande do Sul, Sao Paulo, and Santa Rita de Caldas, Minas Gerais (Fig. 3A). On the other hand, shape clearly differentiates *T. arthurneivai* from Serra do Cipó from *T. wygodzinsky*, and interestingly clusters the insects from Ituparanga with the two *T. wygodzinsky* populations (Fig. 3B). Thus, contradictory results between size and shape were found.

Size and shape are good estimates of genetic variance expressed in the morphological characters but size is more affected by the environment where the individual grows while shape is a more stable character (Monteiro et al., 2002). Triatominae is not an exception to this statement. *Panstrongylus geniculatus* showed a progressive reduction in size, at least by five generations, from sylvatic insects to its descendants in laboratory while it was not seen any discernible change in the shape in five generations of laboratory cultures (Jaramillo et al., 2002). *P. rufotuberculatus* showed similar results (data no published). A carefully study of classical taxonomic characters, made by Dr. Ana Laura Carbajal de la Fuente, brings near the individuals from Ituparanga, Sao Paulo to *T. wygodzinskyi*, and not to *T. arthurneivai* from Serra do Cipó (where holotype specimens of this species are from). Corium pigmentation and phallosome were wery informative.

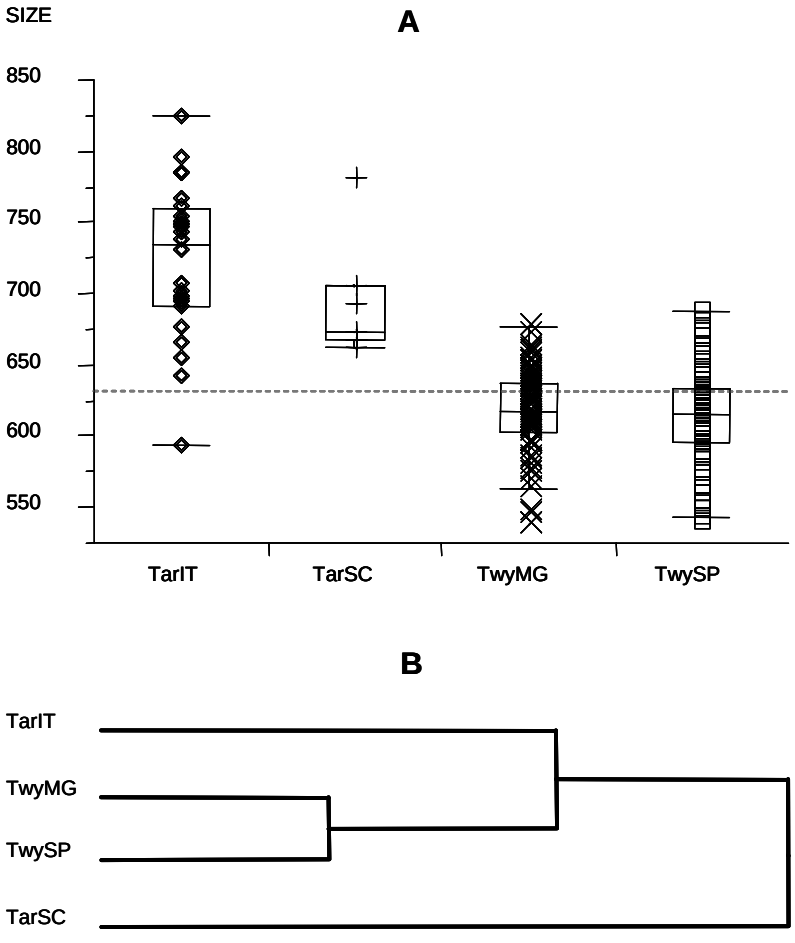


Figure 3:
Size (A) and Shape (B) Variation of *Triatoma arthurneivai* and *T. wygodzinsky*

TarSC
Triatoma arthurneivai from Serra do Cipó, MG; TarIT: wrongly identified *T. arthurneivai* from Ituparanga, SP; TwyVG and

TwySR
T. wygodzinsky from Vargem Grande do Sul, SP and from Santa Rita de Caldas, MG,

Finally, the results suggest that *T. arthurneivai* could be an endemic species from Serra do Cipó; an area that exhibits atypical high endemism for a big number of plant and animal species. In conclusion, our results suggest that the *T. "arthurneivai"* records of those from Serra do Cipó could be misidentifications, and signal the importance of revising the taxonomy of some museums collections.

These are some examples of the works performed in our research laboratory of Chagas disease at the Universidad de Antioquia. The intention is to show some possibilities and directions of research for the next future. A better knowledge of population dynamics and taxonomy of Triatominae will improve control programs. Geometric morphometrics will surely contribute to this, helping to understand the domestication processes and solving taxonomic difficulties that are present day to day in field and museum collections.

Acknowledgments

The works here presented benefited from the international collaboration through ECLAT network. Support was from CDIA project and Specific Support Action - American Trypanosomiasis Update Project (Contract No: INCO-SA 515942); "Dirección General de Cooperación para el Desarrollo, Presidencia de Gobierno de la Generalitat Valenciana", Valencia, España (Expediente 2000/3042); CNPq and FAPERJ, Brazil; Colciencias, Colombia (project 1115-0414-387) and the "Comité para el Desarrollo de la Investigación – CODI", Universidad de Antioquia, Colombia.

References

Dos Santos SM, Lopes CM, Dujardin JP, Panzera F, Pérez R, Carbajal de la Fuente AL, Pacheco R, Noireau F. 2007. Evolutionary relationships based on genetic and phenetic characters between *Triatoma maculata*, *Triatoma pseudomaculata* and morphologically related species (Reduviidae: Triatominae). *Infection, Genetics and Evolution* 7: 469-475.

Dujardin JP, Muñoz M, Chávez T, Ponce C, Moreno J, Schofield CJ. 1998. The origin of *Rhodnius prolixus* in Central America*. Medical and Veterinary Entomology* 12: 113-115.

Dujardin JP, Steindel M, Chávez T, Machane M, Schofield CJ. 1999. Changes in the Sexual Dimorphism of Triatominae in the Transition from Natural to Artificial Habitats. *Memórias do Instituto Oswaldo Cruz* 94: 565-569.

Feliciangeli MD, Sánchez-Martín M, Marrero R, Davies C, Dujardin JP. 2007. Morphometric evidence for a possible role of *Rhodnius prolixus* from palm trees in house re-infestation in the state of Barinas (Venezuela). *Acta Tropica* 101:169-177.

Galvão C, Rocha DS, Jurberg J, Carcavallo RU. 2001. Início da atividade de vôo em *Triatoma infestans* (Klug, 1834) e *T. melanosoma* Martínez, Olmedo & Carcavallo, 1987 *(Hemiptera, Reduviidae). Memórias do Instituto Oswaldo Cruz.* 96: 137-140.

Jaramillo N, Castillo D, Wolff M. 2002. Geometric morphometric differences between *Panstrongylus geniculatus* from field and laboratory. *Memórias do Instituto Oswaldo* Cruz 97: 667-673.

López DC, Jaramillo C, Guhl F. 2007. Estructura poblacional y variabilidad genética de *Rhodnius prolixus* (Hemiptera: Reduviidae) procedente de diferentes áreas geográficas de Colombia. *Biomédica* 27 (Supl. 1): 28-39.

Monteiro L, Diniz-Filho J, dos Reis S, Araujo E. 2002. Geometric Estimates of Heritability in Biological Shape. *Evolution* 56: 563-572

Rohlf FJ, Marcus LF. 1993. A revolution in morphometrics. *Trends in Ecology and Evolution* 8: 129-132.

Schofield CJ, Dujardin JP. 1999. Theories on the evolution of *Rhodnius*. *Actualidades Biológicas* 21: 183-197.

Advances in Community Surveillance of *Trypanosoma cruzi* Transmission

Elsa L. Segura[1], Graciela Palavecino[2], Teresa Pereira[5], Marcelo Abril[3], Ana Saavedra[4], Gustavo Barbieri[6]

1. CONICET, en Instituto Nacional de Parasitología "Dr. Mario Fatala Chabén", ANLIS, Ministerio de Salud, Argentina, Av. Paseo Colón 568, 1063 Buenos Aires

2. Servicio Nacional de Chagas, Av. 9 de Julio 356, 5000 Córdoba.

3. Fundación Mundo Sano, Av. Libertador 1068, Ciudad de Buenos Aires.

4. Hospital Regional de Añatuya, Santiago del Estero

5. Programa Anti-Chagas, Ministerio de Salud, Santiago del Estero.

6. Laboratorio de Chagas Hospital Regional de Santiago del Estero

The presence of *Triatoma infestans* vector of *Trypanosoma cruzi* in the south of South America has been, either actually or potentially, delimited between 40º north and 45º south latitudes. Specimens have been found at altitudes of up to 3,000 meters above sea level. Natural human transmission of the disease has been observed from the south of the United States, (where some few moderate cases occurred), down up to the province of Chubut, Argentina.

Since the 1950s and 1960s onwards, several studies have shown a perfect correlation between the presence of triatomism and the prevalence of *T. cruzi*, and between this and the heart disease attributed to Chagas disease (17). Additionally, recent migration processes have shown that no region is exempted from the occurrence of a local autochthonous transmission caused through any of the following routes: vectorial, transfusional, congenital transmission, organ transplant, or by the use of needles shared by injecting drug users (13).

Considering the major transmission ways, the main control strategies consist in monitoring the vector presence, controlling it by using insecticides, controlling blood to be transfused, and detecting *T. cruzi* transmission from infected mothers to their newborns in order to provide them medical treatment.

In the conference held in Alma-Ata in 1978, the WHO renewed its call for turning community participation (CP) into a key element for individual and community health, where responsibility is equally shared by the members of the community and the healthcare professionals. Transference to community is a strategy that leads to a change of habits in behavioral patterns within the health-disease correlation.

Primary Health Care (PHC) strategy was applied in developing countries as a vertical, distance-action program (11) with virtually no components of transference to the community and far from stimulating the community's participation in healthcare actions (7). Communities were engaged as mere supportive manpower, in most of these cases, Argentina also used this faulty modality, although with few honorable exceptions.

The surveillance of the *T. cruzi* transmission project presented here has involved staff Healthcare Agents (HA's), Municipal Agents (MA's), and non-staff people with lesser basic training of the same social origin, residing in communities similar to those of the HA's.

A health promotion procedure was put to test among the rural population. HA's and MA's performances were compared. Acting in "social networks" means acting through a social plan or system that interrelates the different social "stakeholders" -institutions, organizations, or individuals- of a specific community that gathers together to pursue shared interests or values in order to meet their needs, either of goods or services (12, 2, 4). This work shows the results of social network actions taken to support and monitor HA's and MA's work in the community.

At the beginning of PHC practice community participation considered as a magic bullet that solved health-related or political problems, Susan Rifkin (16) suggested a paradigm change. The new paradigm considered community participation as an iterative learning process towards a more eclectic approach. This is one of the most significant orientations when planning the development of a surveillance system of *T. cruzi* transmission involving the community participation applied to almost every type of organization existing in the social-healthcare system.

Methods

The study involved a total of 190 rural dwellings of the Avellaneda district, province of Santiago del Estero (Figure 1) that, were monitored for *T. cruzi* transmission with the community participation and the HAs' within the social network framework. In 360 dwellings of Rio Hondo district, surveillance by participation was not promoted. These dwellings were monitored through the ordinary activity of the health system.

In this work, the network promotion stages completed were: summons of population, work proposal elaboration, goals and objectives setting, and action plan development that took 5-6 months. The following aspects of Chagas transmission surveillance were dealt afterwards: training for healthcare

centers, HA's, leaders, and professionals; surveillance implementation, spreadsheet management, work kinetics, and supervision-related concepts. Afterwards, during surveillance, activities were supervised while specific aspects of the network performance, as well as monitoring and surveillance performance, were evaluated externally. Once these activities were concluded, household heads were summoned to participative workshops on Chagas disease control. These workshops were repeated 4 times in 2 years in the forms of meetings in each operative area, and of workshops in the sectors, and were summoned from their respective healthcare centers or schools. Supervision started as the HA's and MA's -according to healthcare organization- visited the dwellings.

Here, the selected research methodology used within the Primary Health Care strategies, is the one primarily applied in the field of disease prevention programs, specifically for Chagas disease. Thus, this methodology was intended to be used with support provided by the operative levels where community action takes place.

This study comprised all the dwellings of the 2 PHC districts involved in Avellaneda.

The work of the HA's procedure with the community members consisted on:

a. Elaboration of the census and cartography of dwellings.
b. The application of the survey was conducted to analyze the population knowledge about Chagas disease risk and surveillance, before the intervention and 32 months after the beginning of the intervention.
c. The treatment with insecticides of the dwellings was by two groups of houses.
d. The evaluation applied was: entomologic survey[1], serology survey[2], and **e.** monitoring of field actions performed by the social network members.

The intervention of promotion made by the HA's started after the entomologic evaluation and chemical insecticide spraying on dwellings (1) in the studied areas.

Serological studies were performed for anti-*T. cruzi* antibodies detection in the population of children under 14 years of age, and the information obtained was submitted to the local and central healthcare services. Collections of children blood were performed using Serokit®, collecting 100 µ l of blood, which was analyzed using ELISA and HAI (19). The confirmation was performed by studying the same reactions in blood taken from the antecubital fossa vein.

[1] Performed by the National and Provincial Chagas Services
[2] Performed by the National Chagas Service of Catamarca

Social Evaluation of Interactions among the Social Networks Members within the Community.

This evaluation instrument was administered during our first contact with the community before receiving any kind of training. The only requirement for its administration was the existence of a principle of horizontal dialogue between the professional team and the community. The social network workshop was conducted after the application of an instrument consisting in placing Chagas contacts in a concentric fashion according to acquaintance and contact frequency with the interviewees, with the purpose of determining the progress of the network members' socialization. The group of data obtained from this first contact is called "*Evaluation 0*". The evaluation instrument was re-administered 10 months (*Evaluation 1*) and 30 months (*Evaluation 2*) after commencement of the Network operations. The instruments were analyzed qualitatively.

Monitoring

Actions were monitored or supervised by physicians of the research project. A private vehicle was used for these tasks. In each location, supervision was performed as follows:

The Supervisor presented his greetings to the School and Healthcare Center or Municipality. Then, the Supervisor performed the "HA's, MA's, or Teacher's Survey", according to the participant involved in the local control activities.

Knowledge Assessment In general, the survey respondents were the household heads. However, in many occasions it was the family that sat around the interviewer to answer the questions. This modality was respected, as it is customary in the community, and because whenever an issue arises, the family members take decisions jointly to solve it. Some variables have been re-categorized for the analysis of the data collected through the surveillance knowledge survey. In the case of the variable "How do you search your house for vinchucas?", aimed at exploring the population's modality used to search for the vinchuca (*Triatoma infestans*) insect, the group of indicators showing higher disregard for such variable was considered as a "passive attitude" towards *T. infestans* infestation surveillance. Thus, the indicators "Does not search" and "They just show up" represented passive attitudes. Conversely, attitudes which result was the finding of vinchucas, even when such result was not the clear and desired objective, were considered as "active attitudes". This variable includes the indicators: "Cleaning", "Using light/lamps", "Moving the

furniture around", "In holes and corners", "Roof and walls", all of which suggest that the inhabitants dynamically search for the vinchuca insect.

Data Analysis: The presentation of the data description was based on a univariate analysis. The association analysis was based on a bivariate analysis. Proportions were compared using Chi Square and Fisher tests. In all cases the tests were applied for a confidence level of 95.0 %. Data were analyzed using SSPS software.

Results

Social Evaluation of Interactions among the Social Networks Members within the Community: These networks, intentionally promoted for purposes of the participative *T. cruzi* transmission surveillance, require, however, their leaders' voluntary and conscious participation. As any network, this one intends to satisfy the community needs, and implies articulations and connections between groups and individuals around a central objective functioning as a common thread sustaining all such relationships. Interactions increased by 20% from Evaluation 0 to Evaluation 1, and by 40% from Evaluation 1 to Evaluation 2.

Entomologic Evaluation: The baseline evaluation was performed in Avellaneda in 2003, and a control evaluation was performed in 2004. Two groups were considered: surveillance development, and control group, under the responsibility of the provincial healthcare system. A difference between both groups was observed, with a statistically significant reduction in the infestation levels in monitored dwellings. The second evaluation performed in Rio Hondo localities does not show significant differences in comparison to the first one.

Serologic Evaluation: 651 children under 15 years were studied in Avellaneda on March 2002 and 2,879 children in June 2004. Global prevalence was 6.8 and 5.8 respectively. In this work, a decreasing trend was observed in the serology of children living in areas already covered by participative surveillance. Thus, in children from 6 months to 5 years of age in 2004, prevalence was 2 times lower than that of the base control, performed in 2002.

Evolution of *T. cruzi* Transmission Surveillance Knowledge Acquired by the Community in a 4-Year Follow-Up: The applied instrument helped to evaluate the knowledge received along a 2-year period in the participative workshops and through each four-monthly message provided by the HA's strategic allies to each dwelling. The last evaluation was performed two years after the researchers had

left the research area and ceased having contact with the community, which responded with a considerable level of knowledge, significantly higher –from a statistical point of view- than that of the first evaluation performed in 2003. The opposite was observed in Rio Hondo localities, a fact that promoted our surveillance participation.

Discussion

The main strategy for vector control of *T. cruzi* transmission comprises the spraying of the dwellings with residual effect insecticides and the epidemiologic surveillance implementation. The control programs were structured in three successive action phases: preparatory, massive attack with insecticides and surveillance. Vector control using insecticides is effective and it has been proved that it interrupts transmission, since the most anthropophilic species are susceptible to the application of such products. Despite the positive results of the use of insecticides to control insect populations –as disease transmission is interrupted-, the effectiveness of this method is maintained only for 3 years, in absence of epidemiological surveillance. After such period, reinfestation occurs, as well as retransmission (8). So far, the only solution to maintain the control status is the epidemiologic surveillance, i.e., the third phase mentioned above. On the other hand, it is important to extend time spans between one insecticide spraying and the next one, considering the evidence on resistance to insecticides.

Human dwelling reinfestation by *T. infestans* is the main concern in the endemic rural area. The determinant factors of reinfestation include demographic and environmental variables in diverse spatial scales, as well as detection of residual foci at very low insect density (5, 9).

Control using insecticides must begin with the participative surveillance principles, as maintained in this study. Community commitment is the right option to achieve long-standing surveillance. There are previous work experiences we can resort to, which proved that *T. cruzi* transmission is interrupted with the community's participation. With the researchers guidance, sustained community participation grew out of establishing trusted relationships with the affected communities and the local schools. The process included health promotion and community mobilization, motivation, and supervision, in close cooperation with locally nominated leaders (2)

This work included the social network component, which was composed by people who were relevant in resources management or services provision to the community, namely the PHC's coordinating physicians, hospital directors, mayors, teachers, police officers, journalists (6, 18).

This work shows the social networks role as a contention and control framework for epidemiologic surveillance, based on the translation of the HA's knowledge to the community, the effectiveness of 4 visits per year (promotion for the triatomine' detection) to each dwelling, and the effective information flow between HA's and the local healthcare level.

The social networks evaluation shown in this work had to follow the social networks building evolution. Furthermore, the community had to be activated, either by the provincial Health system or the researchers. The global results obtained 10 and 21 months after commencement of the study on the communities involved show that the social networks in general progressed from individual interactions to collective ones. Graphic representations of the Networks global data, previously considered separately for each social network, correspond to the analysis of the initial surveys and of those conducted in average periods of 10 and 21 months after issuance of summons (July 2002) for Social Network promotion.

The mobility implied by community-based work through networks frequently creates new networks that diminish the presence of the oldest leaders. In this work, an example of that was that some of the leaders performed their Evaluation 0 in the Colonia Dora Social Network, and then they constituted a new one in Icaño; or started in Lugones, and then moved to Mailin localities, where they completed Evaluations 1 and 2.

Concerning future perspectives, this qualitative triangulated analysis with a quantitative perspective will permit the acquisition of targeted social indicators, and their possible changes along the program execution term.

So far, the analysis has been performed by an external observer not involved in the work team; but for future studies, we propose the incorporation of a system composed by two external expert judges in order to be able to prepare statistical tests that help to analyze inter-observer consistency, thus increasing the validity and reliability of the results. In order to obtain the real integrating value of the Social Networks actions for *T. cruzi* transmission surveillance, this type of evaluation must be complemented with an evaluation of prevention, spraying, and surveillance performances, like it was developed in this work.

This work was developed on the basis of a working rural healthcare system, coordinated by hospital "radiant-action" physicians in charge of PHC coordination. In the 60's, healthcare services in Argentina started a long-term, constant transfer process to local systems, consisting in the transference of the administrative and financial services to the provinces. PHC functionality at the national level has been

under a rearrangement process for the last three years. As suggested in Alma Ata (1968), it is unthinkable to imagine an activity to be developed at rural level without the participation of the local healthcare organization. A procedure including the healthcare agent as the main communicator of entomologic surveillance knowledge was developed.

All the observations in this work suggest that the central provincial organization should include the epidemiological surveillance of *T. cruzi* transmission and its inherent procedures in the HA's curriculum. Especially, the HA's should know how to translate HA's knowledge to the community inhabitants in order to achieve long-term sustainability for all the actions. Another observation regarding HA's operations is the need to establish the monitoring of HA's activities. Currently, radiant-action physicians perform technical supervision, but they cannot supervise procedure compliance. In this work it is suggested that the local social network provides the local healthcare system with a framework that is both participative and of social commitment.

We have pointed out the different serological results observed in two areas of the Rio Hondo district (3) province of Santiago del Estero, in 1991: in 120 dwellings studied in the area controlled by the HA's with the provincial organization, 4 children whose results in a previous evaluation had been negative were found infected; and in 100 dwellings studied in the area in which the researchers participated, only one child that had been out of the area periodically with his/her mother, was found infected (4). In this sense, a surveillance follow-up conducted in Amamá (Moreno district, province of Santiago del Estero) indicated that the serology showed a correlation with abundant *T. infestans*, although abundance in one of the described cases was minimal (8).

Lastly, the interest in researching the hypothesis of community participation as a means to the community attainment of the solutions to its problems (16) entails the risk pointed out by many authors regarding the sustainability of the control actions (7). Sharing Rifking's hypothesis (16), in this work we insisted on the advantages of engaging the HA's in a commitment to work together with their communities in a steady and constant way, accompanied by the social network.

Acknowledgement

We thank Dr. Silvia Gold, Fundación Mundo Sano, Misses B. H. de Bravo and Ing. E Bravo for helpful cooperation. This work was financed by ANLIS Malbrán, Ministerio de Salud de la Nación, Fundación Mundo Sano and Ministerio de Salud y Desarrollo Social, de Santiago del Estero (2002-March 2005).

References

1. Blanco SB, Spillmann CA,Canale D, Ripoll C, Audicio MI, Marzotín F, Programa Nacional de Chagas 2001, Ministerio de Salud de la Nación Argentina, Secretaría de Atención Sanitaria, Coordinación Nacional de C oordinación de Vectores, VIG+ A.
2. Cardinal MV, Lauricella MA, Marcet PL, Orozco MM, Kitron U, Gürtler RE. Impact of community-based vector control on house infestation and Trypanosoma cruzi infection in *Triatoma infestans*, dogs and cats in the Argentine Chaco, *Acta Trop* 2007 Sep; 103 (3): 201-11
3. Chuit R, Gürtler RE, Mac Dougall L, Segura EL, Singer B, Chagas Disease- Risk assessment by an environmental approach in northern Argentina. *Revista de Patologia Tropical* 30 (2): *193-207 (2001).*
4. Chuit, R., Paulone, I. Wisnivesky-Colli, C. Bo, R. Pérez, A.C. Sosa-Estani, S. and Segura E. L. Results of a first step toward community-based surveillance of transmission of Chagas'disease with appropiate technology in rural areas. *Am. J. Trop Med. Hyg.* 46 (4) 444-450 (1992).
5. Chuit, R.; Subias, E.; Perez, A.C.; Paulone, I.; Wisnivesky- Colli, C. and Segura, E.L. Usefulness of serology for the evaluation of *Trypanosoma cruzi* transmission in endemic areas of Chagas' Disease. *Rev da Soc Brasileira de Medicina Tropical* 22 (3): 119-124, (1989).
6. Esquivel ML, Gomez AO, Salomón OD, Sosa Estani S y Segura EL, La participación comunitaria en el control de Chagas, *Medicina* (Buenos Aires), 55 (Supl III): 34-35 (1997).
7. Glickman SW, Baggett KA, Krubert CG, Peterson ED, Schulman KA. Promoting quality: the health-care organization from a management perspective. *Int J Qual Health Care* 2007, Oct 18.
8. Gürtler, RE,Cecere MC, Lauricella MC, Petersen RM, Chuit R, Segura EL, Cohen JE, Incidence of *Trypanosoma cruzi* infection among children following domestic reinfestation after insecticide spraying in rural northwestern Argentina. *Am. J. Med. Hyg* 2005: 95-103
9. Gürtler, R.E.; Cecere, M.C.; Castañera, M.B.; Canale, D.; Lauricella, M.A.; Chuit, R.; Cohen, J.E.; and Segura, E.L. Probability of infection with *Trypanosoma cruzi* of the vector *Triatoma infestans* fed on infected humans and dogs in northwest Argentina. *Am. J. Trop. Med. Hyg* 55 (1): 24-31 (1996).
10. Gürtler R. E., Cohen J. E., Lauricella M. A., Chuit R., y Segura E. L. Influence of humans and domestic animals on the household prevalence and density of *Triatoma infestans* populations in northwest Argentina. *Am. J. of Trop. Med. Hyg* 58: 748-758 (1998).
11. Hall JJ, Taylor R. Health for all beyond 2000: the demise of the Alma-Ata Declaration and primary health care in developing countries. *Med J Aust*. 2003 Jan 6;178 (1): 17-20
12. Jodetet S. *Representaciones social.* 1993, Buenos Aires, Editorial Paidós.
13. Montero, M. Teoría y práctica de la psicología comunitaria. La tensión entre comunidad y sociedad. Buenos Aires, 2003, Editorial Paidos.
14. Leiby DA, Herron RM Jr, Read EJ, Lenes BA, Stumpf RJ. Trypanosoma cruzi in Los Angeles and Miami blood donors: impact of evolving donor demographics on seroprevalence and implications for transfusion transmission. *Transfusion*. 2002 May;42(5):549-55.
15. Paulone I., Chuit R., Perez A.C., Canale D., and Segura E.L. The Status of transmission of *Trypanosoma cruzi* in an endemic area of Argentine prior to control attempts, 1985. *Ann. Trop. Med. and Parasit.* 85: 23-29 (1991).
16. Rifkin SB, Hartley SD, Learning by doing: teaching qualitative methods to health care personnel. *Educ Health* (Abingdon). 2001;14 (1): 75-85.
17. Rosenbaum MB, Cerisola J A. Epidemiology of Chagas' disease in the Argentine. Republic. *Hospital* (Rio de Janeiro). 1961 Jul; 60: 55-100.
18. Segura, E. L.; Esquivel, M. L.; Salomón, D.; Gómez, A.; Sosa Estani, S.; Luna, CA.; Tulián, L.; Hurvitz, A.; Blanco, S.; Andrés, A.; Zárate, J.; Chuit, R. Participación comunitaria en el Programa Nacional de Control de la Transmisión de la Enfermedad de Chagas. *Medicina* (Buenos Aires), 54 (Nº 5/2) 610-611 (1994).
19. Segura E L, Robertazzi M, Sosa Estani S, Vaccari L, Gómez A, Palavecino G, Aranda de Pereira T, Saavedra A, *Redes Sociales para la Vigilancia de la transmisión del* Trypanosoma cruzi *(Chagas).* CONAPRIS, Ministerio de Salud y Ambiente de la Nación, Buenos Aires, Argentina, pp. 129, 2005.

New Triatomine Control and Detection Tools: Use Perspectives

Antonieta Rojas de Arias
Vector Control National Consultant
Pan-American Health Organization, Asunción, Paraguay

The evaluation of sensors for detecting the presence of triatomines after chemical interventions started in the first decades of Chagas disease control. Some of them were the box of Gomez Nuñez in Venezuela (Gómez Nuñez, 1965), still used in some research studies (Cuba et al, 2003), bamboo sensors (García-Zapata, 1978; Marsden & Penna, 1982), filter paper sheets used for early detection of triatomines presence by the recognition of the feces impregnating the papers (Schofield et al., 1978) and the more recent Bioadvanced sensor boxes in Argentina, widely used by the national program of Chagas (Wisnivesky-Colli et al 1987) and also in field research in Paraguay, Uruguay and Chile (Oliveira Filho, 1997). In Bolivia, there were others sensors incorporated to control assays like the Golden Box, the already mentioned attractant pheromones (Cohen, 1998) and the traps baited with yeast cultures with attractant capacity for *T. infestans* and other triatomine species (Lorenzo et al., 1998; Pires et al., 2000). Special artificial shelter units with black plastic were also evaluated (De Marco et al. 1999).

The studies about the response of triatomines to chemicals have been developed for more than 40 years (Cruz López et al, 2001). It is important to point out that little experimental research has been performed about the chemical ecology of triatomines and few of these studies have reached the phase of field tests.

In relation to sex or copulation pheromones, many authors have suggested their presence in triatomines based in behavioral and electrophysiological studies. For example, Baldwin et al. (1971) observed that volatile compounds obtained from the copulation of *Rhodnius prolixus* attracted male *Rhodnius* in the absence of females. However, these compounds were not isolated. On the other hand, Ordaza et al. (1986) showed that hexane extracts collected from volatile compounds remaining in glass capsules containing *Triatoma mazzottii* female specimens attracted other females of the same species. Again, there were not any attempts to isolate these compounds definitely.

Manrique and Lazzari (1995) showed that *T. infestans* male specimens aggregated around a copulating pair, suggesting the presence of sex pheromones. This fact was later confirmed by De Brito Sánchez et al. (1995) who showed an increase of the excitation of olfactory cells of the antennas of male

T. infestans in presence of copulating pairs. It is thought that these compounds are released by Briddley´s glands (Hack et al, 1980; Juárez & Brenner, 1981)

Other trapping system based on the sensory physiology of triatomines has been evaluated according to literature related to host odors (Taneja & Guerin 1995, Guerin et al. 2000), CO2 (Taneja & Guerin 1995, Barrozo & Lazzari, 2004), and ammonia released from triatomine feces (Taneja & Guerin 1997).

The percentages of sensitivity of these instruments is variable (2% to 96%) depending on the triatomines density of the study area and the comparative methods used to determine their sensitivities (Gurtler et al., 1995,1999; Rojas de Arias et al., personal communication).

A project carried out by our group provided important contributions to the knowledge of *T. infestans* semio-chemicals and their attractant capacity. A series of new compounds showing attractant capacity were identified such as hexanal, heptanal, nonanal, dipropylsulphoxide, methylbutanol and/or methylbutanol and benzaldehyde (INCO DC, 2002).

The laboratory bioassays preceding field assays showed significant attraction responses when these compounds were placed in adult females, especially aliphatic aldehydes that showed that the attraction level was highly dose-dependant. However, the results had lower magnitude if the adult was male (INCO-DC, 2000, 2002; Fontan et al., 2002). It is important to point out that hexanal is the most abundant aliphatic aldehyde and could have activity at low doses (Cork et al., 2001, Cork et al., 2001a, Rojas de Arias et al., 2002).

An experimental field study with pre and post-interventions processes was carried out to measure the presence or absence of triatomines in dwellings by the use of traps with and without attractants (triatomine pheromones). Post-intervention measures were carried out at 1, 3 and 6 months after the placement of traps in dwellings confirmed negative by manual search of triatomines carried out by the project staff through capture of insects during one hour/man in dwellings and peridomicile when this exists. The 1, 3 and 6 months exposures of baited traps with and without aldehydes showed sensitivity in the traps containing aldehydes from 80% to 94% at 3 months of exposure.

In general, the most common detection method to assess the prevalence and intensity of infestation of domestic and peridomestic areas of dwellings are timed manual collections (TMC) and TMC with an irritant spray (Rabinovich et al., 1995). Other tools commonly used in field assays that

allow triatomine captures in the forest and peridomestic areas are the Mouse-baited traps with sticky tape (Noireau traps). Light-trap stations are currently used as well, on each of two perpendicular transects across villages, to assess invasion by triatomine bugs (Vazquez-Prokopec et al., 2006) and in nearby sylvatic areas. The literature mentioned that the incorporation of chemicals to the traps used for the capture of wild insects to make them more sensitive would give a great impulse to behavior studies of secondary potential vector species (Abach Franch et al, 2000; Noireau et al., 2002).

The most remarkable gaps that can be mentioned in vector control area are the ignorance of the factors that determine a process of rapid reinfestation and the origin of post-spraying triatominae populations despite a good insecticide coverage. Thus, evidence about the dynamics of vector scattering is necessary to adjust and identify strategies that facilitate the establishment of a sustainable triatomines monitoring system. Therefore, the development of this new system as well as the assessment of genetic markers before and after spraying processes will give control programs the necessary evidences to identify the most appropriate control strategies.

References

Abad-Franch, F.; Noireau, F.; Paucar, C.A. *et al.* 2000. The use of live-bait traps for the study of sylvatic *Rhodnius* populations (Hemiptera, Reduviidae) in palm trees. *Trans. Roy. Soc. Trop. Med. Hyg* 94: 629-630

Baldwin WF, Knight AG, Lynn KR. 1971. A sex pheromone in the insect *Rhodnius prolixus (Hemiptera Reduviidae) The Canadian Entomologist* 103: 18-22.

Barrozo RB, Lazzari CR, 2004. The response of the blood-sucking bug *Triatoma infestans* to carbon dioxide and other host odours. *Chem Senses* 29: 319–329

Cohen HL. 1998. Six-Month Field Test of the Golden Box™ to control the vector of Chagas disease in Bolivia, December 1996 through June 1997. *Bol Entomol Venez* 13 (2): 93-121.

Cork A, Alzogaray R, Farman DI, González Audino P, Camps F, Fontán A, Martínez A, Masuh H, Santo Orihuela P, Rojas de Arias A, Zerba E. 2001. Development of an odour-baited trap for use in control of a vector of Chagas disease, *T. infestans*. *International Society of Chemical Ecology, 18th Annual Meeting*, Lake Tahoe, July 7-12.

Cork A, Alzogaray R, Farman DI, González Audino P, Camps F, Fontán A, Martínez A, Masuh H, Santo Orihuela P, Rojas de Arias A, Zerba E. 2001a. Towards the development of an odour-baited trap for use in control of a vector of Chagas disease, *T. infestans*. Royal Entomological Society International Symposium, Insects, Disease and Entomology. University of Aberbdeen, 10-12 September.

Cruz-López, L., A. Malo, JC Rojas, & ED Morgan. 2001. Chemical Ecology of triatomine Bugs: Vectors of Chagas Disease. *Medical & Veterinary Entomology* 15: 351-357.

Cuba Cuba, C., F. Vargas; J. Roldan; C. Ampuero. 2003. Domestic *Rhodnius ecuadoriensis (Hemiptera, Reduviidae)* infestation in Northern Peru: a comparative trial of detection methods during a six-month follow-up. *Rev. Inst. Med. Trop*. *São Paulo* 45 (2).

De Brito Sanchez MG, Manrique G, Lazzari CR. 1995. Existence of a sex pheromone in *T. infestans (Hemiptera Reduviidae):* II Electrophysiological correlates. *Mem. Inst. Oswaldo* Cruz 90 (5): 649-651.

De Marco RJ, Gürtler RE, Salómon OD, Chuit R. 1999. Small-scale field trial of a sensing device for detecting peridomestic populations of *Triatoma infestans (Hemiptera: Reduviidae)* in northwestern Argentina. *J Med Entomol* 36: 884-887

Fontán A, González Audino P, Martínez A., Alzogaray R, Zerba E, Camps F. and Cork A. 2002. Attractant volatiles released by female and male *Triatoma infestans (Hemiptera: Reduviidae),* a vector of Chagas disease: chemical analysis and behavioural bioassay. *Journal of Medical Entomology* 39 (1): 191-197.

Garcia-Zapata, MT.1985. *Vigilancia epidemiológica no controle do* Triatoma infestans *em duas áreas no Estado de Goiás, Brasil.* Brasilia. (Disertação de Mestrado, Universidade de Brasilia)

Gómez-Núñez, J.C. 1965. Desarrollo de un nuevo método para evaluar la infestación intradomiciliar por *Rhodnius prolixus. Acta Cient. Venez* 16: 26-31.

Gorla, D. 2004. Encuentro Regional. Avances en la Vigilancia de la enfermedad de Chagas en el Cono Sur. CDIA E/IICS, *Libro de resúmenes. El componente espacial en la Ecología poblacional del Triatoma infestans analizado a diferentes escalas geográficas*, p. 28.

Guerin PM, Kröber T, McMahon CP, Guerenstein P, Grenacher S, Vlimant M, Diehl PA, Steullet P, Syed Z. 2000. Chemosensory and behavioural adaptations of ectoparasitic arthropods. *Nova Acta Leopoldina* 83: 213-229

Gurtler, RE. 2004. Encuentro Regional. Avances en la Vigilancia de la enfermedad de Chagas en el Cono Sur. CDIA E/IICS, *Libro de resúmenes. Eco epidemiología de la enfermedad de Chagas en el Noroeste de Argentina: Aplicación*

de Imágenes satelitales de Alta resolución y Morfometría de Alas para Análisis espacial y Control de Triatoma infestans, pp. 26-27.

Gürtler, RE.; Chuit, R.; Cecere, MC. & Castañera, MB. 1995. Detecting domestic vectors of Chagas disease: comparative trial of six methods in north-west Argentina. *Bull. Wld. Hlth. Org*. 73: 487-494.

Gürtler, RE.; Cecere, MC.; Canale, DM.et al. 1999. Monitoring house reinfestation by vectors of Chagas disease: a comparative trial of detection methods during four-year follow-up. *Acta Trop.* (Basel) 72**:** 213-234.

Hack WH, Riccardi AIA, Oscherov B, Oliveti de Bravi MG 1980. Composición de la secreción de las glándulas de Bridndley en triatomineos. *Medicina* 40: 178-180.

INCO DC. 2000. *Development of an odour-baited trapping system for control of the vector of Chagas disease.* Second Annual Report. Contract Number ERB18 CT980356 (period 1999–2000).

INCO DC. 2002. *Development of an odour-baited trapping system for control of the vector of Chagas disease.* Final Report. Contract Number ERB18 CT980356 (period 1998–2001).

Juárez P, Brenner RR 1981. Biochemistry of the evolutive cycle of *T. infestans* V. Volatile fatty acids emission. *Acta Physiologica Latinoamericana* 31: 113-117.

Juarez P. 2004. *Encuentro Regional. Avances en la Vigilancia de la enfermedad de Chagas en el Cono Sur. CDIA E/IICS, Libro de resúmenes. Hidrocarburos del Complejo sordida. Aplicaciones en taxonomía Química*, p. 31., Asunción, 9-11 junio.

Lorenzo MG, Reisenman CE, Lazzari CR. 1998. *Triatoma infestans* can be captured under natural climatic conditions using yeast-baited traps. *Acta Trop.* 3070 (3): 277-84.

Manrique G & Lazzari CR 1995. Existence of a sex pheromone in *T. infestans (Hemiptera Reduviidae)* I. Behavioural Evidence. *Mem. Inst. Oswaldo Cruz* 90: 645-648.

Marsden, P.D. & Penna, R.A. 1982. A 'vigilance unit' for households subject to triatomine control. *Trans. Roy. Soc. Trop. Med. Hyg* 76: 790-792

Noireau F., F. Abad-Franch, S. Vaelnte, A. Dias Lima et al. 2002. Trapping *Triatominae* in Sylvatic habitats. 2002. *Mem. Inst. Oswaldo Cruz* 97 (1): 61-63.

Oliveira Filho, AM. 1997. Uso de Nuevas Herramientas para el Control de Triatominos en diferentes Situaciones Entomológicas en el Continente Americano. *Rev. Soc. Bras. Med. Trop.* (30): 41-46.

Ondarza RN, Guitierraz Martínez A & Malo EA. 1986. Evidence of the presence of sex and pheromones from *T. mazzotii (Hemiptera Reduviidae) Journal of Economic Entomology* 79: 688-692.

Pires HH, Lazzari CR, Diotaiuti L, Lorenzo MG. 2000. Performance of yeast-baited traps with *Triatoma sordida, Triatoma brasiliensis, Triatoma pseudomaculata, and Panstrongylus megistus* in laboratory assays. *Rev Panam Salud Publica* 7 (6): 384-388.

Rabinovich JE, Gürtler RE, Leal JA, Feliciangeli D. 1995. Density estimates of the domestic vector of Chagas disease, *Rhodnius prolixus* Stål *(Hemiptera: Reduviidae),* in rural houses in Venezuela. *Bull World Health Organ* 73: 347-357

Rojas de Arias, A, Canale D, Alzogaray R, Cork A, Fontán A, Masuh H, Secaccini R, Stariolo R, Zerba R. 2002. Desarrollo de una trampa insecticidas cebada con atractantes para el control de *T. infestans*. Evaluación en pre-campo y campo. *V Congreso Argentino de Entomología*, Buenos Aires, marzo.

Schofield, C.J. 1978. A comparison of sampling techniques for domestic populations of Triatominae. *Trans. Roy. Soc. Trop. Med. Hyg* 72**:** 449-455.

Silveira AC & O. Sánchez. Guía *para muestreo en actividades de vigilancia y control vectorial de la enfermedad de Chagas*. OPS/DPC/CD/276/03.

Taneja J, Guerin PM. 1995. Oriented responses of the triatomine bug *Rhodnius prolixus* and *Triatoma infestans* to vertebrate odours on a servosphere. *J Comp Physiol A* 176: 455–464-

Taneja J, Guerin PM. 1997. Ammonia attracts the haematophagous bug *Triatoma infestans*: behavioural and neurophysiological data on nymphs*. J Comp Physiol A* 181: 21–34.

Vázquez-Prokopec GM, Ceballos LA, Marcet PL, Cecere MC, Cardinal MV, Kitron U, Gürtler RE 2006. Seasonal variations in active dispersal of natural populations of *Triatoma infestans* in rural north-western Argentina. *Med Vet Entomol* 20: 273-279

Wisnivesky-Colli, C.; Paulone, I.; Perez, A. *et al.* 1987. A new tool for continuous detection of the presence of triatomine bugs, vectors of Chagas disease, in rural households. *Medicina* (Buenos Aires) 47**:** 45-50.

Insecticide Impregnated Curtains and Bed Nets for Control of Cutaneous Leishmaniasis and Chagas Disease In Venezuela

Dr. Elci Villegas Avila, MSc, PhD
Universidad de los Andes, Núcleo Universitario "Rafael Rangel"
Instituto Experimental "José Witremundo Torrealba"
Trujillo, Venezuela

Abstract

The use of insecticide treated materials for the control of Chagas disease transmission is potentially cost-effective and sustainable where vectors are sylvatic and enter houses at night. A randomized trial was undertaken including all houses in two communities in a regions endemic for this disease (96 houses). After a baseline study (including a short questionnaire survey, entomologic assesment and Chagas disease serology), each household was randomly allocated to either the intervention group, which used pyrethroid curtains and bed nets, or the control group, which used unimpregnated curtains and bed nets. Serologic analysis of children in the baseline study showed active transmission of Chagas disease (10.7% of 103 children were seroreactive). The vectors were sylvatic (mainly *Rhodnius robustus*) and entered the houses at night. This randomized trial showed that the users of impregnated curtains and bed nets were well protected from vector bites. The long-term effect on the community was high vector mortality. All vectors detected died within 72 hours of contact with impregnated bed nets and curtains. In houses that used unimpregnated nets, only 24.5 % (13 of 53) of the vectors died and only 20% (6/30) of vectors died, probably due to rough handling, in the houses with non-impregnated curtains. The vectors most likely came from infected palm trees that maintained transmission of the disease in these communities (28.1% of 6,229 *R. robustus* were positive for *Trypanosome cruzi*). It is concluded that pyrethroid-impregnated curtains and bed nets are good protectors and represent an important option for the reduction or even elimination of man-vector contact and thus of Chagas disease transmission in areas infested with *R.prolixus* and *R. robustus*. We also carried out a community randomised trial in urban area of Venezuela where indoor transmission of cutaneous leishmaniasis occurred mainly with an annual incidence of 4%. We assessed any reduction in abundance of sandflies indoors and clinical cases in areas with houses protected by curtains impregnated with pyrethroid insecticide compared with areas with houses using non impregnated o with no curtains at all. Curtains are preferred to bed nets in urban areas. The mean number of sandflies per trap per night was 16. After the follow-up, the 12 month incidence of cutaneous leishmaniasis was 0% in the intervention group and 9% in the six pairs of the control group that received unimpregnated curtains. The number of sandflies entering houses was reduced in houses with impregnated curtains. In conclusion, pyrethroid impregnated curtains provided a high degree of protection against indoor transmission of cutaneous leishmaniasis.

Eradication of Chagas Disease: What Are Its Possibilities?

João Carlos Pinto Dias, MD, PhD
Oswaldo Cruz Foundation, René Rachou Institute, Brazil
jcpdias@cpqrr.fiocruz.br

In July 2007, Dr. Roberto Salvatella (from PAHO Staff) declared in Geneva (Salvatella 2007):

"In the recent WHA 51.14 Resolution, the health authorities declared that the elimination of Chagas Disease (CD) could be attained in 2010, by means of continuous control and the pertinent surveillance in the endemic areas. Analyzing this proposition, a panel formed by experts of the American Intergovernmental Initiatives against CD decided to clarify the following points:

- ✓ CD, an endemic infectious disease, was formerly a primitive enzootic phenomenon, which in Latin America suffered an evolution to a widespread anthropozoonosis. This disease has a lack of effective treatment in all its phases and no immunoprophylaxis is available. Moreover, the natural circulation of Trypanosoma cruzi is largely spread in sylvatic ecotopes all over the American Continent. Because of these reasons, it cannot be considered eradicable, since **eradication** means the definitive interruption of the transmission, to be completely maintained in the absence of any control or surveillance action. On the other hand, elimination could be understood as the absence of transmission (or of a determined vector species) in a determined geographical space, being dependent of the maintenance of continuous control and/or surveillance actions.
- ✓ By another angle, a residual contingent of at least 12 million of already infected individuals remains living in endemic and non endemic countries, generating the possibilities of new cases production, by means of alternative mechanisms of T. cruzi transmission, mainly throughout the congenital and transfusion routes.
- ✓ In addition, the persistence of sylvatic foci has been more and more involved in the so called oral transmission of human CD, with about one hundred of new cases being reported yearly in different ecological situations.
- ✓ Nevertheless, during the last two decades, the transmission of human CD has been effectively controlled in several endemic areas, carried on by means of massive vector control activities followed by continued surveillance and by the regular serological selection of blood donors.

- ✓ The interruption of parasite transmission to new generations, plus the dramatic reduction of domestic triatomines in a worked region, generated the pragmatic concept of **elimination**, which has a temporal and geographical meaning, since the control actions are maintained in a sustainable way.
- ✓ In the particular case of introduced and restrict domestic triatomines (such as *Triatoma infestans* in several countries and *Rhodnius prolixus* in Central America and México), the concept of elimination can also be applicable."

Our concern for human CD to the next future will depend of both political and scientific processes, considering its ecological complexity and that its social weight is still very high. For practical purposes CD must be considered as a neglected disease closely linked with human poverty, with the following three questions and their respective answers (Dias et al. 2002, Salvatella 2007):

- ✓ ***Neglected***: It must be responded to with attention.
- ✓ ***Poverty***: It must be responded to with development policies.
- ✓ ***Diseases***: They must be responded to with health policies, not disease policies.

By such a point of view, the problem of a sustainable control of the disease will basically be dependant of the whole public policies of the endemic countries. It is not a simple situation, considering the complexity of the political context of Latin America. So, the current affirmation concerning CD elimination in some endemic areas must be analyzed according the following possibilities and interrogations:

- ✓ Is it a realistic possibility?
- ✓ Does it mean a demagogic proposition?
- ✓ Does it represent an inconspicuous optimism?
- ✓ Could the best results represent a stimulating effect to not yet controlled Countries?
- ✓ Could the proclamation of the best results emulate the weakness of current control programmes?

In the reality, each one of these questions has some reason to be true, depending on different circumstances. For instance, the epidemiological results observed in the last years in Brazil must be considered very positive in terms of *T. infestans* elimination and blood banks control, with a substantial impact on incidence reduction. In such circumstances, the transmission of human CD has been highly minimized in the country, almost reaching the point of its elimination. The morbidity and the mortality due to the disease are also falling down in the country (Dias 2006, Prata 2001). In terms of Latin America,

from 1990 to 2006 the decreasing of some epidemiological indicators followed the implementation of objective control actions, as shown in a recent WHO workshop (Salvatella 2007):

Table 1: Changes in Some Epidemiological Parameters Following the Transmission Interruption of Chagas Disease, 1999–2006

Epidemiologic Parameters	1990	2000	2006
Annual deaths	>45,000	21,000	12,500
Human cases of infection	30 million	18 million	15 million
Annual incidence	700,000	200,000	41,200
Population under risk	100 million	40 million	28 million
Distribution	21 countries	19 countries	18 countries

Source: TDR/WHO, PAHO, WHO

In spite of the fact that American Trypanosomiasis cannot be eradicated, a dramatic and consistent reduction of human CD transmission has been observed in several endemic areas under regular vector and blood bank control. Even so, an important epidemiologic residual of incidence and morbidity still exists. Since the theoretic and idiomatic dilemma between CD eradication and elimination does not exist, the main problem for scientists and governments remains in the sustainability of its control. Several evidences state that this is an attainable goal to other endemic areas, since the main tools and strategies to accomplish it are available. The current situation of the Regional Latin American Initiatives was recently summarized by Salvatella (2007):

- ✓ Uruguay, Chile and Brazil with interruption of main vector transmission in all the country. Argentina, Paraguay and Bolivia with partial interruption.
- ✓ Guatemala, Honduras y El Salvador with *Rhodnius prolixus* elimination areas.
- ✓ CA with different grades of *Triatoma dimidiata* control.
- ✓ Andean Countries with Chagas Programs in progress.
- ✓ Amazonian Surveillance System in progress.

- ✓ México National Chagas Programme in progress;
- ✓ Non-endemic countries: a new "Initiative" is being proposed, to face the consequences of human migration and the possibilities of transmission by means of congenital, blood transfusion and organ transplantation routes.

In terms of Public Health, an effective control of human CD is undoubtedly possible, but the sustainability of programme actions must be maintained during several years. In order to reinforce the optimistic perspectives, some data registered in Brazil can be taken in consideration (Dias 2006):

- ✓ The number of *T. infestans* positive municipalities decreased from 711 (1980) to 2 or 3 (2006);
- ✓ The proportion of intradomestic triatomine foci decreased from 25% (1980) to less than 2% (2006);
- ✓ The proportion of controlled blood banks increased from 13 % (1980) to 99% (2006);
- ✓ The proportion of chagasic candidates to blood donation is progressively decreasing (4% in 1980 to 0.4% in 2006);
- ✓ The risk of congenital disease has been usually low and tends to decrease because the number of infected fertile women is progressively decreasing.

From another angle, there are different reasons showing that the control task is not finished, involving the consolidation of control activities in terms of programme implementation as much as of continuous surveillance (Dias 2006, Prata 2002). For example, in different Latin American regions, current data show that the work must be extended for several years more, because of different reasons such as:

- ✓ The numerous enzootic cycles existing along the Continent, including those involving sylvatic foci of *T. infestans* in some parts of Bolivia;
- ✓ The existence of at least eight or nine secondary vector species which can colonize human dwellings, chiefly in Brazil and Mexico;
- ✓ The occurrence of ecological difficulties for vector control in several parts of the Continent, such as in Chaco and Amazonian regions;
- ✓ The detection of vector resistance against the current pyrethroid insecticides in some Argentine and Bolivian regions;
- ✓ The occurrence of dwelling invasion and disease transmission by adult vectors, without colonization, in some regions of Panama, Ecuador and Brazil;

- ✓ The existence of at least two million of infected individuals who need medical and social attention and can transmit the disease throughout different alternative epidemiological routes;
- ✓ The detection of more than 100 new acute cases per year, resulting from oral transmission mechanism.

.

Analyzing the whole frame, the central question seems to remain in terms of political will, sustainable control programmes and technical expertise. Additional research is required to face some problems of control improvement and some new epidemiological situations, as well as the unpredictable occurrence of oral transmission (Dias & Schofield 1999, Dias et al 2002, Schofield & Dias 1999). A responsible attitude is required from the side of scientific community. The subject is not finished and much remains to be done. The greatest danger in such good news lies in inconsequential triumphalism and slackening of control measures. In addition, it must be remembered that the decentralization of Health Programmes all around Latin America, during the last twenty years, transformed radically the classic vertical vector control activities in municipal programmes. An additional problem arises from this decentralized model, since generally the municipalities have not tradition in vector control and epidemiological surveillance. In such circumstances, very strong central and sub regional technical teams are required to accomplish the articulation, motivation, qualification and regular supervision of the municipal unities. Another correlated problem emerges from the usual political instability of local (municipal) governments, leaving under risk the sustainability of surveillance activities. In terms of vector control, if epidemiological vigilance were to be prematurely relaxed, the consequences could be severe due the progressive re-establishment of foci of active transmission, not only because of vector population recovery but also as a possible result from housing invasion by secondary and sylvatic species. (Dias et al 2002).

Other biological and contextual situations will be involved in the future of CD and its control. For example, an important and speculative question emerges from the possible introduction of different *T. cruzi* groups in a determined area, as a result of ecological and anthropic changes, may be promoting different patterns of morbidity (Dias 2006, Prata 2001). From the sociological side, a very fundamental obstacle for disease overcoming will remain in the so called "sick house" (*La casa enferma*), so well described by Briceño León (1990): infested rural huts mean an isolated context of poverty; low political profile and very low quality of life. The sick house does not stimulate self reliance, as well as does not bring happiness. The same context can be discussed in the case of rural-urban migration of chagasic people; in which the condition of the slum houses is quite similar (or worse) to their original huts.

At the side of blood transfusion, the programmes in endemic are going on correctly, with very few chances for the occurrence of new cases of transfusion CD. The improvement of blood donors selection is still required in some parts of Bolivia, Costa Rica and Mexico, according WHO (Salvatella 2007). Considering the progressive decreasing of infected blood donors as a medium term consequence of vector control, it is expected that the blood banks control must persist for about twenty years more, in endemic countries. For this period, a series of challenges and constraints must be present in the working agenda of Health Authorities and researchers. The key point will concern with the maintenance of donor control, in terms of cost benefit, besides the desirable improvement of the serological tests, in terms of the maximum sensibility & specificity. A new perspective for universal chemoprophylaxis is also in the expectative of the researches (Dias & Schofield 1998, Moraes-Souza 2000, Pirard et al. 2005, Schmunis et al. 2001).

In terms of congenital transmission, the expected tendency seems to be the progressive exhaustion of infected fertile women in those areas with an effective and continuous vector control. This tendency is clearly observed in Brazil and Uruguay, where the incidence of congenital disease is becoming lower and lower, in parallel with the displacement of the infection to higher age groups of mothers (Dias et al. 2002).

Finally, considering morbidity and mortality, several data are indicating the reduction of the disease medical impact, in the last decades. The reasons for this fact seem to be complex and multi factorial, deserving careful and multicentric analysis. The urbanization of millions of infected individuals can be involved with better medical attention, reduced physical efforts, interruption of exogenous reinfection and other possible causative parameters. Nevertheless, the still existing 12 million of infected individuals do not permit a merely contemplative attitude of health professionals and authorities in the next decades. One of the more urgent tasks in the new millennium just remains in the provision of medical care to chagasic people, mainly for those suffering of chronic cardiopathy (Dias 2007, WHO 2002).

In terms of future situations, the next two decades probably will be characterized by the progressive reduction and focalization of domestic vector population, following predictable changes of sylvatic ecotopes (puzzle and concentration) and the reduction of rural human population. The transfusion transmitted disease probably will be completely controlled (consolidation), but eventual cases of congenital transmission still will occur, in spite of progressively being reduced. Oral transmitted cases seem to continue their appearance in different ecological situations, generally being linked with natural foci of American Trypanosomiasis. A progressive reduction of CD prevalence is expected in endemic

areas, correlated with control activities and with the mortality of already infected individuals. As a consequence, the social and political visibility of the disease must be reduced, as well as the priorities for control and research (Dias 2007, Dias et al. 2002).

As a conclusion, the virtual elimination of human CD can be considered an attainable goal, but we still are under risk. A series of additional questions will be crucial in the next future, such as:

- ✓ Is it possible to maintain the critical mass of research and service? How long?
- ✓ Is it possible the occurrence of a new *malaria-like* situation in this history?
- ✓ How will be maintained the higher references for National Programmes, considering the health decentralization, the political weakness and the priority decreasing?
- ✓ Who will be the main protagonists and what will be the central agenda for the next two decades?
- ✓ What have been the principal success and miscarriage lessons from this history of control, health services and research?
- ✓ What must be our best legacy to the next generations of citizens, researchers and politicians?

References

Briceño-León R 1990. *La Casa Enferma. Sociología de la Enfermedad de Chagas.* Ediciones Capriles, Caracas, 153 p.

Dias JCP 2006. Chagas disease: successes and challenges. Editorial. *Cad. S. Públ.* 22: 2021.

Dias JCP & Schofield CJ 1998. Controle da transmissão transfusional da doença de Chagas na Iniciativa do Cone Sul. *Rev. Soc. Bras.Med. Trop*. 31: 373-383.

Dias JCP & Schofield CJ 1999. The evolution of Chagas Disease (American Trypanosomiasis) control after 90 years since Carlos Chagas discovery. *Mem. Inst. Oswaldo Cruz* 94 (Suppl. I): 103-121.

Dias JCP, Silveira AC & Schofield CJ 2002. The impactof Chagas Disease control in Latin America. A Review. *Mem. Inst. Oswaldo Cruz* 97: 603-612.

Moraes-Souza H 2000. Transmissão transfusional da doença de Chagas. *Rev.Patol. Trop.* 29 (Supl. 1) :91-100.

Pirard M, Lihoshi N, Boelaert M, Basanta P, Lopez F, Van der Stuyft P 2005. The validity of serologic tests for *Trypanosoma cruzi* and the effectiveness of transfusional screening strategies in a hyperendemic region. *Transfusion* 45: 554-561.

Prata AR 2001. Clinical and epidemiological aspects of Chagas disease. *Lancet Infect Dis 7*: 572-100.

Salvatella R 2007. *Achievements in controlling Chagas disease in Latin America*. Conference in Geneva (WHO) July 6, 2007. In Press.

Schmunis GA, Zicker F, Cruz JR, Cuchi P 2001. Safety of blood supply for infectious diseases in Latin American countries. *Am J Trop Med Hyg* 65: 924-930.

Schofield CJ, Dias JCP 1999. The Southern Cone Initiative against Chagas Disease. *Adv. Parasitol.* 42: 2-29.

Senior K 2007. Chagas disease: moving towards global elimination. *Lancet Infect Dis* 7: 572.

José Rodrigues Coura

Laboratório de Doenças Parasitárias, Instituto Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brasil

Determinants and Pathogenesis of Chagas disease

The determinants of Chagas disease result from the quantity of parasites in the initial infection; the infecting forms in the initial inoculum (number of trypomastigotes); the lineage of *T. cruzi* inoculated (I, II, Z3 or hybrid Z1/Z3); reinfection; the quality of the strains and clones (biodema); the specific clonal-histotropic receptors of the host; and the patient's initial and late immune response (Coura 1988, Macedo & Pena 1998, Andrade et al. 2006, Teixeira et al. 2006).

The parasites deposited on skin wounds or mucosa stimulates a local inflammatory reaction (inoculation chagoma and or Romaña's sign) with a lymphoreticular response. The circulating trypomastigotes enveloped by macrophages are taken to the liver, spleen, lymphatic ganglia and skeletal and cardiac muscles to form pseudocysts of amastigotes. With the rupture of the pseudocysts in the myocardium or myenteric plexuses, acute myocarditis mediated by $TCD4^+$ and $TCD8^+$ cells and interleukins (mainly IL2 and IL4) occurs. The inflammatory reaction leads to muscle and neuron cell destruction, which is maintained by the presence of *T. cruzi* or its fragments and by the DNA of the parasite, with a late hypersensitivity reaction, dilatation of the microcirculation and fibrosis, thus inducing dilated chronic myocardiopathy, arrhythmia, dysperistalsis, megaesophagus and megacolon.

Three characteristics of the presentation of Chagas disease need to be discussed here: the acute or initial phase; the chronic phase with its indeterminate, cardiac and digestive forms; and the congenital form, which presents particular characteristics.

Among the determinants of Chagas disease, the following need to be considered: the inoculum of *T. cruzi* in the initial infection and the opportunities for reinfection; the biological characteristics of the infecting strains and clones, and particularly their histotropism; and the host response at cell and humoral level.

The inoculum in the initial infection is expected to be a factor of great importance in the development of Chagas disease. All the indications from experimental studies are that this inoculum is generally small. In one experimental study (Borges-Pereira et al. 1988), a mean range of 51 to 276 *T. cruzi* per defecation was found in a study on eight species of infected triatomines, although sometimes single defecations containing more than 1500 parasites were found. Even so, the natural inoculum is infinitely smaller than what is used experimentally on laboratory animals, which reaches 10,000 parasites or more for one mouse.

The vast majority of cases of the acute or initial form of Chagas disease in Brazil present few or no symptoms, possibly because of the small inoculum. Among 510 chronic cases in several Brazilian states that we followed up over the last 30 years, we found that less than 1% had a history of an acute phase (Coura et al. 1983). A study following up 544 individuals who were exposed to natural infection by *T. cruzi* over a 16-month period, it was found that 14 (2.57%) became infected, of whom only one third presented symptoms compatible with the acute phase of the disease (Teixeira 1997). On the other hand, a study covering almost three decades of following up cases with a known acute phase, in the municipality of Bambuí, found that the most severe chronic cases originated from cases that have had a severe acute form. From this, it could be inferred that the initial inoculum and/or the infecting strain of *T. cruzi* had an influence on the evolution of the disease (Dias 1982).

Reinfection by *T. cruzi* is expected to be rare because of the concomitant immunity induced by the primary infection. Nonetheless, its existence has already been proven both experimentally and in human cases. A wide-ranging study on the "influence of exposure to reinfection on the evolution of Chagas disease" has even described a case of proven reinfection that led to death during the acute phase (Macedo 1976).

The biological characteristics of the strains and clones of *T. cruzi*, and particularly its tissue tropism, certainly have an important function as determinants of Chagas disease and its clinical form. Different strains of *T. cruzi* have been grouped according to their biological characteristics and cell tropism in mice, to create three characteristic groups (Andrade 1976). In another study, it was found that clones from the same strain produced lesions of differing intensities experimentally (Postan et al. 1983). In a study under our guidance, it was seen that there was a lack of correlation between experimental histopathological findings from mice and 17 strains of *T. cruzi* that were isolated from patients with different clinical forms of human Chagas disease (Schemper et al 1983).

Two pathogenic mechanisms are known for infection by *T. cruzi*: the first, described by Gaspar Vianna in 1911, consists of a local inflammatory reaction with necrosis, tissue destruction and scar formation with fibrosis; the second, which is more complex to understand and prove, is the immunological mechanism or mechanisms (Teixeira et al. 1975, Andrade 1999). However, this auto-immunity is insufficient to explain all the pathogenesis of Chagas disease (Tarleton 2003). Sensitization of TCD4+ and TCD8+ lymphocytes by *T. cruzi*, with the development of anti-myocardial cells, associated with migration and activation of macrophages and the release of platelet aggregation factors, thereby respectively inducing chronic Chagas myocarditis and myocardial ischemic lesions, may explain the findings encountered in cases of chronic Chagas cardiopathy (Higushi 1999). On the other hand, the neuron destruction in the heart, esophagus, colon and other hollow viscera may be explained both by direct inflammatory phenomena and by immunological mechanisms that result in the cardiopathy, megaesophagus, megacolon and other enlargement phenomena seen in Chagas disease (Prata 2001, Teixeira et al. 2006).

The lesions of the acute phase of the disease are characterized by the presence of localized inflammatory reactions, with predominance of mononuclear cells at the foci of the pseudocyst ruptures, occasionally with the formation of granulomas located mainly in muscle and cardiac tissue. In certain cases, there may be lymphoreticular hyperplasia in the lymphatic ganglia, liver and spleen and the presence of macrophages invaded by parasitic cells. In other cases, or concomitantly, there may be diffuse acute myocarditis, with interstitial edema, hypertrophy of myocardial fibers and dilatation of cardiac cavities. The destruction of the cardiac neurons and myenteric plexuses, with reductions in the numbers of neurons, begins in the acute phase and continues in the chronic phase of the disease (Koberle 1961, Andrade 2000, Andrade 2005).

The indeterminate chronic phase (asymptomatic) has practically no anatomopathological translation, except for occasional isolated inflammatory foci in the myocardium and a limited reduction in the numbers of cardiac neurons and myenteric plexuses that is insufficient to produce clinical manifestations (Andrade et al. 1997). On the other hand, in the cardiac form of the chronic phase, there may be extensive myocardial fibrosis, destruction of the conduction system and a large reduction in the numbers of cardiac neurons. It is worth pointing out that, in these cases, isolated foci of acute inflammatory reaction are occasionally present, as if this were a reactivation of the process. In chronic Chagas cardiopathy cases, it is common to find hypertrophy of myocardial fibers and dilatation of the cavities, with the presence of thrombi, fibrosis and thinning of the ventricular apices, particularly in the left ventricle. These are sometimes characterized as true apical aneurysms and are almost always internally carpeted with organized blood thrombi. Also in the chronic phase, there is frequently a large

reduction in the number of neurons in the myenteric plexuses, particularly in the esophagus and colon, which leads to dysperistalsis and dilatation of these organs that is characterized by megaesophagus, megacolon and other enlargements of hollow viscera such as the bladder, ureter, gallbladder and other less common examples (Andrade & Andrade 1966, Lopes & Chapadeiro 1997, Dias & Macedo 2005).

The congenital form of Chagas disease seems to occur solely in pregnant women who have a lesion in the placenta that favors penetration by *T. cruzi* as far as the chorionic villi, where amastigote forms multiply (probably in Hofbauer cells) and subsequently invade the fetal circulation (Bittencourt AL 1963, Carlier & Torrico 2005).

Clinical Phases and Forms: Morbidity and Mortality

Chagas disease presents an initial or acute phase with evident parasitemia seen in direct examination of the blood. In most cases there are no symptoms, but in symptomatic cases there are entry point signs (inoculation chagoma or Romaña's sign, with fever, generalized adenopathy, edema, hepatosplenomegaly, myocarditis and meningoencephalitis in severe cases. This is followed by a chronic phase that in most cases presents as an indeterminate form (asymptomatic, with normal results from electrocardiogram and x-ray of the heart, esophagus and colon), which may evolve to the cardiac or digestive form (megaesophagus and megacolon), or cardiac and digestive forms together. The so-called congenital form mentioned earlier may also occur, by means of transmission across the placenta or through the birth canal during delivery, and this may give rise to abortion, prematurity or organic lesions in the fetus (Bittencourt 1963, Carlier & Torrico 2005). In cases with immunosuppression, the chronic infection may become acute again, thereby producing diffuse myocarditis, lesions of the central nervous system and severe meningoencephalitis.

The clinical phases and forms of Chagas disease can be summarized as follows (Coura et al. 2007):

- ✓ **Acute Phase**
  - Asymptomatic form
  - Moderate form
  - Severe form
- ✓ **Chronic Phase**
  - Indeterminate form
  - Cardiac form

- Digestive form
- Mixed form (cardiac and digestive)
- Neuroautonomic form

✓ **Congenital Form**

- Abortion
- Prematurity
- Organic lesions in the fetus

✓ **Forms in Immunosuppressed Individuals**

- Evident parasitemia
- Lesions of the central nervous system
- Diffuse myocarditis and meningoencephalitis

In the chronic phase, Chagas infection may present as an indeterminate form, in which approximately 40% of the infected individuals remain totally asymptomatic, with anatomically and physiologically normal x-ray results for the heart, esophagus and colon and no changes seen on electrocardiograms. Although they are asymptomatic, these patients present positive serological reactions for *T. cruzi* infection and, for many of these patients, the xenodiagnosis and PCR results may be repeatedly positive for many years. Thus, they display a veritable equilibrium between the parasite and host. There is great regional variation in the morbidity of Chagas disease. The indeterminate form can vary from 40 to 90% of the cases (Coura et al. 1999).

The chronic cardiac form is the most expressive manifestation of Chagas disease, both because of its frequency and because of its severity. It generally appears between the second and fourth decades of life, five to fifteen years after the initial infection. The signs and symptoms of chronic Chagas cardiopathy result from arrhythmia, cardiac insufficiency, auricular-ventricular and branch blockages and thromboembolism. In our first series of 100 cases studied (Coura 1965), we found the following manifestations: effort dyspnea 65%, palpitations 54%, extrasystole 51%, apical systolic murmur 47%, vertigo and/or fainting 37%, lower-limb edema 35%, precordial pain 37%, double second heart sound at the pulmonary focus 24%, hepatomegaly 19%, double first heart sound at the mitral focus 18%, bradycardia 17%, tachycardia 15%, hypophonesis of the heart sounds 10%, gallop rhythm 6%, anasarca 6% and convulsions 5%.

In the chronic digestive form of the disease, the clinical manifestations result from dysperistalsis of the esophagus and colon caused by destruction of the myenteric plexuses, which consequently leads to megaesophagus and megacolon. Although isolated cases of autonomic disorders of the esophagus have

been described in the acute phase of the disease, these occur mostly in the chronic phase, when the dysperistalsis and heart spasms are accompanied by enlargement of the esophagus.

The prognosis for Chagas disease depends on the clinical form and the complications during its evolution. In the acute phase, it depends on the patient's age and the severity and location of the lesions. In general, the acute phase is very severe among children less than two years old, and it is almost always fatal in those with myocarditis, cardiac insufficiency and meningoencephalitis. The prognosis may also be very poor in the congenital form, which may lead not only to abortion and prematurity but also to organic lesions in the liver, spleen, heart and central nervous system, with neurological sequelae and mental deficiency. Many cases may be asymptomatic and remain in the indeterminate form.

In the chronic cardiac form, the prognosis varies considerably from one case to another. Patients with minimal lesions such as blockage of the right branch alone or unifocal ventricular or auricular extrasystole tend to remain stable and most of them survive for long periods and often end up dying for other reasons. Patients with complex arrhythmia, multifocal extrasystole, paroxystic tachycardia, auricular fibrillation, third-degree A-V blockage or cardiac insufficiency have a very poor prognosis. A third group of patients with slightly increased heart area and changeable electrocardiographic findings and clinical manifestations have an uncertain prognosis (Nogueira & Coura 1990).

Studies carried out by our group (Abreu 1977, Coura et al. 1985, Borges Pereira et al. 1985) in field areas in Minas Gerais have shown that the mortality due to Chagas cardiopathy increases progressively from 30 to 59 years of age. In these studies, we showed that the death rate due to Chagas cardiopathy was 8.9% among patients followed up for six years and 17% in another group followed up for ten years. These same authors found that sudden death occurred in two thirds of the cases, while the other third died because of cardiac insufficiency. The prognosis for the digestive and indeterminate forms is generally good, except in cases of the digestive form with complications (esophageal cancer, obstruction with twisting and colon necrosis). None of the cases of the digestive or indeterminate form that we observed over six and ten-year periods progressed to death (Coura & Borges Pereira, 1984).

Chagas Disease in the Amazon Region

Several acute cases of human Chagas disease have been reported from the Amazon region most of by *T. curzi I, Z3* and hybrid ZI/Z3. In the localities where this disease has been reported, the chronic form of this disease is considered to present low endemicity. The first acute cases in the Amazon region were reported by Floch & Tasque (1941) and Floch & Camain (1948) from French Guyana. Shaw et al

(1969) described another four acute cases in Belém, the capital of the State of Pará, in northern Brazil. Since then, more than four hundred acute cases have been reported, most of them from outbreaks probably by oral transmission in the States of Pará, Amapá and Amazonas, Brazil (Valente &Valente 1993, Valente et al. 1999, Pinto et al 2004, Coura 2006). Serological surveys and cross-sectional studies carried out by Fundação Nacional de Saúde from 1975 to 1980 in different states in the Brazilian Amazon region and by Coura et al from 1971 to 2002 in the State of Amazonas showed prevalences ranging from 2.4% to 13.2% (Camargo et al 1984; Coura et al 1999, 2002). Sporadic chronic cases of Chagas disease have also been reported from the Brazilian Amazon region (Albajar et al. 2003, Xhavier et al. 2006).

References

Abreu LL 1977. *Doença de Chagas: Estudo da morbidade no município de Pains, Minas Gerais* (Masters Dissertation). Universidade Federal do Rio de Janeiro.

Albajar P, Laredo SV, Terrazas MB, Coura JR (2003) Miocardiopatia dilatada em pacientes com infecção chagásica crônica. Relato de dois casos fatais autóctones do Rio Negro, Estado do Amazonas. *Rev Soc Bras Med Trop* 36: 401-407.

Andrade SG 1976. Tentative for grouping different *Trypanosoma cruzi* strains in some types. *Rev Inst Med Trop S Paulo* 18: 140-141.

Andrade SG 2005. Biodemas, Zimodemas e Esquisodemas: sua relação com a patologia da doença de Chagas. In: Coura JR (Editor). *Dinâmica das Doenças Infecciosas e Parasitárias*, Guanabara-Koogan, Rio de Janeiro, p. 621-637.

Andrade SG, Andrade Z 1966. Chagas disease and neuronal alterations at the Auerbach' plexus. *Rev Inst Med Trop São Paulo* 8: 219-224.

Andrade SG, Campos RF, Sobral SC, Magalhães JB, Guedes RSP, Guerreiro ML 2006. Reinfections with strains of *Trypanosoma cruzi*, of different biodemes as a factor of aggravation of myocarditis and myosites in mice. *Rev Soc Bras Med Trop* 39: 1-8.

Andrade Z 1999. Immunopathology of Chags disease. *Mem Inst Oswaldo Cruz* 94 (Suppl I): 71-80.

Andrade Z 2000. Patologia da doença de Chagas. In: Brener Z, Andrade Z, Barral-Netto M (Editores). Trypanosoma cruzi *e Doença de Chagas*. 2ª Ed. Guanabara-Koogan; Rio de Janeiro, p. 201-230.

Andrade Z, Andrade SG, Sadigurski M, Wenthold RJ Jr, Hilbert SL, Fernans VJ 1997. The indeterminate phase of Chagas disease: ultrastructural cardiac changes in the canine model. *Am J Trop Med Hyg* 57: 228-236.

Bittencourt AL 1963. Placenta chagásica e transmissão da doença de Chagas. *Rev. Inst. Med. Trop. S. Paulo*, 5: 62-67.

Borges-Pereira J, Willcox HP, Coura JR 1985. Morbidade da doença de Chagas. III. Estudo longitudinal de seis anos, em Virgem da Lapa, MG, Brasil. *Mem Inst Oswaldo Cruz* 80: 63-71.

Borges-Pereira J, Pessoa I, Coura JR 1988. Observações sobre as dejeções e o número de *T. cruzi* eliminados por diferentes espécies de triatomíneos durante a alimentação. *Mem Inst Oswaldo Cruz* 83 (Suppl I): 7.

Camargo ME, Silva GR, Castilho EA, Silveira AC 1984. Inquérito sorológico da prevalência da infecção chagásica no Brasil, 1975-1980. *Rev Inst Med Trop São Paulo* 26: 192-204.

Carlier V, Torrico F 2005. Colóquio Internacional Infección Congênita por *Trypanosoma cruzi*: desde los mecanismos de transmisión hasta una estratégia de diagnóstico y control. *Rev Soc Bras Med Trop* 38 (Supl II): 125-128.

Coura JR 1965. *Estudo da doença de Chagas no Estado da Guanabara* (Free Docent Thesis), Universidade Federal do Rio de Janeiro.

Coura JR 1988. Determinantes epidemiológicos da doença de Chagas no Brasil: a infecção, a doença e sua morbi-mortalidade. *Mem Inst Oswaldo Cruz* 83: 392-402.

Coura JR 2006. Transmissão da infecção chagásica por via oral na história natural da doença de Chagas. *Rev Soc Bras Med Trop* 39 (Supl. IV): 113-117.

Coura JR, Abreu LL, Borges-Pereira J, Willcox HP 1985. Morbidade da doença de Chagas. IV. Estudo longitudinal de dez anos em Pains e Iguatama, Minas Gerais, Brasil. *Mem Inst Oswaldo Cruz* 80: 73-80.

Coura JR, Anunziato N, Willcox HPF 1983. Morbidade da doença de Chagas. I – Estudo de casos procedentes de vários estados do Brasil, observados no Rio de Janeiro. *Mem Inst Oswaldo Cruz* 78: 362-372.

Coura JR, Borges-Pereira J 1984. A follow-up evaluation of Chagas' disease in two endemic areas in Brazil. *Mem Inst Oswaldo Cruz* 79 (Suppl): 107-112.

Coura JR, Borges-Pereira J, Araujo RM 1999. Morbidity and regional variation of Chagas disease in Brazil. *Mem Inst Oswaldo Cruz*, 94 (Suppl II): 26-27.

Coura JR, Junqueira ACV, Boia MN, Fernandes O 1999. Chagas disease: from bush to huts and houses. Is it the case of the Brazilian Amazon? *Mem Inst Oswaldo Cruz* 94 (Suppl. 1): 379-384.

Coura JR, Junqueira ACV, Fernandes O, Valente SAS, Miles MA 2002. Emerging Chagas disease in Amazonian Brazil. *Trends Parasitol* 18: 171-176.

Coura JR, Junqueira ACV, Carvalho-Moreira CJ, Borges-Pereira J, Albajar PV 2007. Uma visão sistêmica da endemia chagásica. In Silveira AC (Ed), *La enfermedad de Chagas a la puerta de los 100 años del conocimiento de una endemia americana ancestral*. Organización Panamericana de la Salud y Fundación Mundo Sano, Buenos Aires, Argentina, pp. 25-35.

Dias JCP 1982. Doença de Chagas em Bambuí, Minas Gerais, Brasil. *Estudo clínico-epidemiológico a partir da fase aguda, entre 1940 e 1982* (PhD Thesis), Universidade Federal de Minas Gerais.

Dias JCP, Macedo VO 2005. Doença de Chagas. In: Coura JR (Editor). *Dinâmica das Doenças Infecciosas e Parasitárias*. Editora Guanabara Koogan, Rio de Janeiro, pp. 557-593.

Floch H, Tasque P 1941. Un cas de maladie de Chagas en Guyane Française. *Bull Soc Path Exot* 36-37.

Floch H, Camaim R 1948. Deux nouveaux cas de maladie de Chagas en Guyane Française. *Bull Soc Path Exot* 47: 22-25

Higushi ML 1999. Human chronic chagasic cardiopathy: participation of parasites antigens, subsets of lymphocytes, cytokines and microvascular abnormalities. *Mem Inst Oswaldo Cruz* 94 (Suppl I): 263-267.

Koberle F 1961. Patología y anatoma patológica de la enfermedad de Chagas. *Bol Ofi Sanit Panamer* 51: 404-428.

Lopes ER, Chapadeiro E 1997. Anatomia Patológica da Doença de Chagas. In: Dias JCP, Coura JR. *Clínica e Terapêutica da Doença de Chagas.* Editora Fiocruz, Rio de Janeiro, p. 67-84.

Macedo VO 1976. Influência da exposição à reinfecção na evolução da doença de Chagas (Estudo evolutivo de cinco anos) *Rev Pat Trop*, 5: 33-116.

Macedo AM, Pena SDJ 1998. Genetic variability of *Trypanosoma cruzi*: implications for the pathogenesis of Chagas disease. *Parasitol Today* 14: 119-124.

Nogueira N, Coura JR 1990. American Trypanosomiasis (Chagas' Disease). In: Warren KS, Mahmoud AAF. (Editors). *Tropical and Geographical Medicine* (2nd ed). Ed Mc Graw-Hill, New York. pp. 281-296.

Pinto AY, Valente SA, Valente VC 2004. Emerging acute Chagas disease in Amazonian Brazil: case reports with serious cardiac involvement. *Braz J Infect Dis* 8: 454-460.

Postan M, Mc Daniel JR, Dvorak JA 1983. Studies on *Trypanosoma cruzi* clones in imbred mice I. A comparison of infection of C3H mice with two clones isolated from a common source. *Am J Trop Med Hyg* 32: 497-506.

Prata A 2001. Clinical and epidemiological aspects of Chagas disease. *Lancet Infect Dis* 1: 92-100.

Shaw J, Lainson R, Fraiha H 1969. Considerações sobre a epidemiologia dos primeiros casos autóctones de doença de Chagas registrado em Belém, Pará, Brasil. *Rev. Saúde Publ.* (S. Paulo), 3: 153-157.

Schlemper Jr BR, Avila CM, Coura JR, Brener Z 1983. Course of infection and histopatological lesions in mice infected with seventeen *Trypanosoma cruzi* strains isolated from chronic patients. *Rev Soc Bras Med Trop* 16: 23-30.

Tarleton RL 2003. Chagas disease: a role for autoimmunity? *Trends Parasitol*, 19: 447-451.

Teixeira ARL, Nascimento RJ, Sturm NR 2006. Evolution and pathology in Chagas disease—A Review. *Mem Inst Oswaldo Cruz* 101: 463-491.

Teixeira ARL, Teixeira ML, Santos-Buch CA 1975. The immunology of experimental Chagas' disease. IV. Production of lesions in rabbits similar to those of chronic Chagas' disease in man. *Am J Pathol* 80: 163-180.

Teixeira MGLC 1997. *Doença de Chagas. Estudo da forma aguda inaparente* (Masters Thesis), Universidade Federal do Rio de Janeiro.

Teixeira MMG, da Silva FM, Marcili A, Umezawa E, Shikanai-Yasuda MA, Cunha-Neto 2006. *Trypanosoma cruzi* lineage I in endomyocardial biopsy from north-eastern Brazilian patient at end-stage chagasic cardiomiopathy. *Trop Med and Intern Health* 2: 294-8.

Valente SAS, Valente VC 1993. Situação da doença de Chagas na Amazônia. *Rev. Soc. Bras. Med. Trop.* (Supl. 2): 68-70.

Valente SAS, Valente VC, Fraiha Neto H 1999. Considerations on the epidemiology of Chagas disease in the Brazilian Amazon. *Mem Inst Oswaldo Cruz* 94 (Suppl. 1): 395-398.

Vianna G 1911. Contribuição para o estudo da anatomia patológica da "Moléstia de Carlos Chagas". *Mem Inst Oswaldo Cruz*, 3: 276-193.

Xavier SS, Sousa AS, Albajar VP, Junqueira ACV, Bóia MN, Coura JR 2006. Cardiopatia chagásica crônica no Rio Negro, Estado do Amazonas. Relato de três novos casos autóctones, comprovados por exames sorológicos, clínicos, radiográficos do tórax, eletro e ecocardiográficos. *Rev Soc Bras Med Trop* 39: 211-216.

Recent Advances in Chemotherapy of American Trypanosomiasis

Roberto Docampo
Center for Tropical and Emerging Global Disease and Department of Cellular Biology
350A Paul D. Coverdell Center, University of Georgia, Athens, GA 30602
Tel.: 706-542-8104; Fax: 706-583-0181, rdocampo@uga.edu

Abstract

Chagas' disease remains an important health problem in the Americas. Advances are being made in parts of South America in blocking transmission from insect vectors or blood transfusion, but more effective chemotherapy is needed for the millions who are already infected. This is especially important since recent results have indicated that treatment is beneficial for the elimination of the chronic course of the disease. The rational development of new drugs depends on the identification of differences between human metabolism and that of the causative parasite, *Trypanosoma cruzi*. Recent developments in the study of the basic biochemistry of the parasite have allowed the identification of novel targets for chemotherapy, such as sterol metabolism, protein prenylation, proteases, and phospholipid metabolism, and these are the subject of this presentation.

The Need for Chemotherapy of Chagas' Disease

In the Americas, from Mexico in the North to Argentina and Chile in the South, there are 16 to 18 million people infected with *Trypanosoma cruzi*, the causative agent of Chagas' disease [1]. Estimated yearly incidence amounts to 561,000 cases [2] not including countries like Argentina, Brazil, Chile and Uruguay where active programs of vector elimination have been in progress for years [3]. Vector elimination in other countries of Latin America has been very difficult because of the sylvatic life cycle of the parasite in several regions. It was estimated that 2 to 3 million individuals have the clinical symptoms that characterize the chronic stage of Chagas' disease, and that 45,000 of them die each year [4]. Even if all the vectors were eliminated the high number of patients already infected makes the search for effective chemotherapy extremely important [5].

Two drugs, nifurtimox and benznidazole, are capable of curing at least 50% of recent infections as demonstrated by the disappearance of symptoms and the negativization of parasitemia and serology [6-9]. However, results of treatment trials for acute infections were not uniform in the different countries [7-8],

probably because of the different drug sensitivity of different *T. cruzi* strains. In addition, both drugs produced side effects that disappeared when treatment was discontinued [7-10]. Side effects were much more common in adults than in children. Treatment duration was another drawback as nifurtimox was given for 30, 60, 90, or 120 days [7-10] and benznidazole for at least 30 days [10]. The usefulness of these drugs for parasitological cure in the indeterminate or chronic stage of the infections has also been questioned; after treatment, serology in most cases remained positive even when parasitemia was absent [7, 10, 11]. In addition, as chronic lesions thought to originate in an immunological phenomenon, it was considered doubtful that elimination of parasites would affect the course of cardiac and/or hollow viscera lesions.

Some reports, however, suggest that anti-parasite treatment of chronic Chagasic patients with benznidazole results in fewer electrocardiographic changes and a lower frequency of deterioration in their clinical condition [12]. Lack of progress in the myocardiopathy correlated well with negativization of serology [12]. Moreover, even when asymptomatic, some children aged 12 years or less, could be parasitologically cured when treated with benznidazole [13]. Side effects were mild and fewer children in the benznidazole-treated group showed myocardiopathy [13]. These findings match well with the fact that benznidazole-treatment of *T. cruzi*-infected mice induces a late regression of lesions in the myocardium and skeletal muscle [14], and that parasitization of heart tissue is both necessary and sufficient for the induction of tissue damage in *T. cruzi* infection [15, 16]. The above findings stress the need for chemotherapeutic agents that are effective against all strains of *T. cruzi*, and with fewer or no side effects than those currently available [17, 18].

Possible Approaches to the Chemotherapy of Chagas' Disease

The costs of drug discovery and development in the pharmaceutical industry have escalated during the past 25 years. As a consequence, pharmaceutical companies have focused their research and development efforts on areas where adequate returns are commensurate with such costs. The result has been the complete withdrawal by the major companies from activities directed towards the discovery of drugs aimed at tropical diseases [19].

The formation of a partnership between the public sector (international and national agencies, non-governmental organizations including charities) and the private sector (especially the pharmaceutical industry) proposed by Gutteridge [19] and recently put forward in the case of malaria (Medicines for Malaria Venture) is one possible approach to develop new drugs against Chagas' disease. The feasibililty of this approach is greatly increased when the following two factors are taken into account:

(1) The close relationship of *T. cruzi* with other parasites (*T. brucei* group, *Leishmania* spp.) that produce diseases of human and veterinary importance, and their similar susceptibility to a number of drugs already available.

(2) The knowledge already gathered concerning possible novel targets in trypanosomatids that have counterparts in other pathogens or in cancer cells and are thereby of potential interest to pharmaceutical companies.

Potential Targets for Further Development of Chemoterapeutic Drugs against Chagas' Disease

A few recent examples could illustrate the potential of this approach.

Sterol Metabolism

Azole-containing compounds have provided a major breakthrough in antifungal therapy in both human and veterinary medicine, and have shown excellent activity against various fungi that are pathogenic to plants [20]. Although several hypotheses on the mode of action have been proposed for both the imidazole and triazole antifungal agents, all of them interfere with sterol biosynthesis and belong with some other nitrogen heterocycle-containing antifungals to the class of ergosterol biosynthesis inhibitors [21]. Since *T. cruzi* contains ergosterol [22, 23], it was not unexpected that, when two of these inhibitors, miconazole and econazole, were initially tested against this parasite, they showed a potent growth inhibitory action parallel to a decrease in its 5,7-diene sterol content [24]. Later studies showed that ketoconazole, and other potent antimycotic azoles were also active in protecting mice against lethal infections with *T. cruzi* [25, 26], in inhibiting intracellular multiplication of the parasites [27-29], and in blocking their biosynthesis of fungal-type sterols [27, 28, 30]. More recent work has been related to the modifications caused by these inhibitors on the plasma membrane of the parasites [31] and to the synergistic effect of combinations of these inhibitors in experimental *T. cruzi* infections [32-35].

It is generally accepted that imidazoles such as ketoconazole and itraconazole are inhibitors of the cytochrome P-450-dependent lanosterol 14α-demethylase, resulting in the accumulation of 14α-methylsterols and decreased availability of ergosterol [20]. Although this enzyme is also present in mammalian cells, it is much less sensitive to the drugs than that present in fungi and trypanosomatids. Although these compounds strongly suppress *T. cruzi* proliferation they are unable to induce radical parasitological cure in most cases [36-38]. On the other hand, ergosterol differs from cholesterol, the predominant mammalian sterol by the presence of a 24-methyl group and Δ^7 and Δ^{22} double bonds. The 3 enzymatic reactions which introduce the extra methyl group and the Δ^{22} double bond of ergosterol have no

counterpart in mammalian sterol biosynthesis, and may be regarded as targets for new antiparasitic agents [39]. In agreement with this hypothesis it has been shown that $\Delta^{24(25)}$sterol methyl transferase inhibitors have a potent antiproliferative effect on *Crithidia fasciculata in vitro* [37] and on *T. cruzi in vitro* and *in vivo* [40].

Several years ago, Ryley and collaborators described the activity of ICI 195,739, an orally active racemic bistriazole against *T. cruzi* infection in mice [41]. On the basis of biochemical and ultrastructural studies Urbina et al. [42, 43] concluded that ICI 195,739 has a dual mechanism of action against *T. cruzi* involving inhibition of the sterol biosynthesis pathway at the level of the cytochrome P-450-dependent C14α-demethylase and a blockade of the cell cycle at cytokinesis. Urbina and coworkers [44] presented evidence that D0870, the *R(+)* enantiomer of ICI 195,739, was able to cure short- and long-term experimental *T. cruzi* infections in mice.

Another promising triazole derivative (SCH 56592 or posaconazole) has also been shown to have potent *in vitro* and *in vivo* trypanocidal activity, even with *T. cruzi* strains naturally resistant to nitrofurans, nitroimidazoles, and conventional antifungal azoles [45, 46] and clinical trials are in progress. It has been reported that either interferon-γ or IL-12 deficiency reduces the efficacy of posaconazole or benznidazole in a mouse model of *T. cruzi* infection and that the anti-*T. cruzi* activity of posaconazole is much less dependent on the activity of interferon-γ than that of beznidazole is [47] is. Other triazole derivatives, such as UR-9825 [48], ravuconazole [49], TAK-187 [50, 51] and albaconazole [52] have also been shown to be extremely effective against *T. cruzi in vitro* and/or *in vivo*.

Combination therapy has been tried recently against experimental models of *T. cruzi* infection. For example, combination of posaconazole with amiodarone [53], and lysophospholipid analogs with ketoconazole [54] are highly effective.

Another potential target in the sterol biosyntesis pathway, is the enzyme squalene synthase. Squalene synthase catalyzes the first committed step in sterol biosynthesis and is currently under intense study as a possible target for cholesterol-lowering agents in humans. Inhibitors of this enzyme, such as quinuclidine derivatives [55-57] and aryloxyethyl thiocyanates [58, 59] have been shown to have anti-*T. cruzi* activity *in vitro* and/or *in vivo*.

Protein Prenylation

The occurrence of protein prenylation in *T. cruzi* and *T. brucei* has been demonstrated [60, 61]. Protein prenylation in mammals and yeast involves the attachment of 15-carbon farnesyl or 20-carbon geranylgeranyl groups to the C-terminal cysteine residues of a subset of cellular proteins. Many of these prenylated proteins are small GTPases, including Ras, Rac, Rab, and Rho that have roles in cellular signal transduction and intracellular vesicle trafficking [62, 63]. The known functions of prenyl groups attached to cellular proteins is to anchor proteins to membranes and to serve as molecular handles for mediating protein-protein interactions [63]. Three enzymes have been identified in eukaryotic cells including those from mammals and plants and in yeast that attach prenyl groups to proteins: protein farnesyl transferase (PFT); protein geranylgeranyl transferase I (PGGT-I) and protein geranylgeranyl transferase II (PGGT-II) [64, 65]. There is particular interest in PFT because selective inhibition of this enzyme suppresses transformation induced by oncogenic forms of Ras that are farnesylated and arrests the growth of human tumors in rodents [66-68]. Over the past several years, hundreds of potent PFT inhibitors have been synthesized with the primary goal of developing anti-cancer drugs [69]. Some of these compounds have been shown to inhibit the *T. brucei* recombinant PFT [69] and to inhibit the growth of *T. brucei* and *T. cruzi* [60] and are potential chemotherapeutic agents. Tipifarnib (R115777), an inhibitor of human protein farnesyltransferase (PFT), was shown recently to be a highly potent inhibitor of *T.cruzi* growth (ED_{50} = 4 nM). Surprisingly, this was due to the inhibition of cytochrome P450 sterol 14-αdemethylase. Homology models of the *T. cruzi* CYP51 were used for the prediction of the binding modes of the substrate lanosterol and of Tipifarnib, providing a basis for the design of derivatives with selectivity for TcCYP51 over human PFT [70].

An essential preliminary step for protein prenylation is the reaction catalyzed by the farnesyl diphosphate synthase. This enzyme has been demonstrated to be the target of bisphosphonates [71-77]. Bisphosphonates are pyrophosphate analogs in which the oxygen bridge has been replaced by carbon and diverse carbon side chains have generated a large family of compounds. Several bisphosphonates are potent inhibitors of bone resorption and are in clinical use for the treatment and prevention of osteoporosis, Paget's disease, hypercalcemia caused by malignancy, tumor metastases in bone, and other diseases [78]. Selective action on bone is based on the binding of the bisphosphonate moiety to the bone mineral. Millions of people have been treated to date with bisphosphonates and since they are already FDA-approved they constitute an attractive group to develop as chemotherapeutic agents against Chagas' disease.

Bisphosphonates inhibit the prenylation of small GTP-binding proteins that control cytoskeletal reorganization, vesicular fusion, and apoptosis, processes involved in osteoclast activation and survival [71, 72]. Nitrogen-containing bisphosphonates have been postulated to act as carbocation transition state analogs for isoprenoid biosynthesis [73]. Recent studies have indicated that they are potent inhibitors of protein prenylation because they inhibit farnesyl diphosphate synthase in a very effective manner [74-79] and have been shown to be active *in vitro* and *in vivo* against *T. cruzi* [80-87]. Interestingly some bisphosphonates also inhibit the glycolytic enzyme hexokinase from *T. cruzi* [88-89].

Proteases

The success of aspartyl protease inhibitors in the chemotherapy of HIV infection has stimulated interest in this type of drug [90, 91]. Protease inhibitors of angiotensin converting enzyme (ACE) are is use for treatment of hypertension, metalloprotease inhibitors have reached Phase II trials for the treatment of human cancers [91], and serine protease inhibitors show promise in treatment of certain pulmonary and allergic diseases [91]. Several trials of inhibitors of cysteine proteases in animal models of parasitic infections have now provided evidence that this fourth class of proteases could be amenable to targeting and drug development [91]. Cruzipain, the major cysteine protease of *T. cruzi* has been the target of extensive structure-based drug design [92]. Successful treatments of animal models of Chagas' disease with inhibitors designed to inactivate cruzipain [93-95] have provided an important 'proof of concept" for the use of these inhibitors *in vivo*, and at least one of these inhibitors is currently under development as a therapeutic agent against Chagas' disease [92].

Phospholipid Metabolism

The recent success of miltefosine (hexadecylphosphocholine) as a new oral agent for the treatment of visceral leishmaniasis [96] and the demonstration of a suppressive effect of this compound on *T. cruzi* infections [97, 98] as well as in proliferations and differentiation of *T. cruzi* [99] have stimulated interest in this class of compounds previously shown to have antiviral and anticancer activities [100]. Miltefosine is used as a topical agent and has been tested by the oral route in phase I and II clinical trials, which have provided valuable information on human tolerance for the drug [101], The mode of action of this drug is unknown although it presumably interferes with phosphatidylcholine biosynthesis [102].

Conclusions

What conclusions can be drawn from these studies? Perhaps the most important is that sterol biosynthesis inhibitors, protein prenylation pathway inhibitors, protease inhibitors and phospholipid analogs are important potential chemotherapeutic agents against Chagas' disease. We should expect that

the use of some of these compounds could result in an adequate treatment for the acute and chronic forms of Chagas' disease. The advantage of these compounds is that many of them are under development for other uses by pharmaceutical companies and some are already FDA-approved.

However, even if these drugs are able to eliminate most of the parasites present in the patients chronically infected, will this eliminate the course of the chronic disease? Or will it be beneficial to administer a drug to infected people without symptoms of any disease? These and other questions reveal the complexities involved in the treatment of Chagas' disease. However, the rediscovery of the importance of specific anti-*T. cruzi* treatment in chronic Chagasic patients [12], suggests that the answer to the first question is yes, treatment is beneficial. Findings in children without a symptomatic acute infection who may be parasitologically cured after treatment may be an indication to the public health authorities of those countries, that treatment of infected children even if asymptomatic is indicated. This would be especially true in those countries that are going forward in their attempt to eliminate the vectors [3]. There, there is no possibility of reinfection.

Even if the situation exists that cardiopathy or megas are not prevented in all those treated, eradication of parasitemia will prevent transmission by blood transfusion and, in female children, congenital transmission years later. It is obvious that for treatment of infected children, most of them from the rural areas, a drug that combines proven efficacy with a short period of administration would be extremely advantageous.

Acknowledgments

Work in our laboratory was supported by the U.S. National Institutes of Health (AI068647).

References

1. Anonymous (1991) *Control of Chagas' disease. WHO Tech Rep.* Ser No 811.
2. Hayes, R.J., and Schofield, C. (1990) Estimación de las tasas de incidencia de infecciones y parasitosis crónicas a partir de la prevalencia: la enfermedad de Chagas en America Latina. *Bol. Of. Sanit. Panam.* 108, 308-316.
3. Schmuñis, G.A., Zicker F, and Moncayo A. (1996) Interruption of Chagas' disease transmission through vector elimination. *Lancet* 348 1171.
4. Moncayo, A. (1993) in *Eleventh Programme Report of the UNDP.World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR)*, pp. 67-75, World Health Organization.
5. Docampo, R. (2001) Recent developments in the chemotherapy of Chagas disease. *Curr. Pharm. Des*. 7 1157-1164.
6. Cerisola, J.A. (1969) Evolución serológica de pacientes con enfermedad de Chagas aguda tratados con Bay 2502. *Bol. Chil. Parasitol.* 24, 54-59.
7. Cançado, J.R., and Brener, Z. (1979) in *Trypanosoma cruzi e Doença de Chagas* (Brener, Z., and Andrade, Z., eds), pp. 362-424, Guanabara Koogan.
8. Brener, Z. (1979) Present status of the chemotherapy and chemoprophylaxis of human trypanosomiasis in the Western hemisphere. *Pharmacol. Ther.* 7, 71-90.
9. Schmuñis, G.A., Szarfman A., Coarasa L., Guilleron C., Peralta, J.M. (1980) Anti-*Trypanosoma cruzi* agglutinins in acute human Chagas' disease. *Am. Soc. Trop. Med. Hyg.* 29, 170-178.
10. Barclay, C.A., Cerisola, J.A., Lugones, H., Ledesma, O., Lopez Silva, J., and Mouso, G. (1978) Aspectos farmacológicos y resultados terapéuticos del benznidazol en el tratamiento de la infección Chagásica. *Prens. Med. Arg.* 65 239-244.
11. Ferreira, A.O. (1990) Tratamento da forma indeterminada da doença de Chagas com nifurtimox e benznidazol. *Rev. Soc. Bras. Med. Trop.* 23 209-211.
12. Viotti, R., Vigliano, C., Armenti, H., and Segura, E.L. (1994) Treatment of chronic Chagas' disease with benznidazole: clinical and serologic evolution of patients with long-term follow up. *Am. Heart. J.* 127 151-162.
13. Andrade, A.L. ., Zicker, F., Ameidab e Silva, S., Luquetti, A., Travassos, L.R., Almeida, I.C., Andrade, S.S., Andrade, J.C., and Martelli, C.M. (1996) Randomized trial of efficacy of benznidazole in treatment of early *Trypanosoma cruzi* infection. *Lancet*, 348 1407-1413.
14. Andrade, S.G., Freitas, L.A.R., Peyrol, S., Pimentel, A.R., and Sadigursky, M. (1991) Experimental chemotherapy of *Trypanosoma cruzi* infection: persistence of parasite antigen and positive serology in parasitologically cured mice. *Bull. Wld. Hlth Org.* 69: 191-197.
15. Tarleton, R.L., Zhang, L., and Downs, M.O. (1997) "Autoimmune rejection" of neonatal heart transplants in experimental Chagas disease is a parasite-specific response to infected host tissue. *Proc. Natl. Acad. Sci. USA* 94: 3932-3937.
16. Tarleton, R.L., and Zhang, L. (1999) Chagas disease etiology: autoimmunity or parasite persistence. *Parasitol. Today* 15 94-99.
17. Docampo, R., and Moreno, S.N.J. (1985) Biochemical toxicology of antiparasitic drugs used in the chemotherapy and chemoprophylaxis of American trypanosomiasis (Chagas' disease). *Rev. Biochem. Toxicol.* 7 159-204.
18. Urbina, J.A., and Docampo, R. (2003) Specific chemotherapy of Chagas disease: controversies and advances. *Trends Parasitol*. 19, 495-501.
19. Gutteridge, W.E. (1997) Designer drugs: pipe-dreams or realities? *Parasitology* 114 S145-S151.
20. Van den Bossche, H., Willemsens, G., Marichal, P., Coold, W., and Lauwers, W. (1984) The molecular basis for the antifungal activities of *N*-substituted azole derivatives. Focus on R 51 211, in *Mode of Action of Antifungal Agents* (Trinci, A.P.J., and Ryley, J.F., eds.), pp. 321-341, British Mycological Society.

21. Van de Bossche, H. (1985) Biochemical targets for antifungal azole derivatives: hypothesis on the mode of action, in *Current Topics in Medical Mycology*, (McGinnis, M.R., ed.), pp. 313-351, Springer-Verlag.

22. Korn, E.D., von Brand, T., and Tobie, E.J. (1969) The sterols of *Trypanosoma cruzi* and *Crithidia fasciculata*. *Comp. Biochem. Physiol.* 30: 601-610.

23. Dixon, H. Ginger, C.D., and Williams, J. (1972) Trypanosome sterols and their metabolic origins**.** *Comp. Biochem. Physiol.* 41B, 1-18.

24. Docampo, R., Moreno, S.N.J., Turrens, J.F., Katzin, A.M., Gonzalez-Cappa, S.M., and Stoppani, A.O.M. (1981) Biochemical and ultrastructural alterations produced by miconazole and econazole in *Trypanosoma cruzi*. *Mol. Biochem. Parasitol.* 3: 169-180.

25. McCabe, R.E., Remington, J.S., Araujo, J.S. (1984) Ketoconazole inhibition of intracellular multiplication of *Trypanosoma cruzi* and protection of mice against lethal infection with the organism. *J. Infect. Dis.* 150: 594-601.

26. Raether, W., and Seidenath, H. (1984) Ketoconazole and other potent antimycotic azoles exhibit pronounced activity against *Trypanosoma cruzi*, *Plasmodium berghei* and *Entamoeba histolytica* in vivo. *Z. Parasitenkd.* 70: 135-138.

27. Beach, D.H., Goad, L.J., and Holz, G.C. (1986) Effects of ketoconazole on sterol biosynthesis by *Trypanosoma cruzi* epimastigotes. *Biochem. Biophys. Res. Commun.* 136: 851-856.

28. Goad, L.J., Berens, R.L., Marr, J.J., Beach, D.H., and Holz Jr., G.G. (1989) The activity of ketoconazole and other azoles against *Trypanosoma cruzi*: biochemistry and chemotherapeutic action *in vitro*. *Mol. Biochem. Parasitol.* 32: 179-190.

29. McCabe, R.E., Remington, J.S., and Araujo, F.G. (1986) *In vitro* and *in vivo* effects of itraconazole against *Trypanosoma cruzi*. *Am. J. Trop. Med. Hyg.* 35: 280-284.

30. Larralde, G., Vivas, J., and Urbina, J.A. (1988) Concentration and time dependence of the effects of ketoconazole growth and sterol synthesis by *Trypanosoma (Schizotrypanum) cruzi* epimastigotes. *Acta Cient. Venez.* 39: 140-146.

31. Urbina, J.A., Vivas, J., Ramos, H., Larralde, G., Aguilar, Z., and Avilán, L. (1988) Alteration of lipid order profile and permeability of plasma membranes from *Trypanosoma cruzi* epimastigotes grown in the presence of ketoconazole. *Mol. Biochem. Parasitol.* 30: 185-196.

32. Urbina, J.A., Vivas, J., Ramos, H., Larralde, G., Aguilar, Z., and Avilán, L. (1988) Antiproliferative synergism of the allylamine SF 86-327 and ketoconazole on epimastigotes and amastigotes of *Trypanosoma (Schizotrypanum) cruzi*. *Antimicrob. Agents Chemother.* 32: 1237-1242.

33. Lazardi, K., Urbina, J.A., and De Souza, W. (1990) Ultrastructural alterations induced by two ergosterol biosynthesis inhibitors, ketoconazole and terbinafine, on epimastigotes and amastigotes of *Trypanosoma (Schizotrypanum) cruzi*. *Antimicrob. Agents Chemother.* 34: 2097-2105.

34. Urbina, J.A., Lazardi, K., Marchan, E., Visbal, G., Aguirre, T., Piras, M.M., Piras, R., Maldonado, R.A., Payares, G., and De Souza, W. (1993) Mevinolin (Lovastatin) potentiates the antiproliferative effects of ketoconazole and terbinafine against *Trypanosoma (Schizotrypanum) cruzi*: *in vitro* and *in vivo* studies. *Antimicrob. Agents Chemother.* 37: 580-591.

35. Maldonado, R.A., Molina, J., Payares, G., and Urbina, J.A. (1993) Experimental chemotherapy with combinations of ergosterol biosynthesis inhibitors in murine models of Chagas' disease. *Antimicrob. Agents Chemother.* 37, 1353-1359.

36. McCabe, R.E. (1988) Failure of ketoconazole to cure chronic murine Chagas' disease. *J. Infect. Dis.* 158: 1408-1409.

37. Moreira, A.A.B., de Souza, H.B.W.T., Amato Neto, V., Matsubara, L., Pinto, P.L.S., Tolenzano, J.E., Nunes, E.V., and Okumura, M. (1992) Avaliação da atividade terapéutica do itraconazol nas infeçoes crónicas, experimental e humana, pelo *Trypanosoma cruzi*. *Rev. Inst. Med. Trop. Sao Paulo* 34: 177-180.

38. Brener, Z., Cançado, J.R., Galvao, L.M.D.C., Da Luz, Z.M.P., Filardi, L.D.S., Pereira, M.E.S., Santos, L.M.T., and Cançado, C.B. (1993) An experimental and clinical assay with ketoconazole in the treatment of Chagas' disease. *Mem. Instituto Oswaldo Cruz* 88: 149-153.

39. Rahman, M.D., and Pascal, R.A. (1990) Inhibitors of ergosterol biosynthesis and growth of the trypanosomatid protozoan *Crithidia fasciculata*. *J. Biol. Chem.* 265: 489-4996.

40. Urbina, J.A., Vivas, J., Lazardi, K., Molina, J., Payares, G., Piras, M.M., and Piras, R. (1996) Antiproliferative effects of $D^{24(25)}$ sterol methyl transferase inhibitors on *Trypanosoma (Schizotrypanum) cruzi*: *in vitro* and *in vivo* studies. *Chemotherapy* 42: 294-307.

41. Ryley, J.F., McGregor, S., and Wilson, R.G. (1988) Activity of ICI 195,739- a novel, orally active bistriazole- in rodent models of fungal and protozoal infections. *Ann. N.Y. Acad. Sci.* 544: 310-328.

42. Urbina, J.A., Lazardi K., Aguirre T., Piras M.M., Piras R. (1991) Antiproliferative effects and mechanism of action of ICI 195,739, a novel bis-triazole derivative, on epimastigotes and amastigotes of *Trypanosoma (Schizotrypanum) cruzi*. *Antimicrob Agents Chemother.* 35: 730-736.

43. Lazardi, K., Urbina J.A., de Souza W. (1991) Ultrastructural alterations induced by ICI 195,739, a bis-triazole derivative with strong antiproliferative action against *Trypanosoma (Schizotrypanum) cruzi*. *Ant.imicrob Agents Chemother.* 35: 736-740.

44. Urbina, J.A., Payares G., Molina J., Sanoja C., Liendo A., Lazardi K., Piras M.M., Piras R., Perez N., Wincker P., Ryley J.F. (1996) Cure of short- and long-term experimental Chagas' disease using D0870. *Science (Wash. D.C.)* 273: 969-971.

45. Urbina, J.A., Payares, G., Contreras, M., Liendo, A., Sanoja, C., Molina, J., , Piras, M.M., Piras, R., Perez, N., Wincker, P., and Loeberberg, D. (1998) Antiproliferative effects and mechanism of action of SCH 56592 against *Trypanosoma (Schyzotripanum) cruzi*: *in vitro* and *in vivo* studies. *Antimicrob. Agents Chemother.* 42: 1771-1777.

46. Molina, J., Martins-Filho, O., Brener, Z., Romanha, A., Loebenberg, D., and Urbina, J.A. (2000) Activities of the triazole derivative SCH 5692 (Posaconazole) against drug-resistant strains of the protozoan parasite *Trypanosoma (Schizotrypanum) cruzi* in immunocompetent and immunosuppressed murine hosts. *Antimicrob. Agents Chemother.* 44: 150-155.

47. Ferraz, M.L., Gazzinelli, R.T., Alves, R.O., Urbina, J.A., and Romanha, A.J. (2007) The Anti-*Trypanosoma cruzi* activity of posaconazole in a murine model of acute Chagas' disease is less dependent on gamma interferon than that of benznidazole. *Antimicrob. Agents Chemother*. 51: 1359-1364.

48. Urbina, J.A., Lira, R., Visbal, G., and Bartroli, J. (2000) In vitro antiproliferative effects and mechanism of action of the new triazole derivative UR-9825 against the protozoan parasite *Trypanosoma (Schizotripanum) cruzi. Antimicrob. Agents Chemother.* 44: 2498-2502.

49. Urbina, J.A., Payares, G., Sanoja, C., Lira, R., and Romanha, A.J. (2003) *In vitro* and *in vivo* activities of ravuconazole on *Trypanosoma cruzi*, the causative agent of Chagas disease. *Int. J. Antimicrob. Agents* 21: 27-38.

50. Urbina, J.A., Payares, G., Sanoja, C., Molina, J., Lira, R., Brener, Z., and Romanha, A.J. (2003) Parasitological cure of acute and chronic experimental Chagas disease using the long-acting experimental triazole TAK-187. Activity against drug-resistant *Trypanosoma cruzi* strains. *Int. J. ANtimicrob. Agents* 21: 39-48.

51. Corrales, M., Cardozo, R., Segura, M.A., Urbina, J.A., and Basombrio, M.A. (2005) Comparative efficacies of TAK-187, a long-lasting ergosterol biosynthesis inhibitor, and benznidazole in preventing cardiac damage in a murine model of Chagas' disease. *Antimicrob. Agents Chemother*. 49: 1556-1560.

52. Guedes, P.M., Urbina, J.A., de Lana, M., Alfonso, L.C., Veloso, V.M., Tafuri, W.L., Machado-Coelho, G.L., Chiari, E., and Bahia, M.T. (2004) Activity of the new triazole derivative albaconazole against *Trypanosoma (Schizotrypanum) cruzi* in dog hosts. Antimicrob. Agents Chmeother. 48: 4286-4292.

53. Benaim, G., Sanders, J.M., Garcia-Marchan, Y., Colina, C., Lira, R., Caldera, A.R., Payares, G., Sanoja, C., Burgos, J.M., Leon-Rossell, A., Concepcion, J.L., Schijman, A.G., Levin, M., Oldfield, E., and Urbina, J.A. (2006) Amiodarone has intrinsic anti-*Trypanosoma cruzi* activity and acts synergistically with posaconazole. *J. Med. Chem*. 49: 829-899.

54. Santa-Rita, R.M., Lira, R., Barbosa, H.S., Urbina, J.A, de Castro, S.L. (2005) Anti-proliferative synergy of lysophospholipid analogues and ketoconazole against *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae): cellular and ultrastructural analysis. J. Antimicrob. Chemother. 55: 780-784.

55. Urbina, J.A., Concepcion, J.L., Rangel, S., Visbal, G., and Lira, R. (2002) Squalene synthase as a chemotherapeutic target in *Trypanosoma cruzi* and *Leishmania mexicana*. *Mol. Biochem. Parasitol.* 125, 35-45.

56. Urbina, J.A., Concepcion, J.L., Caldera, A., Payares, G., Sanoja, C., Otomo, T., and Hiyoshi, H. (2004) In vitro and in vivo activities of E5700 and ER-119884, two novel orally active squalene synthase inhibitors, against *Trypanosoma cruzi. Antimicrob. Agents Chemother*. 48: 2379-2387.

57. Orenes Lorente, S., Gomez, R., Jimenez, C., Cammerer, S., Yardley, V., de Luca-Fradley, K., Croft, S.L., Ruiz Perez, L.M., Urbina, J., Gonzalez Pacanowska, D., and Gilbert, I. (2005) Biphenylquinuclidines as inhibitors of squalene synthase and growth of parasitic protozoa. *Bioorg. Med. Chem.* 13: 3519-3529.

58. Urbina, J.A., Concepcion, J.L., Montalvetti, A., Rodriguez, J.B., and Docampo, R. (2003) Mechanism of action of 4-phenoxyphenoxyethyl thiocyanate (WC-9) against *Trypanosoma cruzi*, the causative agent of Chagas' disease. Antimicrob. *Agents Chemother*. 47: 2047-2050.

59. Linares, G.G., Gismondi, R., Codesido, N.O., Moreno, S.N., Docampo, R., and Rodriguez, J.B. (2007) Fluorine-containing aryloxyethyl thiocyanate derivatives are potent inhibitors of *Trypanosoma cruzi* and *Toxoplasma gondii* proliferation. *Bioorg. Med. Chem. Lett*. 17: 5068-5071.

60. Field, H., Blench, I., Croft, S., Field, M.C. (1996) Characterisation of protein isoprenylation in procyclic form *Trypanosoma brucei*. *Mol. Biochem. Parasitol*. 82: 67-80.

61. Yokoyama, K., Trobridge, P., Buckner, F.S., Scholten J., Stuart, K.D., Van Voorhis W.C., and Gelb, M.H. (1998) The effects of protein farnesyltransferase inhibitors on trypanosomatids: inhibition of protein farnesylation and cell growth *Mol. Biochem. Parasitol*. 94: 87-97.

62. Glomset, J.A., Gelb, M.H., Farnswort, C.C. (1990) Prenyl proteins in eukaryotic cells: a new type of membrane anchor. *Trends Biochem. Sci*. 15: 139-142.

63. Glomset, J.A., and Farnsworth, C.C. (1994) Role of protein modification reactions in programming interactions between ras-related GTPases and cell membranes. *Annu Rev. Cell. Biol.* 10: 181-205.

64. Yokoyama, K., Goodwin, G.W., Chomashchi, F., Glomset, J., and Gelb, M.H. (1992) Protein prenyltransferases. *Biochem. Soc. Trans*. 20: 479-484.

65. Casey, P.J., and Seabra, M. (1996) Protein prenyltransferases *J. Biol. Chem*. 271: 5289-5292.

66. Njoroge, F.G., Doll, R.J., Vibulbhan, B., Alvarez, C.S., Bishop, W.R., Petrin, J., Kirschmeier, P., Carruthers, N.I., Wong, J.K., Albanese, M.M., Piwinski, J.J., Catino, J., Girijavallabhan, V., and Ganguly, A.K. (1997) Discovery of novel nonpeptide tricyclic inhibitors of Ras farnesyl protein transferase. *Bioorg. Med. Chem.* 5, 101-113.

67. Koblan, K.S., Hohl, N.E., Omer, C.A., Anthony, N.J., Conner, M.W., se Solms, S.J., Williams, T.M., Graham, S.L., Hartman, G.D., Oliff, A., and Gibbs, J.B (1996) Farnesyltransferase inhibitors: a new class of cancer chemotherapeutics. *Biochem. Soc. Trans*. 24: 688-692.

68. Sun, J., Qian, Y., Mailton, A.D., and Sebti, S.M. (1998) Both farnesyltransferase and geranylgeranyltransferase I inhibitors are required for inhibition of oncogenic K-Ras prenylation but each alone is sufficient to suppress human tumor growth in nude mouse xenografts. *Oncogene* 16: 1467-1473.

69. Leonard, D.M. (1997) Ras farnesyltransferase: a new therapeutic target. *J. Med. Chem*. 40, 2971-2990.

70. Hucke, O., Gelb, M.H., Verlinde, C.L., and Buckner, F.S. (2005) The protein farnesyltransferase inhibitor Tipifarnib as a new lead for the development of drugs against Chagas disease. *J. Med. Chem*. 48: 5415-5418.

71. Buckner, F.S., Yokoyama, K., Nguyen, L., Grewal, A., Erdjument-Bromage, H., Tempst, P., Strickland, C.L., Xiao, L., Van Voorhis, W.C., and Gelb, M.H. (2000) Cloning, heterologous expression, and distinct substrate specificity of protein farnesyltransferase from *Trypanosoma brucei*. *J. Biol. Chem.* 275: 21870-21876.

72. Fisher, J.E., Rogers, M.J., Halasy, J.M., Luckman, S.P., Hughes, D.E., Masarachia, P.J., Wesolowski, G., Russell, R.G.G., Rodan, G.A. and Reszka, A.A. (1999) Alendronate mechanism of action: geranylgeraniol, an intermediate in the mevalonate pathway, prevents inhibition of osteoclast formation, bone resorption, and kinase activation in vitro. *Proc. Natl. Acad. Sci. USA* 96: 133-138.

73. van Beek, E., Pieterman, E., Cohen, L., Löwik, C. and Papadopoulos, S. (1999) Nitrogen-containing bisphosphonates inhibit isopentenyl pyrophosphate isomerase/farnesyl pyrophosphate synthase activity with

relative potencies corresponding to their antiresorptive potencies in vitro and in vivo. *Biochem. Biophys. Res. Commun*. 255: 491-494

74. Martin, M.B., Arnold, W., Heath III, H.T., Urbina, J.A. and Oldfield, E. (1999) Nitrogen-containing bisphosphonates as carbocation transition state analogs for isoprenoid synthesis. *Biochem. Biophys Res Commun* 266: 754-758.

75. Cromartie, T.H., Fisher, K.J., and Grossman, J.N. (1999) The discovery of a novel site of action for herbicidal bisphosphonates. *Pesticide Biochem. Physiol*. 63: 114-126.

76. van Beek, E., Petermand, E., Cohen, L., Lowik, C., and Papapouos, S. (1999) Farnesyl pyrophosphate synthase is the molecular target of nitrogen-containing bisphosphonates. *Biochem. Biophys. Res. Commun*. 264: 108-111.

77. Bergstrom, J.D., Bostedor, R.G., Masarachia, P.J., and Reszka, A.A. (2000) Alendronate is a specific, nanomolar inhibitor of farnesyl diphosphate synthase. *Arch. Biochem. Biophys*. 373: 231-241.

78. Grove, J.E., Brown, R.J., Watts, D.J. (2000) The intracellular target for the antiresorptive aminobisphosphonate drugs in *Dictyostelium discoideum* is the enzyme farnesyl diphosphate synthase. *J. Bone and Min. Res*. 15: 971-981.

79. Rodan, GA. (1998) Mechanisms of action of bisphosphonates. *Ann. Rev. Pharmacol. Toxicol.* 38: 375-388.

80. Urbina, J.A., Moreno, B., Vierkotter, S., Oldfield, E., Payares, G., Sanoja, C., Bailey, B.N., Yan, W., Scott, D.A., Moreno, S.N.J. and Docampo, R. (1999) *Trypanosoma cruzi* contains major pyrophosphate stores and its growth *in vitro* and *in vivo* is blocked by pyrophosphate analogs. *J. Biol. Chem*. 274: 33609-33615.

81. Martin, M.B., Grimley, J.S., Lewis, J.C., Heath, H.T. 3rd, Bailey, B.N., Kendrick, H., Yardley, V., Caldera, A., Lira, R., Urbina, J.A., Moreno, S.N., D0campo, R., Croft, S., and Oldfield, E. (2001) Bisphosphonates inhibit the growth of *Trypanosoma brucei, Trypanosoma cruzi*, *Leishmania donovani, Toxoplasma gondii*, and *Plasmodium falciparum*: a potential route to chemotherapy. *J. Med. Chem.* 44: 909-916.

82. Montalvetti, A., Bailey, B.N., Martin, M.B., Severin, G.W., Oldfield, E., and Docampo, R. (2001) Bisphosphonates are potent inhibitors of *Trypanosoma cruzi* farnesyl pyrophosphate synthase. *J Biol Chem* 276: 33930-33937.

83. Gabelli, S.B., McLellan, J.S., Montalvetti, A., Oldfield, E., Docampo, R., and Amzel, L.M. (2006) Structure and mechanism of the farnesyl diphosphate synthase from *Trypanosoma cruzi:* implications for drug design. *Proteins* 62:80-88.

84. Szajnman, S.H., Ravaschino, E.L., Docampo, R., and Rodriguez, J.B. (2005) Synthesis and biological evaluation of 1-amino-1,1-bisphosphonates derived from fatty acids against *Trypanosoma cruzi* targeting farnesyl pyrophosphate synthase. *Bioorg. Med. Chem. Lett.* 15: 4685-4690.

85. Garzoni, L.R., Waghabi, M.C., Baptista, M.M., de Castro, S.L., Meirelles, M. de N., Britto, C.C., Docampo, R., Oldfield, E., and Urbina, J.A. (2004) Antiparasitic activity of risedronate in a murine model of acute Chagas' disease. *Int J Antimicrob Agents* 23: 286-90.

86. Garzoni, L.R., Caldera, A., Meirelles, M. de N., de Castro, S.L., Docampo, R., Meints, G.A., Oldfield, E., AND Urbina, J.A. (2004) Selective in vitro effects of the farnesyl pyrophosphate synthase inhibitor risedronate on *Trypanosoma cruzi*. *Int J Antimicrob Agents* 23: 273-285

87. Bouzahzah, B, Jelicks, L.A., Morris, S.A., Weiss, L.M., AND Tanowitz, H.B. (2005) Risedronate in the treatment of murine Chagas' disease. *Parasitol Res* 2005; 96: 184-187.

88. Hudock, M.P., Sanz-Rodriguez, C.E., Song, Y., Chan, J.M., Zhang, Y., Odeh, S., Kosztowski, T., Leon-Rossell, A., Concepcion, J.L., Yardley, V., Croft, S., Urbina, J.A., and Oldfield, E. (2006) Inhibition of *Trypanosoma cruzi* hexokinase by bisphosphonates. *J. Med. Chem*. 49: 215-223.

89. Sanz-Rodriguez, C.E., Concepcion, J.L., Pekerar, S., Oldfield, E., and Urbina, J.A. (2007) Bisphosphonates as inhibitors of *Trypanosoma cruzi* hexokinase: kinetic and metabolic studies. *J. Biol. Chem*. 282: 12377-12387.

90. Moyle, G.J., Youle, M., Higgs, C. et al., (1998) Safety, pharmacokinetics, and retroviral activity of the potent, specific human immunodeficiency virus protease inhibitor nelfinavir: results of a phase I/II trial and extended follow-up in patients infected with human immunodeficiency virus. *J. Clin. Pharmacol*. 38: 736-743.

91. McKerrow, J.H. (1999) Development of cysteine protease inhibitors as chemotherapy for parasitic diseases: insights on safety, target validation, and mechanism of action. *Int. J. Parasitol*. 29: 833-837.

92. Brinen, L.S., Hansell, E., Cheng, J., Roush, W.R., McKerrow, J.H., and Feltterick, R.J. (2000) A target within the target: probing cruzain's P1 site to define structural determinants for the Chagas' disease protease. Structure Fold Dev. 15: 831-840.

93. Engel, J.C., Doyle, P.S., Hsieh, I., and McKerrow, J.H. (1998) Cysteine protease inhibitors cure an experimental *Trypanosoma cruzi* infection. *J. Exp. Med*. 188: 725-734.

94. Barr, S.C., Warner, K.L., Konreic, B.G., Piscitelli, J., Wolfe, A., Benet, L., and McKerrow, J.K. (2005) A cysteine protease inhibitor protects dogs from cardiac damage during infection by *Trypanosoma cruzi. Antimicrob. Agents Chemother*. 49: 5160-5161.

95. Doyle, P.S., Zhou, Y.M., Engel, J.C., and McKerrow, J.H. (2007) A Cysteine Protease Inhibitor Cures Chagas' Disease in an Immunodeficient Murine Model of Infection. *Antimicrob. Agents Chemother*. Aug. 13 PMID 17698625.

96. Jha, T.K., Sundar, S., Thakur, C.P., Bachmann, P., Karbwang, J., Fischer, C., Voss, A., and Berman, J. (1999) Miltefosine, an oral agent, for the treatment of Indian visceral leishmaniasis. *New Engl. J. Med*. 341: 1795-1800.

97. Croft, S.L., Snowdon, D., and Yardley, V. (1996) The activities of four anticancer alkyllysophospholipids against *Leishmania donovani, Trypanosoma cruzi* and *Trypanosoma brucei*. *J. Antimicr. Chemother*. 38: 1041-1047.

98. Saraiva, V.B., Gibaldi, D., Previato, J.O., Mendonca-Previato, L., Bozza, M.T., Freire-De-Lima, C.G., and Heise, N. (2002) Proinflammatory and cytotoxic effects of hexadecylphosphocholine (miltefosine) against drug-resistant strains of *Trypanosoma cruzi*. *Antimicrob. Agents Chemother*. 46: 3472-3477.

99. Santa-Rita, R.M., Santos Barbosa, H., Meirelles, M.N., and de Castro, S.L. (2000) Effect of the alkyl-lysophospholipids on the proliferation and differentiation of *Trypanosoma cruzi. Acta Trop*. 75: 219-228.

100. Berger, M.R., Betsch, B., Gebelein, M., Amtmann, E., Heyl, P., and Scherf, H.R. (1993) Hexadecylphosphocholine differs from conventional cytostatic agents. *J. Cancer Res. Clin. Oncol*. 119: 541-548.

101. Fichoux, Y.L., Rousseau, D., Ferrua, B., Ruette, S., Lelievre, A., Grousson, D., and Kubar, J. (1998) Short- and long-term efficacy of hexadecylphosphocholine against established *Leishmania infantum* infection in BALB/c mice. *Antimicrob. Agents Chemother*. 42: 654-658.

102. Urbina, J.A. (2006) Mechanism of action of lysophospholipid analogues against trypanosomatid parasites. Trans. R. Soc. Trop. Med. Hyg. 100: S9-S16.

Trypanosoma cruzi: Congenital and Transfusional American Trypanosomiasis

Myriam Lorca, M.T. MSc., PhD, Post Ph.D
Associated Professor Parasitology
WHO/PAHO Expert in Chagas disease
Faculty of Medicine, University of Chile, Santiago, Chile
clorca@med.uchile.cl

Vector transmission of Chagas disease remains the most frequent mode of infection of people in endemic regions. However in these regions where the vector has been controlled effectively and the blood for transfusion is screened, the congenital route has become the main focus of attention by the scientific community and Public Health authorities. This is particularly evident in areas where the control efforts were directed to vector reduction/control and blood transfusion screening without identifying the relevance of congenital infection.

Transplacental infection of Chagas disease has been studied in detail using patients from different countries in Latin America showing significant prevalence in a few countries in Latin America. However control programs have nor been established to reduce the prevalence in the infected areas. Currently, the prevalence of transplacental infection of Chagas disease in pregnant women ranges from 1 to 50% in endemic areas. An apparent reduction has been detected due to control of vectors in a few countries including Uruguay, Brazil, Chile, and Argentina.

Transplacental transmission to the fetus can occur at any time of the pregnancy, with successive pregnancies, and in twins. In general, there are pathologic effects on the fetus without abortions and the child may be born apparently healthy and adequate body weight or show serious clinical signs that may be fatal in low body weight child, or show hepatosplenomegaly and other signs associated with TORCH.

In Latin America, the incidence of congenital Chagas disease ranges from 1% in Brazil to 28% in Chile. Based on this information, the number of children born with the disease is important in the context of children general health. Modern improvement of the methods of diagnosis has impacted on the numbers of new cases and consequently appears to be a serious disease in some of the countries of the region.

The diagnosis in the pregnant patient is based on conventional serologic tests however the diagnosis in the child is done using direct methods to show the presence of the parasite. The direct or parasite concentration methods include the "slide and coverslip" and blood smears. Other methods used are the "thick drop", MicroStrout, blood culture, and xenodiagnosis. The methods without concentration of the sample show variable sensitivity depending upon the parasitemia of the patient and the experience of the examiner. The results of methods using concentration also depend upon the parasitemia of the child. The methods may be 100% sensitive in children with high parasitemia, however if the infection occurred later during pregnancy, the sensitivity is lower and may result in misdiagnosis.

Standardization of diagnostic methods will impact directly on the effectiveness of the tests and it is correlated with the signs observed in the child. In those countries where a symptomatic disease is most prevalent having a simple and fast method in the hands of well trained personnel will be very effective method of evaluation of disease status. In countries where 80% of the patients do not show signs associated with Chagas disease and low parasitemia, it is necessary to find new and more sensitive methods to be added to TORCH. Conventional methods in the hands of expert personnel report 100% sensitivity [MicroStrout] However, this result is contradicted by other reports published with only a 40% sensitivity. These values are similar to the results obtained using hemoculture and xenodiagnosis. Currently PCR for *T. cruzi* DNA is showing elevated sensitivity and specificity (99%) for the diagnosis of Chagas disease.

Congenital Chagas disease with typical signs deserves special analysis. A few years ago, the frequency of symptomatic cases with low birth weight, premature birth, and positive TORCH was high. Currently such cases are not very frequent and the patient will be asymptomatic (90%), apparently healthy, have adequate body weight for the gestation period and will not be detected in the neonatal examination. However, the patient if infected with *T. cruzi* will develop chronic Chagas disease 15 to 20 years later and this finding will not be associated with vector exposure.

Modern studies of congenital Chagas disease prevalence in Chile have determined a 56% reduction compared with previous evaluations. PCR diagnosis of transplacental transmission produced 21% positive reaction. Since infected children are asymptomatic, it is necessary to conduct systematic studies of children of chagasic mothers and determine transplacental transmission when a disease is not apparent.

Treatment of Chagas disease is conducted obtaining parental consent using nifurtimox or benznidazole. Nifurtimox (Lampit, Bayer) is used in progressive dose for 6 days followed by a dose of 7-

8 mg/kg/day for 60 days after meals. The children are evaluated clinically and with laboratory tests including hemogram and liver tests. No adverse reactions were observed. Benznidazole (Rochagan, Roche) is used at 5 to 10 mg/kg/day for 60 days following a similar protocol described for nifurtimox. The treatment protocols currently available are 100% effective and safe for the patients resulting in cure which has been confirmed by a negative PCR, direct methods and serologic (anti-IgG) tests. Follow-up of treated cases for three years showed 1005 parasitological cure and no adverse effects.

T. cruzi lineage has been studied in blood of congenital infected children, transmitting and non-transmitting mothers. The results show no association between parasite lineage and vertical transmission of the parasite.

The results of the programs demonstrate the relevance of transplacental transmission of *T. cruzi* in Chile and the effects of the implementation of a program for pregnant women and children to diagnose and effectively treat the infection. Having an effective treatment of infected children is justified because will cure the patient and will eliminate the parasite reducing the source for further infection of the arthropod vector of *T. cruzi*.

An intervention program should include mandatory screening of pregnant women and newborn child for levels of *T. cruzi* IgG. If both show positive levels, the newborn should be examined for parasite presence in the blood using MicroStrout and PCR. Alternatively, the child of a Chagas positive mother may be examined using serologic tests at one year of age. A negative result will indicate no exposure to the parasite. A positive result will require a drug treatment. Aggressive treatment of Chagas disease infected children will prevent chronic symptoms of the disease, megaintestinal and cardiac lesions.

Transfusional Chagas Disease

Risk of Chagas disease through transfusions in the Americas has been described long time ago. The safety of blood transfusion depends on a country's laws, decrees and/or regulations concerning the collection, production and use of blood and blood derivatives. It also needs governmental enforcement of those instruments, as well as trained health professionals to obtain blood and produce blood derivatives, following total quality control procedures both at collection and production, and use. By 1998, all Latin American countries had laws, decrees and/or regulations that governed the production and use of blood, with two exceptions (El Salvador and Nicaragua). During the past six decades, economic need in Latin America has promoted migration to urban areas. Consequently, at present time, more than 60% of the population live in cities, which increases the probability of finding blood infected by *T. cruzi* among

donors. Unless all the blood from infected donors is discarded, the possibility of transmitting infection by transfusion remains. Moreover, infection by *T. cruzi* through transfusion is a potential problem in developed countries, now that tens of thousands of individuals from Latin America have migrated to the United States, Canada, Western Europe, Australia and Japan and the migrants can be efficiently transmitted by transfusion. In these non-endemic areas, Chagas disease is imported diseases resulting of traveling to endemic areas and migration of natives from these endemic areas. A recent International Forum showed that in Europe, as well as in the U.S. prevention of transfusion-associated protozoa infections depend mainly on selection of donors using questionnaires. In several countries donors are questioned for risk of *T. cruzi* infection. In some countries donors are excluded when they (or their mothers) were born in South or Central America, if they received a blood transfusion in these areas and if they lived in rural areas in these endemic countries for more than 4 weeks. More research is needed to establish the theoretical risk of transfusion transmission of *T. cruzi* infection in Europe, before preventive measures may be considered. Moreover up to now have been demonstrated a case of a patient with congestive heart failure who was diagnosed with chronic chagasic cardiomyopathy. This is the first case of chagasic dilated cardiomyopathy in Spain demonstrating the risk of Chagas disease in non endemic countries.

When donors are not screened for *T. cruzi*, the risk of transfusing infected blood is greater at higher prevalence rates of infection in the donor population; it also increases with the number of transfusions received by the recipient. In 1993, Bolivia presented the highest risk of receiving infected blood and becoming infected with *T. cruzi*; this country was followed by Colombia, El Salvador and Paraguay. In absolute numbers, the highest potential for occurrence of cases of *T. cruzi* infection was present in Bolivia. Although the situation has improved since 1993, and 100% of donors are being screened for *T. cruzi* in Argentina, Colombia, Ecuador, El Salvador, Honduras, Paraguay, Uruguay and Venezuela, success will only be assured by: total enforcement of the law by governments; implementation of altruistic and volunteer blood donations, exclusively; 100% of donors are screened for communicable diseases; the collection, processing and use of blood strictly follow quality control norms; reagents used in diagnosis are adequate, and the use of blood and blood derivatives is limited to cases where it is only absolutely necessary.

References

Bittencourt AL. Tranmissao vertical da Doenca de Chagas . In: Brener Z, Andrade ZA. *La Doenca de Chagas.* Rio de Janeiro, Brazil: editora Guanabara Koogan SA 2000. pp 16-20.

Cerisola JA, Rabinovich A, Alvarez M, Di Corleto CA, Pruneda J 1972. Enfermedad de Chagas y la transfusión de sangre. *Bol Oficina Sanit Panam 73*: 203221.

Contreras S.,Fernandez M.R.,Agero F., Desse J., Orduna T y Martino O.Enfermedad de Chagas-Mazza congénita en Salta. *Rev. Soc. Bras.Med.Trop.* 1999. 32 (6): 1- 8.

Freijlij H Altech J. Congenital Chagas disease: diagnostic and clinical aspects. *Clinical Infectious Diseases* 1995, 21: 551-555

García A, Bahamonde MI, Verdugo S, Correa J, Pastene C, Tassara R, Lorca M. Infección transplacentaria por *Trypanosoma cruzi*: Situación en Chile. *Rev Méd Chile*. 129 (3). 2001.

Howard JE, Rubio M. Congenital Chagas' disease. I Clinical and epidemiological study of thirty cases. *Bol Chil Parasitol*. 1968, 23: 107.

Howard JE. *La enfermedad de Chagas congénita. Colecc. Monografías Biológicas*. Univ. de Chile. 1962. 55 pp.

Juan M. Burgos, Jaime Altcheh, Margarita Bisio, Tomas Duffy, Helder M.S. Valadares et al. Direct molecular profiling of minicircle signatures and lineages of *Trypanosoma cruzi* bloodstream populations causing Congenital Chagas disease. *International Journal for Parasitology* 2007, 37: 1319–1327.

Lorca M, Child R, Garcia A, Silva MG, Martinez LP, Jerez GM, ToledoI, Mezzano DA 1994. Evaluación de reactivos comerciales empleados en el diagnóstico de la enfermedad de Chagas en bancos de sangre de Chile. II Aplicación rutinaria. *Rev Med Chile* 122: 925-931.

Lorca M, Child R, Garcia AC, Silva MC, Osorio J, Atias AM 1992. Evaluación de reactivos comerciales empleados en el diagnóstico de la enfermedad de chagas en bancos de sangre de Chile. 1. Selección de reactivos. *Rev Med Chile* 120: 420-442.

Lorca M, García A, Bahamonde MI, Fritz A, Tassara R. Certificación serológica de la interrupción de la transmisión de la enfermedad de Chagas en Chile. *Rev Med Chile* 2001, 129: 266-71.

Lorca M, Lorca J, Child R, Atias A, Canales M, Lorca E, Gutierrez J 1988. Prevalencia de la infección por *Trypanosoma cruzi* en pacientes politransfundidos. *Rev Med Chile* 116: 112116.

Lorca M, Thiermann E. Congenital Chagas disease and its serological diagnosis through conventional serology and methods of molecular biology. In: *Biology of Parasitism*. Ehrlich R, and Nieto A. Eds. Ediciones Trilce. Montevideo, Uruguay. 1994, 160-166.

Lorca M, Veloso C, Muñoz P, Bahamonde MI, García A. Diagnostic value of detecting specific IgA and IgM with recombinant Trypanosoma cruzi antigens in congenital Chagas disease. *Am J Trop Med Hyg*. 1995, 52: 512-5.

Luquetti AO. Etiological treatment for Chagas disease. *Parasitology Today* 1997, 13: 127-128.

Moncayo, Alvaro. Chagas disease: current epidemiological trends after the interruption of vectorial and transfusional transmission in the Southern Cone countries *Mem. Inst. Oswaldo Cruz* 98 (5), Rio de Janeiro, July 2003.

PAHO *Consultation on congenital Chagas disease, its epidemiology and manegement.* Montevideo Uruguay, 24-25 June 2004. PAHO doc DPC/CD/ChD/CLAP.2004.

Schmunis GA 1985. Chagas' disease and blood transfusion, p. 127145. In :RV Dodd & LF Barker (eds), *Infection, Immunity and Blood Transfusion,* AR Liss, New York.

Schmunis GA 1989. Chagas' disease and blood transfusion, p. 197-218. In: *Blood Transfusion and Infectious Diseases*, EG Rondanelli, Pichin, Italy.

Schmunis GA 1991. *Trypanosoma cruzi*, the etiologic agent of Chagas disease: status in the blood supply in endemic and non endemic countries. *Transfusion* 31: 547-555.

Schmunis GA 1994. *American trypanosomiasis as a public health problem. Chagas disease and the nervous system. PAHO Sci Pub* 547: 3-29.

Schmunis GA, Zicker F, Pinheiro F, Brandling-Bennett 1998. Risk of transfusion transmitted infectious diseases in Central and South America. *Emerg Infect Dis* 4: 5-11.

Schmunis, Gabriel A. Riesgo de la enfermedad de Chagas a traves de las transfusiones en las Americas Medicina (B.Aires) 59 (supl.2): 125-34, 1999.

Sosa Estani S, Segura E, Ruis A. Efficaccy of chemotheraphy with benznidazole in Chagas disease. *Am. J.Trop.Med.Hyg* 1998, 59 (4): 526-529.

WHO Expert Committee. Control of Chagas disease. *World Health Organ Tech Rep Ser* 2002, 905:i--vi, 1-109.

Conclusions related to American Trypanosomiasis Control and Research

Dr. Lileia Diotaiuti

On the first day of the meeting, the presentations followed the agenda programmed with eight lectures and one conference about Chagas disease in the following order:

1. Dr. Antonio Carlos Silveira, Control of Vector Transmission of chagas disease, Its Limits and the Research of Situations or Areas with Persistent Infestation by Introduced Species

According to this author, control strategies should consider the concepts of *introduced* species and *native* species as well as the complexity of epidemiological scenarios summarized as follows:

1. areas with interrupted transmission;
2. areas of emergent (or greater visibility) peculiar mechanisms of vector transmission (extradomiciliary; domiciliary without colonization; oral, secondarily vectorial);
3. areas with persistent infestation (many actions developed in time).

Additionally, there is a great variety of vectors with distinct biological and ecological characteristics, being the most anthropophilic susceptible to elimination. In this context, it should be considered the conceptual difference between elimination and eradication taking into account that elimination is possible inside a determined geographical space and that the goal is to eliminate transmission and not the vectors. In this way, it is fundamental to review the goal proposed in the 51st World Health Assembly that established that the elimination of Chagas disease would be achieved in 2010, a goal impossible to be fulfilled by the countries that adhered to it. As a governmental initiative, the following goals are proposed as feasible:

1) **Southern Cone Initiative** (1991). To interrupt vector transmission by:
   (a) *T. infestans* elimination;
   (b) Control of domiciliary colonization by native species.
   (c) Interruption of transfusional transmission of *T. cruzi.*
2) **Andean Countries Initiative** (1997). To interrupt vector transmission by:
   (a) Elimination of *T. dimidiata* in Ecuador; *R. ecuadoriensis* in Peru, and *R. prolixus* in Colombia;
   (b) Control of domiciliary colonization by native species;
   (c) To interrupt transfusional transmission of *T. cruzi*.

3) **Central America Initiative** (1997). To interrupt vector transmission by:
   (a) Elimination of *R. prolixus;*
   (b) Control of intradomiciliary colonization by native species.
   (c) To interrupt transfusional transmission of *T. cruzi.*
4) **Amazon Initiative** (2004). To avoid the establishment of endemic transmission at large scale in the sub-region through environment/ vector/human infection surveillance. But within each initiative, the following goals were achieved:

Southern Cone Initiative: Elimination of *T. infestans* in wide areas with transmission interruption in BRA, CHI and URU, parts of ARG and PGY, reduction transmission by secondary species in BRA, coverage of blood screening: ~ 100% (with the exception of BOL and CHI).

Andean Countries Initiative: Actions of **e**ntomological surveillance/vector control were restarted in VEN; awareness of risk in COL, with punctual action of vector control; formal setting up of a regular control program of national scope in ECU; setting up of regular control actions in the MACROSUR region of PER; coverage of blood screening: ~ 100%.

Central America Initiative: Elimination of *R. prolixus* (ESL?) with transmission interruption; transmission reduction by *T. dimidiata*; coverage of blood donors: ~ 100%.

Amazon Initiative: Development and standardization of a surveillance and search model based in cases detection in slides collected for malaria diagnosis; system implemented in BRA, ECU and COL. In relation to *T. infestans*, it can be noticed the persistence of the species in the Gran Chaco region with probable epidemiological origins (occurrence of natural foci) but also due to unsatisfactory responses (related to environmental and/or socio-cultural conditions) and control failures (related to methodological errors). In the case of persistence in Bolivia, a research table was presented with algorithms that could help to understand the causes of this persistence. From that algorithm table, a check list for operational failures and another for environmental determinants were elaborated (both could be associated).

A parallel or subjacent issue that deserves to be mentioned is the lack of continuity of actions almost always determined by the insufficiency of resources due to the lack of priority of the problem.

2) Dr. Marcelo Aguilar, Critical Aspects of T. cruzi Infection and Chagas Disease in the Ecuadorian Amazon

Considerations for other Amazonian areas with probable endemic transmission. The main question was: Why has the transmission of Chagas disease increased so much in the last 15 years in the Amazonian region?, where the disease has historically occurred occasionally. The complexity of the eco-epidemiology of Chagas disease in the region was presented as well as the variety of the Amazonian triatomine fauna as well as the importance of palm trees as sylvatic foci of triatomines, frequently infested and with wide areas of occurrence. In Ecuador, the global infestation rate of palm trees is 48.5%, with an infestation risk by *Rhodnius* related to forest stratum: a) High intervention (grass and crop) +61%, insects average 8.1± 16.9 ; b) Urban areas (+ 43%, insects average 4.7±8.5); c) Secondary forest (+ 34%, insects average 2.4±6). The great novelty is the invasion of houses by triatomines and a seropositivity profile in *Amazonía* compatible to that of an endemic area. Based in the characterization of biophysical and socio-economic variables it was possible to associate the invasion of houses by *Rhodnius* in Ecuador with a deforestation process in the area. *T. cruzi* prevalence in the rural area (3.40%) is higher than in urban area (1.64%). Preliminary data demonstrate that Chagas disease was mainly associated to landscapes made up of a mosaic of secondary forest, crops and forest; the lower urban prevalence was associated to grasses and bushes coverage. Similar situations are being demonstrated in other regions like in Cayenne that presents some important areas of transmission (6-7% prevalence, with specific antibodies increasing with age), and infection of dogs living in houses with good conditions between 2003 and 2005. There are also important stories of transmission without colonization in Colombia. In Brazil, the situation is even more complex due to the existence of different transmission types and endemic profiles with severe cases.

3) François Noireau, Wild Triatoma Infestans: Scientific Curiosity or Potential Threat?

The question refers to the determination of the relation between the persistence of *T. infestans* infestation and the occurrence of sylvatic foci. More recent data demonstrated that these foci are more extensive that first thought between the Andean valleys and the Chaco. Sylvatic foci were recently found in the Argentinean Chaco. The situation acquires more complexity because of the existence of periurban infestation foci, like in Cochabamba, in open growth. An important finding is related to the morphologic plasticity, mainly chromatic, observed between different populations where environmental variations carry weight (altitude, temperature, rocks size, vegetation). The Andean pattern seems to be older;

meanwhile the finding of populations in the Boreal Chaco suggests the existence of native populations in this ecoregion. The finding of an intermediate form between the Andean pattern and that of the Chaco suggest the possibility of the occurrence of other patterns. Little is known about the ecology and epidemiological importance of these sylvatic populations. It has been observed that these rupícolous insects collected in the Andes showed high infestation rates (> 60%), involving small rodents and marsupials. In these studies, it was also observed the occurrence of *T. cruzi* TCI and TCII. The dark populations of the Chaco showed much lower infection rates (2.5%), probably due to the association to birds (parrots nests in tree holes). In the Andean region, studies of populational structure of triatomines suggest the existence of only one annual cycle, in spite of the fact that data of intradomiciliary infestation demonstrated the possibility of the occurrence of two annual cycles; nothing is known about this aspect in the dark populations of the Chaco. Concerning the dispersion capacity of these populations, preliminary data showed the low gene flow between sylvatic and domiciliary populations of Cochabamba, suggesting the little importance of the sylvatic foci in the process of houses recolonization.

4) Dr. Carlota Monroy, Ecosystem Approach Intervention for Long'Term Control of *Triatoma Dimidiata* in Guatemala

At the beginning, Dr. Monroy emphasized the great variability of *T. dimidiata* in Guatemala. Studies comparing populations and using morphometrics of head and genitalia, and molecular markers (ITS and RAPD) not always showed the same results. The question is: Why is there so much diversity in Guatemala? Studies about the geologic formation of this region demonstrated an agglutination of several islands for the formation of the current territory, therefore, having different origins. On the other hand, Honduras and El Salvador would be newer and more homogeneous. Maybe this diversity is related to a larger or smaller easiness for the elimination of *T. dimidiata* by control actions. A work developed in the areas of persistence of the insects, with different ecologic characteristics (also in different wild ecotopes like caverns, trees, rocks, palm trees), determined the risk factors of infestation. Interestingly, the known association of *T. dimidiata* with soil in Costa Ricas was not observed here. It was mentioned that house improvement (periodical wall plastering), especially during Holy Week (when visitors are received), is women's responsibility due to an esthetic reason (cosmetic) without association to the presence of triatomines in the houses. From the identification of these risk factors, the house features that would deserve attention from control perspectives were determined. There were different types of mud that were analyzed in the laboratory to orientate the population to use a more durable formula that included river sand ($1m^2$/house). This project has been performed for three years and the results evaluation showed a decrease of the risk of intradomiciliary infestation and that triatomines concentrated in the coops and other facilities outside of the house. Currently, it is being thought to use *Telenomus* parasitoid while

searching for control complementary alternatives. In this study, it was also evident the higher infestation by *T. dimidiata* in deforested areas that resulted in a work of encouraging the population to plant, mainly fruit trees. The cultivation of mayan bees was also encouraged in this project but it was not successful because there were not flowers in the region and the bees died. This frustrated experience alerts about the need of a better planning of the activities proposed to the community.

5) Dr. Nicolas Jaramillo, Geometric Morphometrics Applied to the Study of Triatominae

At first, general concepts about morphometrics were presented and it was defined as the "study of variation of biological form and size and of their co-variations with other variables". Its objectives are:

a. To detect and describe quantitatively morphological variations,
b. To study the origin and nature of the morphological variation,
c. To study the elements that could alter the morphometric patterns,
d. To collaborate with taxonomic studies especially for populations hard to identify,
e. To detect evolutionary patterns and
f. To support phylogenetic studies.

Some examples of morphometric study of triatomines were presented:

i) *R. prolixus:* Until recently, it was admitted that there were no sylvatic populations of this species and that the insects existent in the palm trees were *R. robustus*. A morphometric study in Venezuela revealed that there was no difference between intradomiciliary and sylvatic populations on the contrary to what had been thought before. Higher sexual dimorphism was observed among sylvatic insects as well as lower variance in the group that would indicate that the flow between populations would be in the sense sylvatic-domestic. In Colombia, *R. prolixus* would be autochthonous in the region of Casanare, and introduced in Serra de Santa Marta, probably from Venezuelan populations of Barinas. The variability observed between different populations of the same species demonstrates the adaptation capacity to different conditions that could also mean different responses to control.

ii) In Brazil, it was observed confusion about the identification of *T. arthurneivai* and *T. wygodzinskii* specimens stocked in collections, a problem originated probably by the wrong identification since the moment they were obtained from nature years ago. The use

of morphometrics allows clarifying that specimens from São Paulo were *T. wygodzinskii* and not *T. arthurneivai*, solving a taxonomical problem and allowing a better analysis of the origin of similar species.

iii) The size change between different generations of *P. geniculatus* in the laboratory was demonstrated; their size decreases with time but they preserve their form.

iv) The comparison between flying and non flying *T. rubrovaria* showed the same size but different conformation for both groups that could be related to the difficulty to fly. Finally, it was considered that the phenotypical plasticity was related to the domiciliation capacity: sylvatic triatomines seem to have less plasticity in relation to domiciliated species. This observation was reinforced by feeding behavioral studies that showed lower ability of triatomines to feed on bird blood. Thus, the adaptation to feeding on different sources present in the intradomicile would be an ability characteristic of species that would adapt to houses.

6) Dr. Elsa Segura, Advances in Community Surveillance of Trypanosoma cruzi Transmission

This is an ongoing project in Argentina; its partial results were presented. The objective is to integrate community and service in order to transfer knowledge to the community that serve to act in the promotion of Chagas disease control. It is recognized that Chagas Program achieved a good control in this country, practically eliminating *T. cruzi* transmission in Santiago del Estero. Unfortunately, this situation has been reverted lately and the return of transmission is being observed. This project was implemented in the Department of Avellaneda where traditional and cultured communities live. Initially, the community was gathered to take part in meeting, generally in schools, to obtain a dialog frame with the population. From the professors, other sectors that act on the community mobilized and a network that met several times resulted, inducing a reflection process about the way the community acted on Chagas disease. A quantitative analysis of the results obtained 31 months after the beginning of the project was made and revealed, in general, a good community interaction. For this type of intervention, planning in terms of activity and financing is necessary. The sanitary agents were qualified to act jointly with the community. The results demonstrated that the prevalence in children decreased, mainly in children under 9 years old. The community is absorbing the knowledge transferred by the health agent. Unfortunately, this work is partially suspended due to some problems of local support from the health systems.

The following conclusions were presented:

1. The partial results suggest the viability of the proposal at the level of the province central organization;
2. The activities supervision by health agents is necessary;
3. This work demonstrated the advantages of the permanent inclusion of health agents in community work accompanied by a social work network.

Finally, Dr. Segura paid homage to "physicians of radiant action" that work in dreadful conditions.

7) Dr. Antonieta Rojas de Arias, New Triatomine Control and Detection Tools: Use Perspectives

After a partial literature review, the project was presented as an important contribution to the knowledge of *T. infestans's* semio-chemicals and their capacity to attract triatomines, with the perspective of developing a trap that eases the capture of triatomines at low densities. Previously, laboratory studies were carried out to identify compounds showing attractant capacity for triatomines (hexanal, heptanal, nonanal, dipropylsulphoxide, methylbutanol and/or methylbutanol and benzaldehyde), mainly aldehyde compounds with a promoted attraction that was highly dose-dependant, especially for females. Pre-field tests were performed in 12 experimental coops in Argentina while the experimental studies were carried out in Paraguay. A field study financially supported by the European Commission and TDR was made using traps with and without attractants (triatomines feromones) and measurements were made at 1, 3 and 6 months in dwellings houses considered negative by manual search as well as in peridomiciles if they existed. Two traps, one with attractant and a control without attractant, were placed in each dwelling. The traps sensitivity using aldehydes as attractants ranged from 80% to 94% at three months of exposure. The attractant response was different for the different compounds. For example, in the case of ammonium, the attractant capacity decreased to 60% at 20 days attracting only adults while nonanal was able to attract nymphs. A limitation of these traps is the feromones liberation system that should be improved as there is a large competition between different odors and the trap inside the house; a great infestation pressure. There is an Argentinean company willing to develop the trap that should be also tested in other countries.

8) Dr. Elci Villegas Ávila, Insecticide-Impregnated Curtains and Bed Nets to Control Cutaneous Leishmaniasis and Chagas Disease in Venezuela

Curtains and bed nets were developed as tools that could reinforce vertical programs. Trujillo state was selected to develop the proposal because of the high incidence of leishmaniosis. The methodologies used included questionnaires, an observational study and an entomological survey. The houses were arranged in three groups: houses with unimpregnated curtains, houses without curtains and houses with lambdacyhalothrin impregnated curtains. The best results were obtained in the houses with the impregnated curtains that provided a high degree of protection against indoor transmission of cutaneous leishmaniasis. For the trial of Chagas disease, the area of Zaragoza, in Trujillo state, was chosen including 80 houses with 74.3% of infestation and 50.8% of infection. *R. robustus* predominated (88.4%) with 48.9% of infection. The infestation survey of palm trees located up to 35 m of the house, showed 100% of infestation by *R. robustus*. In the period of permanence in the area, the entrance of adult triatomines inside the houses was observed, crashing against lights; children infection was around 12-13%. The conclusion was that the impregnated curtains had effect mechanically protecting and killing triatomines by contact.

9) Dr. João Carlos Pinto Dias, Eradication of Chagas Disease: What Are Its Possibilities?

Though the proposed subject was sufficiently discussed in the previous presentations, being clearly established that eradication is impossible, it is important to review the presentations searching for the continuity of control and improvement of the works that Chagas disease offers for the next 30 years. This time mark (30 years) is justified as it is the time expected for the exhaustion of the infected people in the countries where the disease was better controlled. The elimination is possible but as a possibility restricted in time and space, confirmed by the success where the methods proposed since the 1950's were applied. For this, two determinants are indispensable: political will and technical competence but the current reality is other. During 20–30 years of program, financing was not missing for the control of Chagas disease in Brazil but decentralization changed that. This model is an irreversible reality that has to function and search for a solution so that Chagas disease control is maintained and to solve new problems arising. Meanwhile, attention should be paid to the determination of the strategies selected keeping past conquests and searching for the solution of new challenges like the epidemics by oral infection. Today there is concern about the oral infections being considered more important that the consolidations of the epidemiological surveillance in old endemic areas. The program of vector control performed in Brazil was very successful resulting in the reduction of the incidence of acute cases, severity of the disease,

mortality and hospital costs. Probably due to the management of patients, the life expectation increased being the cases displaced to higher age groups. The major consideration is that most vector species still exist in sylvatic ecotopes of all endemic areas. The elimination of these triatomines does not represent a realistic challenge. The only option for the permanent control in the domiciliary environment is the maintenance of vectors in very low densities. The following pragmatic questions about vector transmission were made for Chagas disease in 2007: Which will be the index of reconstitution of domiciliary populations of triatomines in absence of epidemiological surveillance? How long and how will be sustained the epidemiological surveillance in the decentralized health system? Which is the potential of secondary species to replace primary species in areas under control? Which are the clinical and epidemiological consequences of the introduction of different *T. cruzi* groups in a determined area? The conclusion is ambiguous: the elimination is possible but we are still at risk. There is still much to do in the next two or three decades.

10) Dr. José Rodrigues Coura, Advances in Clinics in American Trypanosomiasis

Initially, Dr. Coura described important and classical concepts of the infection course such as the acute and chronic phases, emphasizing the determinants of the disease severity in both phases and on the associated clinical manifestations (forms of the disease). In spite of many studies, the development pattern of the chronic phase is still unknown but is attributed to the inflammatory response (rupture of the pseudocysts of amastigotes that results in fibrosis) and autoimmunity mechanisms. Though indeterminate forms are asymptomatic and present normal ECG and X-rays, new diagnostics methods have shown the occurrence of clinically imperceptible symptoms like Holter or dynamic electrocardiogram (ventricular extrasystoles and partial A-V blockage); autonomic tests stimulated by atropine, pilocarpine, phenylephrine, cold water and Valsalva manoeuvre could show anomalies (ventricular tachycardia/bradicardia, transitory A-V blockage and alteration in the R-R space). Similar alterations could be evaluated by manometric tests of the esophagus and colon. There is an intriguing question about the cardiac Chagas disease: why does it vary so much? The advances in the clinical evaluation of the cardiac form are related to the following methods:

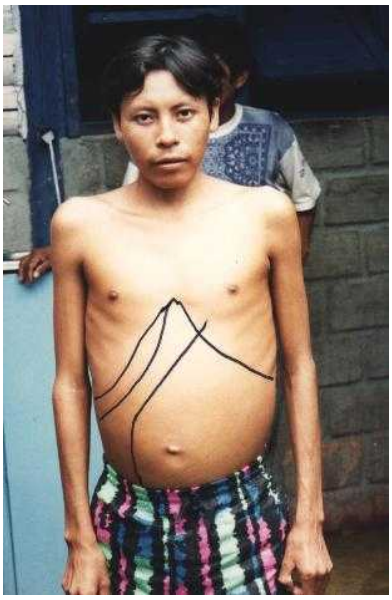
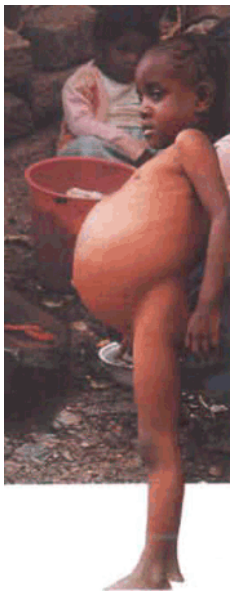
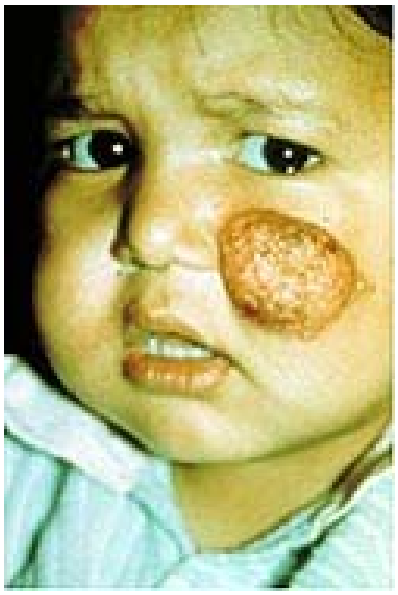
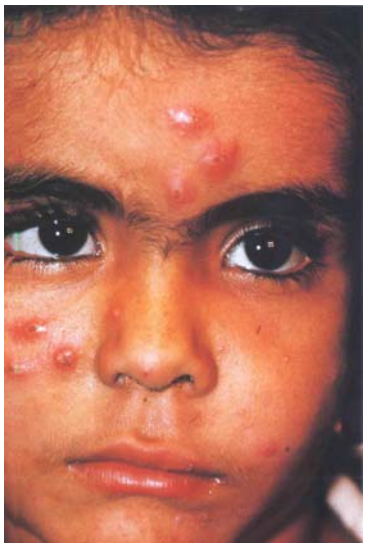
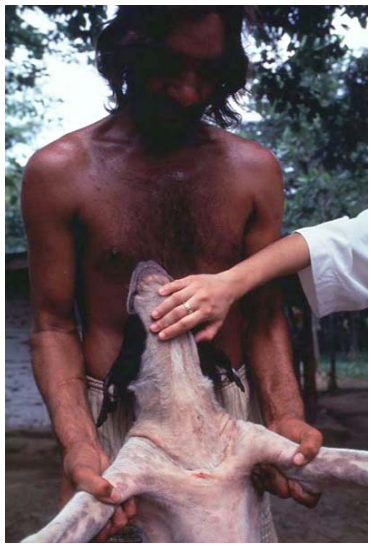
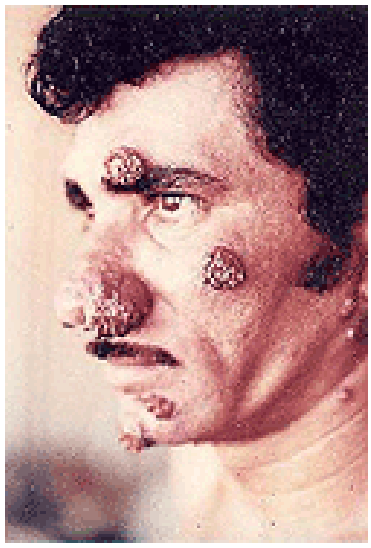
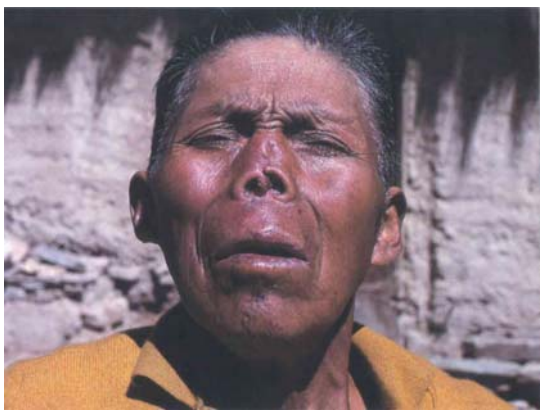
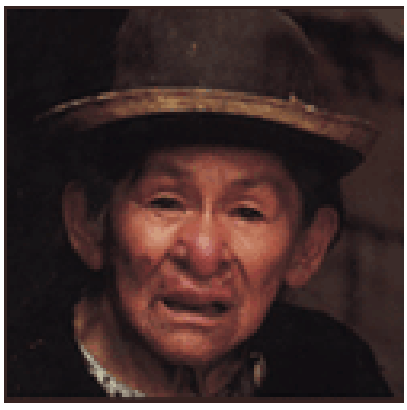
1. Ergometry (functional measurement of the effort capacity, providing information about cardiac rhythm and working capacity);
2. Dynamic electrocardiography (it evaluates the cardiac rhythm at rest or under effort, arrhythmia risks, sudden death and the prognosis of the cardiac disease);

3. Echocardiogram + Doppler (non invasive method to evaluate the morphofunctional structures of the heart);
4. *Myocardial radioisotope scanning (Scintigraphy)* (it reveals important information about myocardium transitory ischemia, necrosis and fibrosis).

The advances in the clinical evaluation of digestive forms include:

a. Teleradioscopy (morphofunctional evaluation of the colon and esophagus);
b. Electromanometry (it evaluates the variation of mobility and pressure in different parts of the esophagus and anorectal region);
c. Telecolonoscopy (it evaluates megacolon complications).

Leishmaniasis

Environmental and Human Leishmaniasis Risk Factors

Roberto Badaró, MD; PhD and Robert T. Schooley, MD
Federal University of Bahia, Brazil
University of California San Diego, USA

Worldwide, 350 million people are at risk for Leishmania infection and 500,000 severe clinical cases are reported annually:

1. *L. chagasi* infection of humans leads to visceral leishmaniasis in a small proportion of infected patients, however, if left untreated it is fatal
2. The rise of HIV infection in highly endemic Leishmania areas where previously the clinical course of leishmaniasis had been controlled has caused an epidemic re-emergence of severe disseminated and exotic forms of leishmaniasis
3. The total number of cases (both asymptomatic and symptomatic) is also believed to be on the rise (1). This is partly due to behaviors, such as mining and timber-related activities in previously unspoiled areas of the Amazon, migration of susceptible peoples into endemic areas, and urbanization (4-6). In light of limited treatment options, there is an urgent need to find new methods by which to control this disease.

Leishmaniasis is a very complex disease phenotype with multiple genetic traits and other risk factors possibly contributing to disease manifestations. In the most severe form of the disease, Visceral Leishmaniasis (VL), the major immunopathological defect appears to be a failure to fully engage intracellular killing mechanisms (7,8). There is increasingly strong evidence from both animal model systems and human studies that a number of genetic determinants play a role in regulating these intracellular killing mechanisms. A number of polymorphisms in genes critical to the induction of innate and adaptive immunity Sic11a1, IL-2, IL4,IL-5,IL-6, IL-10, IL-12,IL-13, IL-15, IL-18;IL-25, IFNGR1, TNFα,TGF-beta, ICOs, CCR6, *IL2RB, P2X7* , CD274 and CD25 have been implicated in modulating pathways involved in engaging these killing mechanisms.

Global Importance of Leishmaniasis

Leishmania is an obligate intracellular parasitic protozoan that causes a wide variety of diseases in man (1). Leishmaniasis consists of a cluster of diseases with widely diverse clinical manifestations, including three major groups of clinical disorders: Visceral Leishmaniasis (known in the old world as Kala-azar), Cutaneous Leishmaniasis, and Mucocutaneous Leishmaniasis (2,9-11). Overall, 350 million people are at risk of Leishmania infection with an incidence of 1,500,000 cases of mucocutaneous disease worldwide. A half million severe clinical cases of visceral Leishmaniasis (VL) are reported yearly worldwide (primarily Brazil), East Africa (primarily Sudan), and Asia (primarily India) (12). HIV infection exacerbates the clinical manifestations of Leishmania infection and increases the proportion of infected individuals who progress to VL. Although initially there was limited geographic overlap between the AIDS epidemic and areas in which Leishmania infection is most common, as the AIDS pandemic spreads to rural areas and leishmaniasis becomes more common in suburban areas, there is an ever greater degree of overlap between the geographical distributions of the two diseases and, in consequence, an increasing incidence of Leishmania/HIV co-infection (3). Visceral Leishmaniasis, one of the most important vector-borne diseases in the world with 50,000 deaths occurring each year In 1990, more than 2 million Disability Adjusted Life Years (DALY) in the world were estimated lost due to VL, second only to malaria among parasitic infections (13).

Parasite-Host Interactions in Visceral Leishmaniasis

Leishmaniasis is transmitted between mammalian hosts by the bite of a sandfly occurring as a zoonosis with transmission from the domestic dog reservoir to humans. Transmission has also been shown to occur through blood transfusions. Visceral leishmaniasis in the Americas is primarily caused by *L. chagasi*. This species is distinct from the anthroponotic form of visceral leishmaniasis found in East Africa and India due to *L. donovani*. Although visceral leishmaniasis has traditionally been associated with rural areas, large outbreaks and epidemics of visceral leishmaniasis have occurred recently in Brazilian cities due to changes in ecology, migration patterns, and epidemiologic conditions (5,6,14,15). Leishmania organisms replicate in phagosomes of inactivated macrophages of patients with knockout Th1 type of cytokine responses (10,16,17).

Clinical Spectrum of Leishmaniasis

Once infection occurs, the clinical manifestations of illness are quite broad. Severity of illness is dependent on a number of parasite and host-derived factors. Individual strains of the parasite manifest specific patterns of disease tropism related to the three major clinical forms: cutaneous, mucocutaneous, or visceral disease (2). In addition, disease manifestations depend on the host immune response (18). Visceral leishmaniasis (VL) varies from an acute disease clinically heralded by profound pancytopenia and massive hepatosplenomegaly to asymptomatic or subclinical infection which evolves over months or years (19-21). In contrast to the overt clinical syndrome of Kala-azar following *L. donovani* infection in which 50% of infected individuals manifest clinical symptoms, *L. chagasi* infection in South and Central America remains subclinical or produces a mild self-healing oligosymptomatic disease in as many as 85% of otherwise healthy individuals (22,23). Indeed, more often than not, infection caused by *L. chagasi* strains is asymptomatic and requires no treatment(19,21,24). The iceberg analogy might represent the spectrum of clinical manifestations of *L. chagasi* infection in South America (Figure 1).

Immune Responses in Human Leishmaniasis

Progression or resolution of Leishmaniasis has been attributed to the difference in responses of Th1 or Th2 CD4+ lymphocytes, respectively. Cytokines produced by mononuclear cells are important regulatory and effector molecules which activate macrophages to kill Leishmania organism (18,32,33). In leishmaniasis several immunological abnormalities have been extensively documented (18,32,34-38).Most with visceral leishmaniasis exhibit deficiencies in delayed type hypersensitivity as manifest by modest or absent lymphoproliferative responses to Leishmania antigen (39). Inadequate production of IL-2, INF-γ,IL-10 and IL-4 during active disease is also documented, reflective of the TH-2 type of Cytokine responses that is characteristic of VL. In addition, a decreased number of T cells, an absence of antigen-reactive cells, and a preferential loss of T regulatory cells that secrete both IFN- γ and IL-10 are observed (16,32,37). In infected humans, the severity of immune dysregulation parallels the degree of disease severity. Infected individuals who control their disease and remain asymptomatic, as well as those successfully treated for VL, exhibit strong Th1 type cytokine responses with high production levels of IL-2 and interferon-γ, as well as strong lymphoproliferative responses to leishmanial antigens (16,34-36,40). The complex cell mediated immune response in visceral Leishmaniasis is T cell dependent (largely CD4) and multi-cytokine driven (16,18,38,41,42). By some unknown mechanism, in the progressive visceral infection Th1 type cellular immune responses fail to mature (38). Suppressive Th2 cell type of cytokine responses, associated with IL4, IL10, IL13 dominate. VL patients usually have very high levels of TNF-α that strongly correlates with disease severity (41). These changes are associated with reduced production

of IL-12 and increased production of TGF-β leading to inhibition of macrophage activation (16,37,43-47). Cytokine-producing cells were compared in Leishmania-activated PBMC cultures from the previous patients and from individuals living in a village where leishmaniasis does not occur. The percentage of IL-10- and IFN-gamma-containing cells was significantly higher in the previous patients than in the controls, indicating that Leishmania-specific T cells producing IL-10 and/or IFN-gamma had been expanded as a result of the infection (48).

Evidence That Genetic Factors Play a Role in Determining Susceptibility to VL

Inherited defects in specific components of the immune system have provided many clues to the immunological mechanisms underlying resistance to microbial infection. Although the mechanisms that underlie the failure to develop an immunologic response capable of controlling the proliferation of Leishmania are incompletely understood, several lines of evidence imply that genetic factors may play a key role. There is epidemiological evidence that visceral leishmaniasis clusters within families in endemic areas. (14) In our previous work in 1986 we were unable to demonstrate clusters within the families of a reported VL cases (24). In a more recent study undertaken in Natal-Brazil, children living in a household with a prior case of visceral leishmaniasis had a 3-fold increased risk of infection. Among 920 children < 11 yrs old, seroconversion was documented in 108 children; the cumulative annual incidence of infection was 4.6%. Of the seroconvertors, 12 (11.1%) developed visceral leishmaniasis during the first 3 years of observation (48) Such observations are interesting but can be easily confounded by environmental factors that are shared by members of the same family. In addition, a comparison of siblings of VL patients with the general population showed that the sibling relative risk of VL was 33.6 (5). A segregation analysis in Brazil showed that genetic factors contribute to the asymptomatic *L. chagasi* infection and was consistent with recessive or additive gene contribution (49). On the other hand, analysis of 87 multi-case pedigrees (824 individuals, 138 nuclear families) also performed in the Northeastern of Brazil demonstrated high relative risk ratio for further siblings of affected pairs (λ_{2s})=34 for *L. chagasi* infection (7).

Human Studies of Genetic Susceptibility Implicated in Leishmaniasis

Genetic studies of susceptibility or resistance to Leishmaniasis has been mainly in new world species (69). Most studies were involving in genotyping candidate genes. Polymorphism genes studies that encode HLA call II and HLA calls III failed to reveal any influence in susceptibility of Visceral Leishmaniasis caused by *L.donovani, L.infantum and L.chagasi* (70-72). Given the broad spectrum of

disease phenotypes in human leishmaniasis it would be expected that genetic polymorphisms affecting the macrophage activation pathway might also be associated with resistance or susceptibility to VL (73). Nramp1 is a transporter protein that pumps divalent cations from intracellular phagosomes that are initially invaded by intracellular organisms (74,75). In humans Nramp1 maps to chromosome 2q35 (76). The role of NRAMP1 in human disease has been most extensively studied in tuberculosis. Although these studies have yielded conflicting results (77,78), a recent study focusing on polymorphic changes in the INT4 and D543N loci of the NRAMP1 gene showed a significant association with severe forms of pulmonary tuberculosis (79). NRAMP1 polymorphisms have also been studied in visceral leishmaniasis, tuberculosis, and leprosy, using multicase family data collected from north-eastern Brazil. This study analyzed the role of candidate genes/regions in determining disease susceptibility. No evidence for linkage between NRAMP1, and susceptibility to tuberculosis or visceral leishmaniasis could be demonstrated in this Brazilian population.(80). On the other hand, another human population study of allelic frequency and heterozygosity for SLC11A1 done in Sudan using multicase families did demonstrate an allelic association between clinical visceral Leishmaniasis and NRAMP1 (81). This study suggested that NRAMP1 (SLC11A1) contributed to but was not fully responsible for the phenotype of *Leishmania* infection. Another study in the Sudanese population more broadly studied possible genetic determinants of VL by focusing on markers located in five chromosomal regions containing the following genes: 2q35 (NRAMP1), 5q31-q33 (Th2 cytokine cluster), 6p21 (HLA/TNF-alpha), 6q23 (INFGRI) and 12q15 (INF-gamma). No evidence of linkage was found with regional microsatellite markers for 5q31-q33 or (HLA/TNF-alpha) and (INFGRI). However, a linkage was found for the 5~CA in the Nramp1 promoter.(82). Polymorphisms linked to IL4/IL9 were also associated with underlying susceptibility to VL. On the other hand, the IFNGR1 gene was not linked to VL but was associated with post kala-azar dermal leishmaniasis (PKDL).(71). In a final study conducted in Brazil, TNF alleles affected disease manifestations. Microsatellite amplification identified 15 different TNFα Microsatellite markers (MSM) alleles in 1,024 individuals. Among 751 individuals included in the association analysis genotyping for the -307 polymorphism, 72 % were homozygous for TNF1 promoter, 26% heterozygous for TNF1:2 and 2% for homozygous for the TNF2 promoter. The strongest association was with individuals with asymptomatic infection (Leishmanin +) favoring the association between asymptomatic infection and the TNF1 promoter (83). Recently in Brazil another gene was implicated in Leishmaniasis, IL-6-174 promoter that influences susceptibility to mucosal but not for localized cutaneous Leishmaniasis. This -174 C allele was demonstrated to have repressive activity for macrophages and was completely overcome by activation of LPS signaling pathway, an important transcription binding sites (NF-κB) for IL-6 promoter(84).

Indeed, genetic susceptibility or resistance appears to play an important role in disease manifestation in Leishmaniasis.

Table 1: Summary of Studies Implicating Specific Genes to Influence susceptibility to Leishmanial or Trypanosomal Infection

Gene (Chromosome)	Study	Parasite	Population	Reference
NRAMP1 (2q35)	Linkage to 5' (CA) repeat in promoter	*L. donovani*	Sudanese	(82)
IL2RB (22q12)	Linkage peak on 22q12	*L. donovani*	Sudanese	(82)
IL4 (5q23.3)	Linkage, association to IL4RP2 and IL4RP1	*L. donovani*	Sudanese	(71)
TNF locus (6p)	Association	*L. chagasi*	Brazilian	(83)
IL- 10	Linkage association to 1q32.1	*T. cruzi*	Brazilian	(94)
IFNGR	Linkage association (6q23)	*L. donovani*	Sudanese	(71)
IL-6 174G/C	Linkage association	*L.brasiliensis*	Brazilian	(84)

Human and Epidemiological Risk Factors

The area of Jacobina, Bahia, Brazil have been extensively observed for potential risk factors for transmission of *L. chagasi* infection as well as progression from asymptomatic infection to full disease (24,30). Various aspects of the zoonotic nature of this disease were temporally explored during the 70 years since Visceral Leishmaniasis was discovered in Bahia (112). In the 1960's the vector and reservoir were explored by Sherlock, I et al (108,113). In the 80's the impact of anemia was revisited (114,115). Subsequent studies have demonstrated that lack of T cell specific immune responses to Leishmania antigens, poor nutritional state and younger age at infection were associated with marked increases in disease rates among the infected population (24,28,35). Although it is clear that immunological disorders associated with nutritional status are important determinants of the progression of *L. chagasi* infection, even when there are overt signs of immunodeficiency not all children progress to frank disease. Several micronutrients, such as Mn+, Cu++, and Fe+ are important minerals related to host defense. Unfortunately, it is almost impossible to estimate intracellular levels of these minerals. We anticipate that indirect observations can be made by looking at the genes that regulate the transport of divalent cations (e.g., Slc11a1 formerly NRAMP1) (116).

Age and nutritional status were clearly demonstrated to be associated with risk of infection and development of diseases (24,28). In the study conducted in Jacobina, Bahia 75% of VL cases occurred in children under five years of age. It was demonstrated that the chance of acquiring Leishmania infection

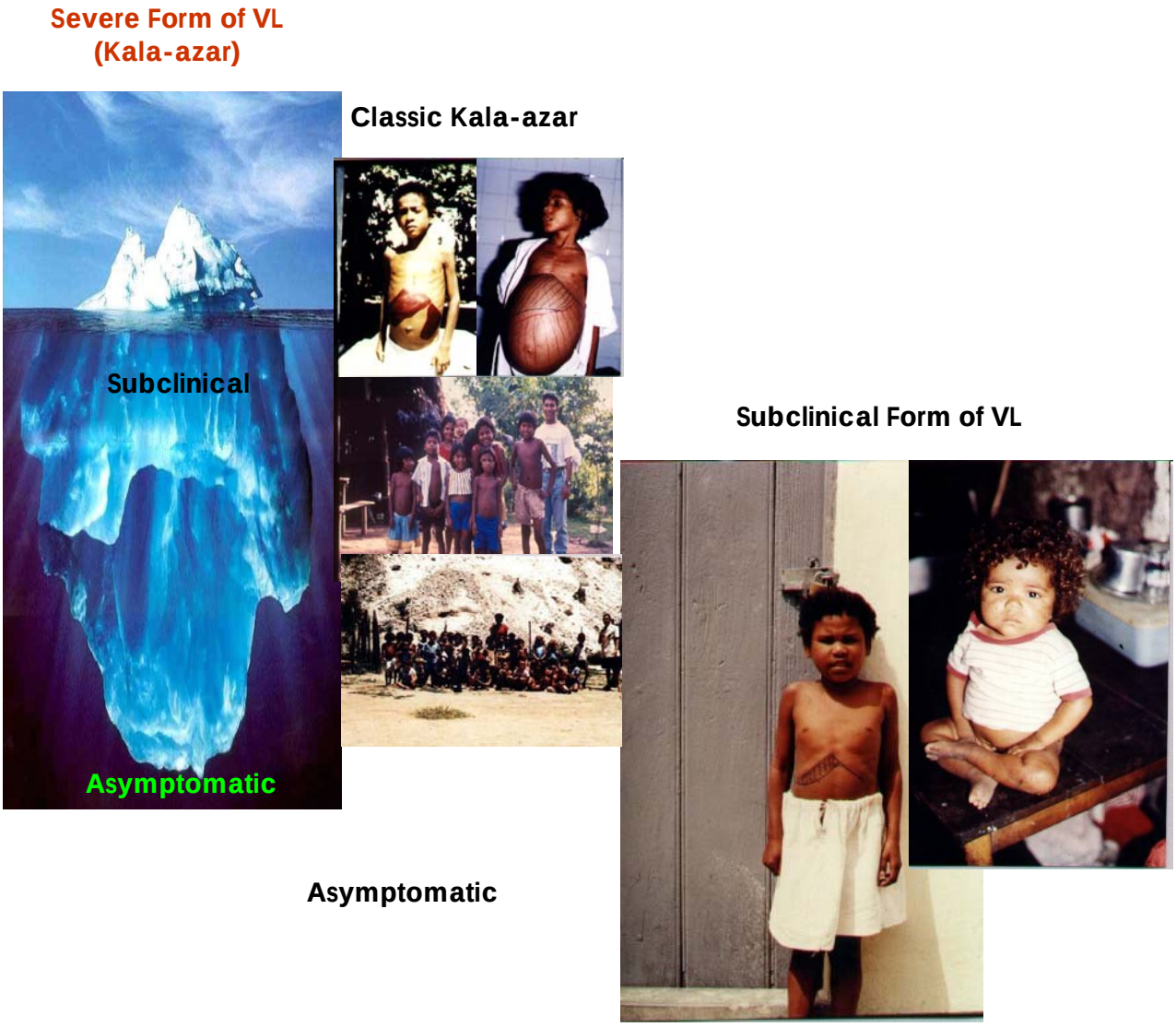
for a two years old child living in the most endemic area was one-in-ten and, if infected, one- in- four for developing disease. If this same child had first degree of malnutrition, the probability of disease was one in two. A seven year old child in the same area had one-in-six chance of infection but, if infected, only one-in-36 chance of disease (24). In the same prospective cohort study it was documented that the annual rate of VL was 8.03 cases/1000 among children with normal nutritional status, compared to 69.44 cases/1000 in children with moderate to severe degree of malnutrition present one year prior to being infected.

Other risk factors include peridomicilary and domiciliary presence of domestic animals such as chickens, cats and dogs (109). An old study from Sherlock, I et al revealed that *L. longipalpis* was easier to catch in domiciles that had animal cages in the back of the house compared to those with no animals in the house (108). It has also been demonstrated elsewhere that physical protection against the vector bite, such as through use of insecticide impregnated bed nets, is associated with significantly lower rates of infection(117). A recent study in Bangladesh found several risk factors associated with infection: age, close proximity to a previous kala-azar patient, bed net use in summer, and cattle density per 1,000 m^2 (118).

In summary, environmental and human risk factors which include a number of parasite host-derived traits are the major determinant of disease manifestation in leishmanial infection. Resistant host remains asymptomatic for the entire life if no acquired immunodeficiency is present. Susceptible host develops a clinical disease, often a severe form of Leishmaniasis and eventually death if not properly treated.

Figure 1:

Clinical Spectrum of Leishmaniasis Following *L. chagasi* Infection in South America



References

(1) Desjeux P. Leishmaniasis. Public health aspects and control. *Clin Dermatol* 1996 Sep;14 (5): 417-23.

(2) Pearson RD, Sousa AQ. Clinical spectrum of Leishmaniasis. *Clin Infect Dis* 1996 Jan 22 (1): 1-13.

(3) Desjeux P, Alvar J. Leishmania/HIV co-infections: epidemiology in Europe. *Ann Trop Med Parasitol* 2003 Oct;97 Suppl 1: 3-15.

(4) Jeronimo SM, Oliveira RM, Mackay S, et al. An urban outbreak of visceral leishmaniasis in Natal, Brazil. *Trans R Soc Trop Med Hyg* 1994 Jul, 88 (4): 386-8.

(5) Arias JR, Monteiro PS, Zicker F. The reemergence of visceral leishmaniasis in Brazil. *Emerg Infect Dis* 1996 Apr 2 (2): 145-6.

(6) Cunha S, Freire M, Eulalio C, et al. Visceral leishmaniasis in a new ecological niche near a major metropolitan area of Brazil. *Trans R Soc Trop Med Hyg* 1995 Mar, 89 (2): 155-8.

(7) Peacock CS, Collins A, Shaw MA, et al. Genetic epidemiology of visceral leishmaniasis in northeastern Brazil. *Genet Epidemiol* 2001 Apr, 20 (3): 383-96.

(8) Skeiky YA, Guderian JA, Benson DR, et al. A recombinant Leishmania antigen that stimulates human peripheral blood mononuclear cells to express a Th1-type cytokine profile and to produce interleukin 12. *J Exp Med* 1995 Apr 1, 181 (4): 1527-37.

(9) Desjeux P. Leishmaniasis: current situation and new perspectives. *Comp Immunol Microbiol Infect Dis* 2004 Sep, 27 (5): 305-18.

(10) Pearson RD, Wheeler DA, Harrison LH, Kay HD. The immunobiology of leishmaniasis. *Rev Infect Dis* 1983 Sep, 5 (5): 907-927.

(11) Desjeux P. Leishmaniasis. *Nat Rev Microbiol* 2004 Sep, 2 (9):692.

(12) Desjeux P. The increase in risk factors for leishmaniasis worldwide. *Trans R Soc Trop Med Hyg* 2001 May, 95 (3): 239-243.

(13) Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet* 1997 May 24, 349 (9064): 1498-1504.

(14) Evans TG, Teixeira MJ, McAuliffe IT, et al. Epidemiology of visceral leishmaniasis in northeast Brazil. *J Infect Dis* 1992 Nov, 166 (5) :1124-32.

(15) Jeronimo SM, Duggal P, Braz RF, et al. An emerging peri-urban pattern of infection with Leishmania chagasi, the protozoan causing visceral leishmaniasis in northeast Brazil. *Scand J Infect Dis* 2004; 36 (6-7): 443-439.

(16) Coffman RL, Correa-Oliviera R, Mocci S. Reversal of polarized T helper 1 and T helper 2 cell populations in murine leishmaniasis. *Ciba Found Symp* 1995, 195: 20-25.

(17) Wilson ME, Pearson RD. Roles of CR3 and mannose receptors in the attachment and ingestion of Leishmania donovani by human mononuclear phagocytes. *Infect Immun* 1988 Feb, 56 (2): 363-369.

(18) Reed SG, Scott P. T-cell and cytokine responses in leishmaniasis. *Curr Opin Immunol* 1993 Aug, 5 (4): 524-531.

(19) Badaro R, Jones TC, Carvalho EM, et al. New perspectives on a subclinical form of visceral leishmaniasis. *J Infect Dis* 1986 Dec, 154 (6): 1003-1011.

(20) Evans T, Reis MF, de Alencar JE, et al. American visceral leishmaniasis (kala-azar). *West J Med* 1985 Jun, 142 (6): 777-781.

(21) Jeronimo SM, Teixeira MJ, Sousa A, Thielking P, Pearson RD, Evans TG. Natural history of Leishmania (Leishmania) chagasi infection in Northeastern Brazil: long-term follow-up. *Clin Infect Dis* 2000 Mar, 30 (3) 608-609.

(22) Thakur CP, Kumar K. Post kala-azar dermal leishmaniasis: a neglected aspect of kala-azar control programmes. *Ann Trop Med Parasitol* 1992 Aug, 86 (4): 355-359.

(23) Thakur CP. Epidemiological, clinical and therapeutic features of Bihar kala-azar (including post kala-azar dermal leishmaniasis) *Trans R Soc Trop Med Hyg* 1984, 78 (3): 391-398.

(24) Badaro R, Jones TC, Lorenco R, et al. A prospective study of visceral leishmaniasis in an endemic area of Brazil. *J Infect Dis* 1986 Oct, 154 (4): 639-649.

(25) Holaday BJ, Pompeu MM, Evans T, et al. Correlates of Leishmania-specific immunity in the clinical spectrum of infection with Leishmania chagasi. *J Infect Dis 1993* Feb, 167 (2): 411-417.

(26) Pearson RD, Cox G, Jeronimo SM, et al. Visceral leishmaniasis: a model for infection-induced cachexia. *Am J Trop Med Hyg* 1992 Jul, 47 (1 Pt 2): 8-15.

(27) Sherlock IA. Ecological interactions of visceral leishmaniasis in the state of Bahia, Brazil. *Mem Inst Oswaldo Cruz* 1996 Nov 91 (6): 671-683.

(28) Cerf BJ, Jones TC, Badaro R, Sampaio D, Teixeira R, Johnson WD, Jr. Malnutrition as a risk factor for severe visceral leishmaniasis. *J Infect Dis* 1987 Dec, 156 (6): 1030-1033.

(29) Badaro R. When Leishmania and HIV Interact, a New Broad Spectrum of Leishmaniasis Occurs. *Braz J Infect Dis* 1997 Jun, 1 (3): 145-148.

(30) Badaro R, Carvalho EM, Rocha H, Queiroz AC, Jones TC. Leishmania donovani: an opportunistic microbe associated with progressive disease in three immunocompromised patients. *Lancet* 1986 Mar 22, 1 (8482): 647-649.

(31) Berenguer J, Gomez-Campdera F, Padilla B, et al. Visceral leishmaniasis (Kala-Azar) in transplant recipients: case report and review. *Transplantation* 1998 May 27, 65 (10): 1401-1404.

(32) Murray HW, Lu CM, Mauze S, et al. Interleukin-10 (IL-10) in experimental visceral leishmaniasis and IL-10 receptor blockade as immunotherapy. *Infect Immun* 2002 Nov, 70 (11): 6284-6293.

(33) Wilson ME, Jeronimo SM, Pearson RD. Immunopathogenesis of infection with the visceralizing Leishmania species. *Microb Pathog* 2005 Apr, 38(4): 147-160.

(34) Carvalho EM, Sampaio D, Bacellar O, Barral A, Badaro R, Barral-Netto M. Immunoregulation in American visceral leishmaniasis. *Mem Inst Oswaldo Cruz* 1988 Nov, 83 Suppl 1: 368-371.

(35) Carvalho EM, Barral A, Pedral-Sampaio D, et al. Immunologic markers of clinical evolution in children recently infected with Leishmania donovani chagasi 133. *J Infect Dis* 1992, Mar, 165(3): 5355-40.

(36) Carvalho EM, Bacellar O, Brownell C, Regis T, Coffman RL, Reed SG. Restoration of IFN-gamma production and lymphocyte proliferation in visceral leishmaniasis. *J Immunol* 1994 Jun 15, 152 (12): 5949-5956.

(37) Ghalib HW, Piuvezam MR, Skeiky YA, et al. Interleukin 10 production correlates with pathology in human Leishmania donovani infections. *J Clin Invest* 1993 Jul, 92 (1): 324-329.

(38) Russo DM, Barral-Netto M, Barral A, Reed SG. Human T-cell responses in Leishmania infections. *Prog Clin Parasitol* 1993, 3: 119-144.

(39) Carvalho EM, Teixeira RS, Johnson WD, Jr. Cell-mediated immunity in American visceral leishmaniasis: reversible immunosuppression during acute infection. Infect Immun 1981 Aug;33(2):498-500.

(40) Bacellar O, Barral-Netto M, Badaro R, Carvalho EM. Gamma interferon production by lymphocytes from children infected with L. chagasi. *Braz J Med Biol Res* 1991, 24 (8): 791-795.

(41) Barral-Netto M, Badaro R, Barral A, et al. Tumor necrosis factor (cachectin) in human visceral leishmaniasis. *J Infect Dis* 1991 Apr, 163 (4): 853-837.

(42) Caldas A, Favali C, Aquino D, et al. Balance of IL-10 and interferon-gamma plasma levels in human visceral leishmaniasis: implications in the pathogenesis. *BMC Infect Dis* 2005, 5:113.

(43) Barral A, Teixeira M, Reis P, et al. Transforming growth factor-beta in human cutaneous leishmaniasis. *Am J Pathol* 1995 Oct, 147 (4): 947-954.

(44) Karp CL, El-Safi SH, Wynn TA, et al. In vivo cytokine profiles in patients with kala-azar. Marked elevation of both interleukin-10 and interferon-gamma. *J Clin Invest* 1993 Apr, 91 (4): 1644-1648.

(45) Murray HW, Hariprashad J, Coffman RL. Behavior of visceral Leishmania donovani in an experimentally induced T helper cell 2 (Th2)-associated response model. *J Exp Med* 1997, Mar 3, 185 (5): 867-874.

(46) Reiner SL, Locksley RM. Cytokines in the differentiation of Th1/Th2 CD4+ subsets in leishmaniasis. *J Cell Biochem* 1993 Dec, 53 (4): 323-328.

(47) Sang DK, Ouma JH, John CC, et al. Increased levels of soluble interleukin-4 receptor in the sera of patients with visceral leishmaniasis. *J Infect Dis* 1999 Mar, 179 (3): 743-746.

(48) Kemp K, Kemp M, Kharazmi A, et al. Leishmania-specific T cells expressing interferon-gamma (IFN-gamma) and IL-10 upon activation are expanded in individuals cured of visceral leishmaniasis. *Clin Exp Immunol* 1999 Jun, 116 (3): 500-504.

(49) Cabello PH, Lima AM, Azevedo ES, Krieger H. Familial aggregation of Leishmania chagasi infection in northeastern Brazil. *Am J Trop Med Hyg* 1995 Apr, 52 (4): 364-365.

(50) Blackwell JM. Genetic susceptibility to leishmanial infections: studies in mice and man. *Parasitology* 1996, 112 Suppl: S67-S74.

(51) Blackwell J, Freeman J, Bradley D. Influence of H-2 complex on acquired resistance to Leishmania donovani infection in mice. *Nature* 1980 Jan 3, 283 (5742): 72-74.

(52) Vidal S, Gros P, Skamene E. Natural resistance to infection with intracellular parasites: molecular genetics identifies Nramp1 as the Bcg/Ity/Lsh locus. *J Leukoc Biol* 1995 Oct, 58 (4): 382-390.

(53) Vidal S, Tremblay ML, Govoni G, et al. The Ity/Lsh/Bcg locus: natural resistance to infection with intracellular parasites is abrogated by disruption of the Nramp1 gene. *J Exp Med* 1995 Sep 1, 182 (3): 655-666.

(54) Blackwell JM, Searle S, Mohamed H, White JK. Divalent cation transport and susceptibility to infectious and autoimmune disease: continuation of the Ity/Lsh/Bcg/Nramp1/Slc11a1 gene story. *Immunol Lett* 2003 Jan 22, 85 (2): 197-203.

(55) Wyllie S, Seu P, Goss JA. The natural resistance-associated macrophage protein 1 Slc11a1 (formerly Nramp1) and iron metabolism in macrophages. *Microbes Infect* 2002 Mar, 4 (3): 351-359.

(56) White JK, Mastroeni P, Popoff JF, Evans CA, Blackwell JM. Slc11a1-mediated resistance to Salmonella enterica serovar Typhimurium and Leishmania donovani infections does not require functional inducible nitric oxide synthase or phagocyte oxidase activity. *J Leukoc Biol* 2005 Mar, 77 (3): 311-320.

(57) Baguet A, Epler J, Wen KW, Bix M. A Leishmania major response locus identified by interval-specific congenic mapping of a T helper type 2 cell bias-controlling quantitative trait locus 4. *J Exp Med* 2004 Dec 20, 200 (12): 1605-1612.

(58) Ansel KM, Greenwald RJ, Agarwal S, et al. Deletion of a conserved Il4 silencer impairs T helper type 1-mediated immunity. *Nat Immunol* 2004 Dec, 5 (12): 1251-1259.

(59) Noben-Trauth N, Paul WE, Sacks DL. IL-4- and IL-4 receptor-deficient BALB/c mice reveal differences in susceptibility to Leishmania major parasite substrains. *J Immunol* 1999 May 15, 162 (10): 6132-6140.

(60) Mason NJ, Artis D, Hunter CA. New lessons from old pathogens: what parasitic infections have taught us about the role of nuclear factor-kappaB in the regulation of immunity. *Immunol Rev* 2004 Oct, 201: 48-56.

(61) Wei XQ, Niedbala W, Xu D, Luo ZX, Pollock KG, Brewer JM. Host genetic background determines whether IL-18 deficiency results in increased susceptibility or resistance to murine Leishmania major infection. *Immunol Lett* 2004 Jun 15, 94 (1-2): 35-37.

(62) Li Y, Ishii K, Hisaeda H, et al. IL-18 gene therapy develops Th1-type immune responses in Leishmania major-infected BALB/c mice: is the effect mediated by the CpG signaling TLR9? *Gene Ther* 2004 Jun, 11 (11): 941-948.

(63) Moore KW, de Waal MR, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001, 19: 683-765.

(64) Kane MM, Mosser DM. The role of IL-10 in promoting disease progression in leishmaniasis. *J Immunol* 2001 Jan 15, 166 (2): 1141-1147.

(65) Groux H, Cottrez F, Rouleau M, et al. A transgenic model to analyze the immunoregulatory role of IL-10 secreted by antigen-presenting cells. *J Immunol* 1999 Feb 1, 162 (3): 1723-1729.

(66) Roberts M, Alexander J, Blackwell JM. Genetic analysis of Leishmania mexicana infection in mice: single gene (Scl-2) controlled predisposition to cutaneous lesion development. *J Immunogenet* 1990 Feb, 17 (1-2): 89-100.

(67) Murphy ML, Wille U, Villegas EN, Hunter CA, Farrell JP. IL-10 mediates susceptibility to Leishmania donovani infection. *Eur J Immunol* 2001 Oct, 31 (10): 2848-2856.

(68) Beebe AM, Mauze S, Schork NJ, Coffman RL. Serial backcross mapping of multiple loci associated with resistance to Leishmania major in mice. *Immunity* 1997 May, 6 (5): 551-557.

(69) Lipoldova M, Demant P. Genetic susceptibility to infectious disease: lessons from mouse models of leishmaniasis. *Nat Rev Genet* 2006 Apr, 7 (4): 294-305.

(70) Meddeb-Garnaoui A, Gritli S, Garbouj S, et al. Association analysis of HLA-class II and class III gene polymorphisms in the susceptibility to mediterranean visceral leishmaniasis. *Hum Immunol* 2001 May, 62 (5): 509-517.

(71) Mohamed HS, Ibrahim ME, Miller EN, et al. Genetic susceptibility to visceral leishmaniasis in The Sudan: linkage and association with IL4 and IFNGR1. *Genes Immun* 2003 Jul, 4 (5): 351-355.

(72) Peacock CS, Sanjeevi CB, Shaw MA, et al. Genetic analysis of multicase families of visceral leishmaniasis in northeastern Brazil: no major role for class II or class III regions of HLA. *Genes Immun* 2002 Sep, 3 (6): 350-358.

(73) Blackwell JM. Genetics and genomics in infectious disease susceptibility. *Trends Mol Med* 2001 Nov, 7 (11): 521-526.

(74) Gruenheid S, Canonne-Hergaux F, Gauthier S, Hackam DJ, Grinstein S, Gros P. The iron transport protein NRAMP2 is an integral membrane glycoprotein that colocalizes with transferrin in recycling endosomes. *J Exp Med* 1999 Mar 1, 189 (5): 831-841.

(75) Tabuchi M, Yoshimori T, Yamaguchi K, Yoshida T, Kishi F. Human NRAMP2/DMT1, which mediates iron transport across endosomal membranes, is localized to late endosomes and lysosomes in HEp-2 cells. *J Biol Chem* 2000 Jul 21, 275 (29): 22220-8.

(76) White JK, Shaw MA, Barton CH, et al. Genetic and physical mapping of 2q35 in the region of the NRAMP and IL8R genes: identification of a polymorphic repeat in exon 2 of NRAMP. *Genomics* 1994 Nov 15, 24 (2): 295-302.

(77) Bellamy R. The natural resistance-associated macrophage protein and susceptibility to intracellular pathogens. *Microbes Infect* 1999 Jan, 1 (1): 23-27.

(78) Bellamy R. NRAMP1 and susceptibility to tuberculosis. *Int J Tuberc Lung Dis* 2002 Sep, 6 (9): 747.

(79) Zhang W, Shao L, Weng X, et al. Variants of the natural resistance-associated macrophage protein 1 gene (NRAMP1) are associated with severe forms of pulmonary tuberculosis. *Clin Infect Dis* 2005 May 1, 40 (9): 1232-1236.

(80) Blackwell JM, Black GF, Peacock CS, et al. Immunogenetics of leishmanial and mycobacterial infections: the Belem Family Study. *Philos Trans R Soc Lond B Biol Sci* 1997 Sep 29, 352 (1359): 1331-1345.

(81) Mohamed HS, Ibrahim ME, Miller EN, et al. SLC11A1 (formerly NRAMP1) and susceptibility to visceral leishmaniasis in The Sudan. *Eur J Hum Genet* 2004 Jan, 12 (1): 66-74.

(82) Bucheton B, Abel L, Kheir MM, et al. Genetic control of visceral leishmaniasis in a Sudanese population: candidate gene testing indicates a linkage to the NRAMP1 region. *Genes Immun* 2003 Mar, 4 (2): 104-109.

(83) Karplus TM, Jeronimo SM, Chang H, et al. Association between the tumor necrosis factor locus and the clinical outcome of *Leishmania chagasi* infection. *Infect Immun* 2002 Dec, 70 (12): 6919-6925.

(84) Castellucci L, Menezes E, Oliveira J, et al. IL6 -174 G/C promoter polymorphism influences susceptibility to mucosal but not localized cutaneous leishmaniasis in Brazil. *J Infect Dis* 2006 Aug 15, 194 (4): 519-527.

(85) Loots GG, Locksley RM, Blankespoor CM, et al. Identification of a coordinate regulator of interleukins 4, 13, and 5 by cross-species sequence comparisons. *Science* 2000 Apr 7, 288 (5463): 136-140.

(86) Smale ST, Fisher AG. Chromatin structure and gene regulation in the immune system. *Annu Rev Immunol* 2002, 20: 427-462.

(87) Bix M, Locksley RM. Independent and epigenetic regulation of the interleukin-4 alleles in CD4+ T cells. *Science* 1998 Aug 28, 281 (5381): 1352-1354.

(88) Kelly BL, Locksley RM. Coordinate regulation of the IL-4, IL-13, and IL-5 cytokine cluster in Th2 clones revealed by allelic expression patterns. *J Immunol* 2000 Sep 15, 165(6): 2982-2986.

(89) Guler ML, Gorham JD, Hsieh CS, et al. Genetic susceptibility to Leishmania: IL-12 responsiveness in TH1 cell development. *Science* 1996 Feb 16, 271 (5251): 984-987.

(90) Haimila KE, Partanen JA, Holopainen PM. Genetic polymorphism of the human ICOS gene. *Immunogenetics* 2002 Mar, 53 (12): 1028-1032.

(91) eu-Nosjean MC, Massacrier C, Vanbervliet B, Fridman WH, Caux C. IL-10 induces CCR6 expression during Langerhans cell development while IL-4 and IFN-gamma suppress it. *J Immunol* 2001 Nov 15, 167 (10): 5594-602.

(92) Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* 2000 Oct 2, 192 (7): 1027-1034.

(93) Fort MM, Cheung J, Yen D, et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies *in vivo*. *Immunity* 2001 Dec, 15(6): 985-995.

(94) Moreno M, Silva EL, Ramirez LE, Palacio LG, Rivera D, rcos-Burgos M. Chagas' disease susceptibility/resistance: linkage disequilibrium analysis suggests epistasis between major histocompatibility complex and interleukin-10. *Tissue Antigens* 2004 Jul, 64 (1): 18-24.

(95) Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. *Genet Med* 2002 Mar, 4 (2): 45-61.

(96) Yang BZ, Zhao H, Kranzler HR, Gelernter J. Practical population group assignment with selected informative markers: characteristics and properties of Bayesian clustering via structure. *Genet Epidemiol* 2005 May, 28 (4): 302-312.

(97) Freedman ML, Reich D, Penney KL, et al. Assessing the impact of population stratification on genetic association studies. *Nat Genet* 2004 Apr, 36 (4): 388-393.

(98) Cardon LR, Palmer LJ. Population stratification and spurious allelic association. *Lancet* 2003 Feb 15, 361 (9357): 598-604.

(99) Azevedo ES, Fortuna CM, Silva KM, et al. Spread and diversity of human populations in Bahia, Brazil. *Hum Biol* 1982 May, 54 (2): 329-341.

(100) Krieger H, Morton NE, Mi MP, Azevedo E, Freire-Maia A, Yasuda N. Racial admixture in north-eastern Brazil. *Ann Hum Genet* 1965 Nov, 29 (2): 113-125.

(101) Azevedo ES. Subgroup studies of black admixture within a mixed population of Bahia, Brazil. *Ann Hum Genet* 1980 Jul, 44 (Pt 1): 55-60.

(102) Pollitzer WS, Azevedo ES, Barefoot J, et al. Characteristics of a population sample of Jacobina, Bahia, Brazil. *Hum Biol* 1982 Dec, 54 (4): 697-707.

(103) Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 1993 Mar, 52 (3): 506-516.

(104) Begue P. [Infection and sickle cell anemia]. *Pathol Biol* (Paris) 1999 Jan, 47 (1): 19-25.

(105) Hand WL. Inhibition of cell-free oxidative bactericidal activity by erythrocytes and hemoglobin. *Infect Immun* 1984 May, 44 (2): 465-468.

(106) Tamouza R, Neonato MG, Busson M, et al. Infectious complications in sickle cell disease are influenced by HLA class II alleles. *Hum Immunol* 2002 Mar, 63 (3): 194-199.

(107) Azevedo E. Historical note on inheritance of sickle cell anemia. *Am J Hum Genet* 1973 Jul, 25 (4): 457-458.

(108) Sherlock IA, Guitton N. [Findings on kala-zar in Jacobina, Bahia. IV. Seasonal and hourly variations of Phlebotomus longipalpis]. *Rev Bras Malariol Doenças Trop* 1969 Oct, 21 (4): 715-727.

(109) Ashford DA, Badaro R, Eulalio C, et al. Studies on the control of visceral leishmaniasis: validation of the Falcon assay screening test—enzyme-linked immunosorbent assay (FAST-ELISA) for field diagnosis of canine visceral leishmaniasis. *Am J Trop Med Hyg* 1993, Jan, 48 (1):1-8.

(110) Ashford RW, Desjeux P, Deraadt P. Estimation of population at risk of infection and number of cases of Leishmaniasis. Parasitol Today 1992 Mar;8(3):104-5.

(111) Badaro R, Pedral-Sampaio D, Johnson WD, Jr., Reed SG. Evaluation of the stability of a soluble intradermal skin test antigen preparation in American visceral leishmaniasis. *Trans R Soc Trop Med Hyg* 1990 Mar, 84 (2): 226-227.

(112) Badaro R. Progress of research in visceral leishmaniasis in the endemic area of Jacobina-Bahia 1934-1989. *Rev Soc Bras Med Trop* 1988 Oct, 21 (4): 159-164.

(113) Sherlock IA. Outbreak of kala-azar in the central zone of the state of Bahia. *Rev Bras Malariol Doencas Trop* 1964 Apr, 16: 157-170.

(114) Pontes de Carvalho LC, Templeman J, Wick G, Roitt IM. The role of self-antigen in the development of autoimmunity in obese strain chickens with spontaneous autoallergic thyroiditis. *J Exp Med* 1982 May 1, 155 (5): 1255-1266.

(115) Pontes de Carvalho LC, Badaro R, Carvalho EM, et al. Nature and incidence of erythrocyte-bound IgG and some aspects of the physiopathogenesis of anaemia in American visceral leishmaniasis. *Clin Exp Immunol* 1986 Jun, 64 (3): 495-502.

(116) Beutler E. Cell biology. "Pumping" iron: the proteins. *Science* 2004 Dec 17, 306 (5704): 2051-2053.

(117) Bern C, Joshi AB, Jha SN, et al. Factors associated with visceral leishmaniasis in Nepal: bed-net use is strongly protective. *Am J Trop Med Hyg* 2000 Sep, 63 (3-4): 184-188.

(118) Bern C, Hightower AW, Chowdhury R, et al. Risk factors for kala-azar in Bangladesh. *Emerg Infect Dis* 2005 May, 11 (5): 655-662.

(121) Sherlock IA, Guitton N. Findings on kala-azar in Jacobina, Bahia. 3. Various data on *Phlebotomus longipalpis*, most important vector. *Rev Bras Malariol Doenças Trop* 1969 Jul, 21 (3): 341-348.

(122) Badaro R, Benson D, Eulalio MC, et al. rK39: a cloned antigen of Leishmania chagasi that predicts active visceral leishmaniasis. *J Infect Dis* 1996 Mar, 173 (3): 758-761.

(123) Murray HW. Treatment of visceral leishmaniasis in 2004. *Am J Trop Med Hyg* 2004 Dec, 71 (6): 787-794.

(124) Reed SG, Badaro R, Masur H, et al. Selection of a skin test antigen for American visceral leishmaniasis. *Am J Trop Med Hyg* 1986 Jan, 35 (1): 79-85.

Research on Vector of Leishmaniasis: Trends and Questions

Oscar Daniel Salomón

CeNDIE-ANLIS- Ministry of Health/CONICET, Argentina

Trends

The research on vectors of leishmaniasis has to deal with two levels of complexity, the leishmaniases as a human construct/biological entity, and the vector diversity itself. The former involves a broad spectra of clinical diseases, immune host responses, parasite species-subspecies-variants, primary-secondary-permissive vectors, host/reservoirs, eco-epidemiological scenarios, transmission cycles, and human risky cultural-rooted behaviors. The latter includes vector cryptic species/clades, each one with different ecological niches and adaptability to environment and climate changes, and vector a-vector b-non vector sand flies-host-parasite interactions. Therefore, the research requires to be addressed by multi/inter/transdisciplinary eco-systemic approaches, while the vector topics need time-space accurate scale definitions, to frame any conclusion beyond focus results. Further, any proposal on research priorities should also try to avoid: a) "to follow what is fashionable and technologically advanced (...diverting) funds away from the goals of disease control" (Ashford in WHO 2004), b) the biases due to the consultant own field of expertise, and c) what Stendhal defined in The Charterhouse of Parma as science as the meeting of keen fools and shrewd hypocrites (*une réunion de nigauds enthousiastes et d 'hypocrites adroits*).

The incidence of leishmaniases increased worldwide during the last 20 years, so these diseases were included in the WHO-TDR as category 1. The Scientific Working Group on insect vectors met in 2002 (WHO, 2003) provided a research agenda on leishmaniases for the period 2003-2008. The "Report on Leishmaniasis" suggested also research lines up to the year 2010 (WHO 2004), 3 out of the 22 high priorities selected were vector-related. These two milestone documents urged to develop molecular tools, predictive models, and operational research to assure effective implementation of control strategies (Table 1).

Table 1: Research on Vectors of Leishmaniases, Priorities by WHO Documents and Papers in NCBI (PubMed) Database by *Lutzomyia/Phlebotomus/Phlebotominae* Search (up to 500-10/10/2007) distributed by topic worldwide [Ww] and related to Latin Americas' parasite or disease [LA])

	WHO 2002*	WHO 2004**	Ww (%)	LA (%)	LA/Ww (%)
			New species 38 (7.6)	24 (9.0)	63.2
			Physiobiology 46 (9.2)	19 (7.1)	41.3
Taxonomy, Genetics, Genomics, Proteomics	*High priority* Molecular taxonomy	*High priority* Molec. Taxonomy	Genetics, species-population structure 34 (6.8)	23 (8.6)	67.6
	High priority Molecular population structure, dynamics	Genomic Epidemiological tools			
	Medium priority Primary vectors genome project		Genomics/proteomics 16 (3.2)	10 (3.8)	62.5
Ecoepidemiology	*High priority* Predictive models	*High priority* Enviromental risk factors studies	Ecoepidemiology 191 (38.2)	105 (39.5)	55.0
		Climate, land use/cover changes, disease expansion			
Surveillance, Control	*Medium priority* Community-based strategies	*High priority* ITNs evaluation	Control 45 (9.0)	11 (4.1)	34.4
		Delthametrin-collar dogs			
	Medium priority Control exhophilic vector tools	Implementation of control strategies			
	Tools monitoring Insecticide resistance	Tools insecticide resistance	Insecticide resistance 2 (0.4)	(0)	0
Natural infection	Molecular tools to assess infection rates	*High priority* Tools to natural infections	Natural infection 39 (7.8)	28 (10.5)	71.8
Vector-parasite-host interaction	*Medium priority* Vector saliva vaccine	Vector saliva vaccine	Saliva-Vaccine 34 (6.8)	21 (7.9)	61.8
	Vector-parasite interaction	Vector-parasite interaction	Vector-Parasite-Host 46 (9.2)	18 (6.8)	39.1
			Pheromone 9 (1.8)	7 (2.6)	77.8
Total			500 (100)	266 (100)	53.2

* Research priorities according to WHO 2003 rated as high priorities, medium priorities, or emphasized out of the priorization scheme.

** Research priorities according to WHO 2004 rated as high priorities, or emphasized out of the priorization scheme in the annexes of the document without priority label.

Table 2: Papers in NCBI (PubMed) database by Leishmaniasis search (up to 1500-10/10/2007) distributed by topic worldwide (Ww) and related to Latin Americas' parasite or disease (LA)

		Ww (%)	LA (%)	LA/Ww %
Immunology Pathology	Cutaneous leishmaniasis	171 (11.4)	76 (15.3)	44.4
	Visceral leishmaniasis	131(8.7)	25 (5.0)	19.1
	Vaccine cutaneous leishmaniasis	25 (1.7)	10 (2.0)	40.0
	Vaccine visceral leishmaniasis	48 (3.2)	19 (3.8)	39.6
Diagnosis Treatment	Case description	234 (15.7)	43 (8.7)	18.4
	Serologic tools, treatment protocol	200 (13.3)	48 (9.7)	24.0
Parasitology	Leishmanicidal drugs *in vitro*	104 (6.9)	34 (6.8)	32.7
	Parasite biology, isolation, culture, animal models,	78 (5.2)	29 (5.8)	37.2
Epidemiology	Without vectors	191 (12.7)	50 (10.1)	26.2
	Reservoirs	26 (1.7)	17 (3.4)	65.4
Genetics Genomics Proteomics Diagnostic tools		112 (7.5)	41 (8.2)	36.6
Social sciences		6 (0.4)	4 (0.8)	66.7
Vectors		174 (11.6)	101 (20.3)	58.0
Total		1500 (100)	497 (100)	33.1

However, the papers attached to WHO 2004 described also the lack of reliable and validated field-based data due to "the diversity of biotopes, hosts and vectors (...and) changes in epidemiological pattern (...) emphasizing the necessity for regular updating" (Guizani in WHO 2004), while the PAHO/WHO Expert Meeting on America Visceral Leishmaniasis recommended a record system of phlebotominae at village locality, insecticide and control standardization, and cost-effectivity studies (Panaftosa 2006). In fact, the papers published recently are still mainly focused in clinical, immunological or ecological oriented work with lab-based or field-based traditional tools (Tables 1 & 2). These tables deserves an in- depth analysis by topic and geographical region, but here we only will discuss the papers about Latin America leishmaniases' vectors during the reviewed period (58 out of 266 in Memorias do Instituto Oswaldo Cruz).

Questions

Phlebotominae Species Identification

American Visceral Leishmaniasis (AVL): *Lu longipalpis* was defined as a species complex or complex of incipient species with 2 to 5 clades, besides *Lu. pseudolongipalpi* and *L. cruzi* already a different species. The criteria used were based on differences about morphological characters, ecological distribution, isoenzymes, male lovesongs, sex pheromone, genetic and molecular markers, and the saliva vasodilator maxadilan (Arrivillaga et al 2002, Bottecchia et al 2004, Hamilton et al 2005, Lainson and Rangel 2005, Watts et al 2005, de Queiroz et al 2006, Bauzer et al 2007).

American Cutaneous Leishmaniasis (ACL): *Lu. intermedia* from two distinct eco-epidemiologic regions were reported with different genetic population structures, domestic/peridomestic different level of variability, and a clustering tendency by micro-habitat (RAPDs) (de Souza Rocha et al 2007). Males from domestic/peridomestic/sylvatic habitats seems to belong to the same gene pool with at least 50 individuals per generation migrating between the three habitats (MLEE), but polymorphic markers showed two clusters domestic/peridomestic and sylvatic (RAPD-PCR) (Meneses et al 2005). *Lu. intermedia* is sympatric and allopatric with its sibling species *Lu. neivai* (Andrade Filho et al, 2007), and with *Lu. whitmani*, with behavioral and morphological differences among them (Souza et al 2005). *Lu. intermedia-Lu.whitmani* shares a high number of polymorphisms, and the introgression in their period nuclear gene is possible (Mazzoni et al 2006). *Lu. whitmani* spatial clusters were also reported, with genetic flow between populations from different landscapes, diverse peridomestic and climate adaptability, and degree of anthropophily (de Souza 2004, Margonari et al 2004, Costa et al, 2007a). On the other hand, morphometric differences due to the environment could be overimposed to the geographic/genic variants (Dujardin et al 2003, Dujardin and Le Pont 2004).

The taxonomic status of the different inter/intra population molecular clusters is an ongoing current controversy, but the epidemiological meaning of these cryptic/sibling species/subspecies/clades should also be discussed. Further, although the molecular approaches contributed to discriminate species (Escovar et al 2002), and to support hypothesis of dispersion, the results were based on very heterogeneous procedures, size and diversity of samples, molecular and analytical tools. Therefore, as new species in the Americas are described every year by phenetic taxonomy, a new cladistic logic as that proposed by Galati (2003) with a comprehensive data-source, a general agreement, and an accurate description of the epidemiological roles of each entity is yet required. In this topic there are also a need of research on polymorphic markers of parasites and vectors that allow molecular epidemiology/population dynamics studies at focus scale (Rodriguez et al 2005, Silva et al 2005, Ochsenreither et al 2006, Rotureau et al 2006a,b, Montoya et al 2007).

Vector incrimination

Natural infections reported by PCR or PCR-hybridization genotyping:

1. *Leishmania spp.*: *Lu. whitmani* (0.4%) (Oliveira-Pereira et al 2006), *Lu. neivai* (9,1%) (Cordoba-Lanus et al 2006), *Lu. ininii* (Fouque et al 2007), *Lu. longipalpis* (rDNA and mini-exon genes 10.39%, dissection 2.6%) (do Nacimento et al 2007), *Lu. longipalpis* (1.1%) (Silva et al 2007), and *Lu. hartamani* (5/319 cytochrome b gene mass screening, sand flies by PCR-RFLP 18S rRNA gene) (Kato et al, 2007).
2. *L. braziliensis*: *Lu. spinicrassa* (nuclear DNA 11.6%) (Perruolo et al 2006), *Lu. intermedia* (1.6%), *Lu. migonei* (PCR multiplex 6%) (de Pita-Pereira et al 2005), *Lu. ovallesi* and *Lu. gomezi* (PCR multiplex) (Jorquera et al 2005), and *Lu. edwardsi* (Sucen 2005).
3. *L. panamensis: Lu. trapidoi* (Santamaría E et al, 2006).
4. *L. guyanensis*: *Lu. flaviscutellata* (2.32%) (Fouque et al 2007).
5. *L. peruviana: Lu. ayacuchensis* (Caceres et al 2004), *Lu. peruensis* (1/75 PCR-RFLP) (Perez et al 2007).
6. *L. mexicana: Lu. ayacuchensis* (3.3% mass screening, 3.5% dissection) (Kato et al 2005), *L. ovallesi* (PCR multiplex) (Jorquera et al 2005).

The infection rates were computed from individual and pool samples, with different protocols, and from different transmission scenarios. Therefore, in order to improve the assessment of natural infection rates we need affordable standardized techniques for vector and parasite identification from individual samples, easily collected and stored in field actual conditions. An attempt was made by Paiva et al (2006, 2007). However, even with a standardized technique, and with an eventually revised criteria of vector incrimination, it should be taking into account the difference between a parasite genetic fragment in the gut and infective parasites in the stomodeal valve ready to be transmitted, and also the quality of the sample as the infected sand flies usually cluster in time and space.

Other issues related with vector incrimination are: Role of secondary vectors to sustain transmission? Primary/secondary vectors or vector communities? Human ACL requires a zoophilic species together with a prevalent anthropophilic one? (Chaves and Anez 2004). Sand flies adjust eating habits to host availability (Barata et al 2005), indoor vectors had relative higher rates of rodent blood (Afonso et al 2005), and wild rat blood produce the highest infection rates in the sand fly *Lu. migonei* by *L. braziliensis* and *L. amazonensis* (Nieves and Pimenta 2002), so what is the influence of the blood source in the infection rate?

Vector Physiology, Parasite-Vector-Host Interaction

Vector physiology studies were focused mainly in the factors that allow species-specific survival and maturation of the parasite, and enhance pathology or protection in the host. *Lu. longipalpis-L. chagasi* interactions and *Lu. longipalpis* salivary components in mammals were the most studied models.

Once ingested by the sand fly the *Leishmania* should survive to digestive enzymatic activity and the peritrophic matrix trapping, and so the structure and blood regulation of these steps are essential to an effective parasite transmission. *Lu longipalpis* cDNA library revealed putative proteins involved in the barrier function of the gut, in digestive physiology, and in the immune response that might be associated with the response of the vector to the development of the parasite (Dillon et al 2006). Blood-feed regulated putative midgut trypsins, vacuolar ATPase subunit C, larval proteases, and peritrophic matrix structure-chemical composition-timing were also reported (Secundino et al 2005, Fazito do Vale et al 2007, Telleria et al 2007, Ramalho-Ortigão et al 2007). Transfection of embryonic cells of *Lu. longipalpis* with double-stranded viral RNAs was performed and natural nucleic acid-induced non-specific antiviral response was observed (Pitaluga et al 2007).

Vector-parasite species specificity was attributed to parasite species and stage- specific lipophosphoglycan (LPG), exposed from the membrane, and complementary to sand fly lectins-like molecules exposed to the gut lumen. Antibodies against LPG were evaluated to reduce parasite development in the sand fly (Pinto-da-Silva et al 2005, Anjili et al 2006). However, this mechanism is far to be universal, the report of phlebotominae midgut glycoprotein bearing terminal N-acetyl-galactosamine together with the occurrence of a lectin-like activity on *Leishmania* surface, as the heparin binding proteins in the promastigote surface of *L. braziliensis,* open a new area for the study of new interactions and permissive vectors (Azevedo-Pereira et al 2007, Myskova et al 2007).

Finally, the vector-parasite-host interactions were also studied. During the differentiation of *Leishmania* to infective stages the parasite secretes a gel-like plug of filamentous proteophosphoglycan that increases the vector biting persistence (re-feeding after interruption), promotes feeding on multiple hosts, and damages the stomodeal valve inducing regurgitation (Volf et al 2004, Rigers and Bates 2007). Sand fly saliva maxidilan up regulates the cytokines associated with a type 2 response (IL-10, IL-6, and TGF-beta) and down regulates type 1 cytokines (IL-12p70 and TNF-alpha) and nitric oxide. This immuno-modulation increased the chance of *Leishmania* transmission, early survival in host macrophages, leukocyte recruitment and exacerbated the clinical disease (Brodie et al 2007, Monteiro et

al 2007, Rohousova and Volf 2006). On the other hand, a host repeatedly exposed to sand fly not-infective bites seems to be protected against *Leishmania* infection, and reduces the fecundity and longevity of the sand flies that bite on him, so some experimental vaccines include saliva components (Oliveira et al 2005, Vilela et al 2006, Rogers et al 2006, Andrade et al 2007). The sand fly digestion, fecundity and oviposition were also influenced by the source of blood, *Lu. ovallesi* fed on chicken has the higher rates of these parameters, so chickens could have a main role in vector dynamics even when they are neither reservoirs nor hosts of *Leishmania* (Noguera et al 2006, Marassá et al 2006, Alexander et al 2002). The molecular control of circadian clocks in *Lu. longipalpis* was also studied (Meireles-Filho et al 2006).

Insecticide resistance, a priority research line in the WHO documents, was scarcely represented in the papers in the reviewed period. There are one report of esterases level from Sri Lanka, and the AceI gene from *Lu longipalpis* was partially characterized, and so the possibility for the development of a resistant phenotype to organophosphates and carbamates suggested (Coutinho-Abreu et al 2007). However, as we will discuss there are still much to do to evaluate insecticide effective measures in the Americas as a first step in the control topic.

Ecoepidemiology

The risk factors usually associated with leishmaniasis in the larger scales are conjectures or "ecological epidemiology", but there is a lack of multidisciplinary investigations, time and space properly designed, to inputs predictive operational models (Correa et al 2007). Therefore, risk assessment and impact monitoring leishmaniasis-focused should be included in many mega-projects that involves environment modifications (e.g. eco-tourism supported by the Amazon Cooperation Council OTCA, the initiative for Integration of Regional Infrastructure in South America IIRSA, mineral resources exploration-exploitation, urbanization management, etc.). Further, as captures during selective logging in native forest, and within and around national parks in the region usually found competent vectors (Curi et al 2006, Afonso et al 2007, Pessoa et al 2007).

Landscape destruction was associated with leishmaniasis in the short time by the ephemeral interaction of man and the zoonotic sylvatic cycle (Ashford 2007). In fact, it could be an immediate increase in incidence among deforestation-related workers, new settlers in the deforested area, and in closer villages by vector/reservoir dispersion during a short period. However, there are also eventual medium term and long term associations to be taken into account in the impact evaluations: concentration of vectors and reservoirs in residual patches (hot spots), cultural interactions with these patches (animal management),

domestication of permissive vectors, survival of vectors in fragmented landscapes, and microclimate variations (Shaw J 2007, Volf and Myskova 2007).

Lu. longipalpis according to the focus remains associated to AVL sylvatic cycles, it could invade or colonize houses with chickens and dogs in the forest fringe, in rural villages, in periruban environments, and recently generates urban transmission cycles, with different overlapping degrees among these scenarios (Lainson and Rangel 2005, Feliciangeli et al 2006). But, how *Lu. longipalpis* an insect with low mobility, with limited flight range, populations of fairly static nature, and improbable unintentionally human transportation of infected adults, eggs or immature stages could generate progressively new urban foci of AVL from the north of Brazil to Argentina in less than 15 years? (Lainson and Rangel 2005, Salomon and Orellano 2005, Bauzer et al 2007) Contributed the deforestation and gas-duct building in Mato Grosso (Araujo e Silva et al 2007)?

Many modeling approaches associated cases *a posteriori* with broad ENSO or poverty indexes (Chaves and Pascual 2006, Cardenas et al 2006, Bavia 2005, Peterson and Shaw 2003), or the multilevel analysis exclude sand fly changing dynamics in space and time (Werneck et al 2007). The vector abundance and infection rates may be the better time and space indicator of transmission, as the human cases have different incubation-perception-diagnosis-reporting periods and infection-living sites. In some AVL foci up to 84% of the human cases were related with canine cases (Marognari et al 2006), in others there is a high correlation only with vector abundance (Franca-Silva et al 2005).

We still need to understand the distribution of AVL but also ACL vectors local variants at foci level (seasonality, breeding and resting sites). *Lu. neivai* metapopulation patterns with source populations and peridomestic local populations were suggested (Salomon et al 2004), mark-release-recapture experiments in an endemic rural area recaptured 7.58% of the sand flies, 90% up to 70 m, and 16 % released in forest edge were caught in the peridomestic habitat (Casanova et al 2005). Thus, to face the leishmaniases emergence it is required field-based information from micro-scale distribution (lower and upper temperature thresholds, growing degree days parameter), to the dynamics of dispersion at continental level, where the experience of ecological niche and forecasting models from agriculture pests could be useful (Kasap and Alten 2005, Malone et al 2006, Pasotti et al 2006).

In conclusion, there are a significant number of papers that performed transversal and longitudinal sand fly trapping, that associates the vector abundance and diversity with climate, sociodemographic and landscape variables, human incidence, and eco-epidemiological scenarios (Ximenes et al 2006, Salomon

et al 2006). These studies proposed recommendations to improve surveillance and control strategies, but there are almost none operational research about implementation in actual pilot programmatic activities.

On the other hand, any atypical transmission report (without reservoirs, without incriminated vectors, out of the endemic area), if the data are fairly acceptable should be investigated as an early warning sentinel data of changing or unknown transmission patterns or alternative vectors (Lainson and Rangel 2005, de Carvalho et al 2007, Dias et al 2007, Shaw J 2007).

Surveillance and Control

AVL: The well known deltamethrin-treated collar dog controlled trial in Iran provided collars to all the dogs for two transmission seasons reducing the odd of serum conversion in dogs 61% and in children 53% each year, without statistical differences between years (Davies in WHO 2004), but the transmission season there lasts 2-3 months, and in the Americas it could be an all year round transmission season. In Amazonian 25% deltamethrin EC-treated bednets (ITNs) reduced human landing (80%), and increased the 24-h vector mortality (98%) (Courtenay et al 2007). Intradomestic spraying with the lambda-cyhalothrin 25 mg/m^2 plus spatial fogging with fenitrothion 30 g/ha around the houses significantly reduced vector populations with residual effect on the wall for about 3 months (Feliciangeli et al 2003b). Alpha-cypermethrin spraying in Campo Grande (MS) at four-month intervals reduced sand fly abundance (Araujo e Silva et al 2007), while a randomized controlled trial performed in Teresina (Piuaí) showed that culling dogs decreased incidence 80% more than blocks with only household spraying, and peridomestic spraying may increase the dispersion of sand flies and human-vector effective contacts (Costa et al 2007b). Thus, immediate and peripheral impact of spraying should also be included in the trial designs.

ACL: Vectorial control in even more difficult due to the complexity of ACL epizoology, and the few options available for control of exophilic/exophagic vectors. Deltamethrin impregnated bednets, repellent (20% DEET, 0.5% permethrin), modification of sand fly resting sites, and health education were evaluated in a group-randomized trial in Colombia, the intervention reduced 58% the incidence but the small number of cases renders the conclusions imprecise (Rojas et al 2006). In Parana, Brazil, neither screening the windows, nor the peridomestic cleaning of organic materials or the spraying with cypetrmethrin 125 mg/m^2 has significant effectivity (Teodoro et al 2006). Lambda-cyhalothrin 25 mg/m^2 house spraying in *Lu. ovallesi* infested area of Miranda, Venezuela, recovered the abundance of control sites after 7-11 weeks (females-males) (Feliciangeli et al 2003a). On the other hand, the "control is not possible" statement of researchers about ACL exophilic vectors had a negative effect on control program

managers, while for each eco-epidemiological scenario could be developed and evaluated specific control measures based on risk factors and risk human behaviors.

Sexual pheromones studies, mainly of *Lu. longipalpis,* for intraspecific characterization and to develop an eventual lured trap began a couple of years ago. A pheromone with a recruitment effect in females and males, and the host odor synergistic and activating effect on the pheromone's attraction on virgin females were reported (Spiegel et al 2005, Bray & Hamilton 2007). However, the attractants are still far to be used in baited traps mainly due to its short range and the shape of the dose-response curve. Biological control or molecular control are on prelimary stage, few papers were published on microsporidae, nematodes, and *Wolbachia* (biological driver), mainly in *Phlebotomus* species (Matos et al 2006). Biocides via feces of host animals, and azadirachtin effect on larval diet was evaluated (Andrade Coelho et al 2006), but the actual breeding-larval growing site is unknown for many species or it is too disperse to think in immature stage control (Feliciangeli 2004).

In conclusion, control trials diverse methods, protocols and outcomes measured (incidence of cases, infection, sandfly density) does not allow again to discuss the contradictory results in a comparative way. The eco-epidemiology of the vector is as important to design a control strategy as the insecticide dose or active principle. There are few examples of scaling up procedures from experimental houses to pilot controlled studies at village level, and none on surveillance methods. The diversion effect on sand fly populations should be included in the protocols, but also cost-effectiveness and acceptability assessment, and even the human time schedule of risky behaviors or dogs-owner interaction (AVL) (Oliveira et al 2006, Gouvêa et al, 2007). A community-based surveillance and control strategy was recommended (WHO 2003), however only four papers related to social sciences appear in this period about knowledge and attitudes (Pardo et al 2006), health education and social representation (dos Resis et al 2006), validation of ethnographic methodology for construction of socio-cultural scenarios related to leishmaniasis (Garcia Guevara 2007), and analyzes of audiovisual production about health education in Brazil (Pimenta et al 2007).

Remaining Trends, Questions, and Possible Answers

a. *Heterogeneity of leishmaniases transmission patterns*: Minimum standard protocols for each topic defined by consensus of experts from all the countries involved (handbook with periodical updates). Research networks to synergize different capabilities (Leish-Med). Multicentric networks to replicate the standard protocols and so validate or frame conclusions in different eco-epidemiological *Leishmania*/vector scenarios.

b. *Complexity of interacting factors in leishmaniasis transmission*: Integrated inter/multi/transdisciplinaries approaches. In intervention trials should be included at least controls, economic and acceptability evaluations.

c. *Leishmaniases human incidence epidemic trend and vector domestication associated to environmental modifications, from multinational mega-projects to village peripheral growing*: Research projects to assess risk, propose and validate mitigation measures, and monitoring short to long term impact of the anthropic interventions in the environment. The risk assessment/impact evaluation required by the financial agencies, when it has epidemiological meaning, should include the topic of leishmaniases (human, vectors and reservoirs) explicitly, and with time and space consistency between the variables measured and the conclusions expected.

d. *Scarce inputs from research to actual surveillance and control strategies*: Pilot projects that scales up research-based promising proposals (standard protocols).

e. *Specific questions*:

   i. *Species identification-vector incrimination*: Validated standard protocols adequate to field conditions. Vector and parasite taxonomic identification and infections rates assessment. Regular monitoring and evaluation of epidemiological role of each species/subspecies/clades-variants

   ii. *Parasite-vector-host interactions*: Molecular and eco-epidemiological factors (blood source, plant diet, parasite offering, seasonality) that modulates the species-specific competent-permissive-refractoriness and so the infectivity outcome. Vector saliva host protection and infection enhancement.

   iii. *Eco-epidemiology*: Vector population-parasite transmission structure and dynamics from focus to regional levels, including vector biology and behavior (exo/endophilic, exo/endophagic, breeding/resting sites, adaptation to periomestic/fragmentary environments, survival and biting rates). Impact monitoring of atypical transmission reports, and developmental mega-projects. Dispersion studies from AVL continental

dispersion to microfoci dispersion (vector and parasite markers/techniques appropriate to study molecular epidemiology at focus level). The minimum standard protocols should be cultural-sensitive, and validated for different eco-epidemiological scenarios. The ecological niche-spatial analysis-dynamic risk map models, and descriptive-predictive tools should be oriented to bring actual operational answers.

iv. *Surveillance and control*: The standard protocols should include cost-effective and acceptability evaluations, diversion effect up to the border of the intervention, risky behavior human schedule, and other minimal inputs to be determined by the experts. Progressive scale-up of the most effective strategies, validating each step up to an actual programme level. Vector surveillance community-based strategies remain to be developed.

References

Afonso MM, Gomes AC, Meneses CR, Rangel EF 2005.Studies on the feeding habits of *Lutzomyia (N.) intermedia (Diptera, Psychodidae),* vector of cutaneous leishmaniasis in Brazil. *Cad Saude Publica* 21: 1816-1820.

Afonso MM, Costa WA, Azevedo AC, Costa SM, Vilela ML, Rangel EF 2007.Data on sand fly fauna *(Diptera, Psychodidae, Phlebotominae)* in Itatiaia National Park, Rio de Janeiro State, Brazil. *Cad Saude Publica* 23: 725-730.

Alexander B, de Carvalho RL, McCallum H, Pereira MH 2002.Role of the domestic chicken (Gallus gallus) in the epidemiology of urban visceral leishmaniasis in Brazil. *Emerg Infect Dis* 8:1480-1485.

Andrade BB, de Oliveira CI, Brodskyn CI, Barral A, Barral-Netto M 2007. Role of sand fly saliva in human and experimental leishmaniasis: current insights. *Scand J Immunol* 66: 122-127.

Andrade Coelho CA, de Souza NA, Feder MD, da Silva CE, Garcia Ede S, Azambuja P, Gonzalez MS, Rangel EF 2006. Effects of azadirachtin on the development and mortality of *Lutzomyia longipalpis* larvae *(Diptera: Psychodidae: Phlebotominae). J Med Entomol* 43:262-266.

Andrade Filho JD, Galati EA, Falcão AL 2007. *Nyssomyia intermedia* (Lutz & Neiva, 1912) and *Nyssomyia neiva*i (Pinto, 1926) *(Diptera: Psychodidae: Phlebotominae)* geographical distribution and epidemiological importance. *Mem Inst Oswaldo Cruz* 102: 481-487.

Anjili C, Langat B, Ngumbi P, Mbati PA, Githure J, Tonui WK 2006. Effects of anti-*Leishmania* monoclonal antibodies on the development of *Leishmania major* in *Phlebotomus duboscqi (Diptera: Psychodidae). East Afr Med* J 83: 72-78.

Araujo e Silva E, Andreotti R, Honer MR 2007. Comportamento de *Lutzomyia longipalpis*, vetor principal da leishmaniose visceral americana, em Campo Grande, Estado do Mato Grosso do Sul. *Rev Soc Bras Med Trop* 40: 420-425.

Arrivillaga JC, Norris DE, Feliciangeli MD, Lanzaro GC 2002.Phylogeography of the neotropical sand fly *Lutzomyia longipalpis* inferred from mitochondrial DNA sequences. *Infect Genet Evol* 2: 83-95.

Ashford RW 2007. Disease as a stabilizing factor in the protection of landscape: the leishmaniases models. *EcoHealth* 4: 99-103.

Azevedo-Pereira RL, Pereira MC, Oliveria-Junior FO, Brazil RP, Cortes LM, Madeira MF, Santos AL, Toma L, Alves CR 2007.Heparin binding proteins from *Leishmania (Viannia) braziliensis* promastigotes. *Vet Parasitol* 145: 234-239.

Barata RA, Franca-Silva JC, Mayrink W, Silva JC, Prata A, Lorosa ES, Fiuza JA, Goncalves CM, Paula KM, Dias ES 2005. Aspectos da ecologia e do comportamento de flebotomíneos em área endêmica de leishmaniose visceral, Minas Gerais. *Rev Soc Bras Med Trop* 38: 421-425.

Bauzer LG, Souza NA, Maingon RD, Peixoto AA 2007. *Lutzomyia longipalpis* in Brazil: a complex or a single species? A mini-review. *Mem Inst Oswaldo Cruz* 102: 1-12.

Bavia ME, Carneiro DD, Gurgel Hda C, Madureira Filho C, Barbosa MG 2005. Remote Sensing and Geographic Information Systems and risk of American visceral leishmaniasis in Bahia, Brazil. *Parasitologia* 47: 165-169.

Bottecchia M, Oliveira SG, Bauzer LG, Souza NA, Ward RD, Garner KJ, Kyriacou CP, Peixoto AA 2004. Genetic divergence in the cacophony IVS6 intron among five Brazilian populations of *Lutzomyia longipalpis*. J Mol Evol 58:754-61.

Bray DP, Hamilton JG 2007. Host odor synergizes attraction of virgin female *Lutzomyia longipalpis (Diptera: Psychodidae). J Med Entomol* 44: 779-787.

Brodie TM, Smith MC, Morris RV, Titus RG 2007.Immunomodulatory effects of the *Lutzomyia longipalpis* salivary gland protein maxadilan on mouse macrophages. *Infect Immun* 75: 2359-2365.

Cáceres AG, Villaseca P, Dujardin JC, Banuls AL, Inga R, López M, Arana M, Le Ray D, Arévalo J 2004. Epidemiology of Andean cutaneous leishmaniasis: incrimination of *Lutzomyia ayacuchensis (Diptera: psychodidae)* as a vector of *Leishmania* in geographically isolated, upland valleys of Peru. *Am J Trop Med Hyg* 70: 607-612.

Cardenas R, Sandoval CM, Rodriguez-Morales AJ, Franco-Paredes C 2006.Impact of climate variability in the occurrence of leishmaniasis in northeastern Colombia. *Am J Trop Med Hyg* 75: 273-237.

Casanova C, Costa AI, Natal D 2005. Dispersal pattern of the sand fly *Lutzomyia neivai (Diptera: Psychodidae)* in a cutaneous leishmaniasis endemic rural area in Southeastern Brazil. *Mem Inst Oswaldo Cruz* 100: 719-724.

Chaves LF, Anez N 2004.Species co-occurrence and feeding behavior in sand fly transmission of American cutaneous leishmaniasis in western Venezuela. *Acta Trop* 92: 219-224.

Chaves LF, Pascual M 2006. Climate cycles and forecasts of cutaneous leishmaniasis, a nonstationary vector-borne disease. *PLoS Med*. 2006 Aug, 3 (8): e295.

Cordoba-Lanus E, De Grosso ML, Pinero JE, Valladares B, Salomon OD 2006. Natural infection of *Lutzomyia neivai* with *Leishmania spp*. in northwestern Argentina. *Acta Trop* 98: 1-5.

Correa Antonialli SA, Torres TG, Paranhos Filho AC, Tolezano JE 2007.Spatial analysis of American Visceral Leishmaniasis in Mato Grosso do Sul State, Central Brazil. *J Infect* 54: 509-514.

Costa SM, Cechinel M, Bandeira V, Zannuncio JC, Lainson R, Rangel EF 2007a. *Lutzomyia (Nyssomyia) whitmani s.l.* (Antunes & Coutinho, 1939) *(Diptera: Psychodidae: Phlebotominae):* geographical distribution and the epidemiology of American cutaneous leishmaniasis in Brazil-mini-review. *Mem Inst Oswaldo Cruz* 102: 149-153.

Costa CH, Tapety CM, Werneck GL 2007b. Controle da leishmaniose visceral em meio urbano: estudo de intervenção randomizado fatorial. *Rev Soc Bras Med Trop* 40: 415-419.

Courtenay O, Gillingwater K, Gomes PA, Garcez LM, Davies CR 2007. Deltamethrin-impregnated bednets reduce human landing rates of sandfly vector *Lutzomyia longipalpis* in Amazon households. *Med Vet Entomol* 21: 168-176.

Coutinho-Abreu IV, Balbino VQ, Valenzuela JG, Sonoda IV, Ramalho-Ortigao JM 2007. Structural characterization of acetylcholinesterase 1 from the sand fly *Lutzomyia longipalpis (Diptera: Psychodidae). J Med Entomol* 44: 639-650.

Curi NH, Miranda I, Talamoni SA 2006.Serologic evidence of *Leishmania* infection in free-ranging wild and domestic canids around a Brazilian National Park. *Mem Inst Oswaldo Cruz* 101: 99-101.

de Carvalho MR, Lima BS, Marinho-Junior JF, da Silva FJ, Valenca HF, Almeida Fde A, da Silva AL, Brandao-Filho SP 2007. Phlebotomine sandfly species from an American visceral leishmaniasis area in the Northern Rainforest region of Pernambuco State, Brazil. *Cad Saude Publica* 23: 1227-1232.

de Pita-Pereira D, Alves CR, Souza MB, Brazil RP, Bertho AL, de Figueiredo Barbosa A, Britto CC 2005.Identification of naturally infected *Lutzomyia intermedia* and *Lutzomyia migonei* with *Leishmania (Viannia) braziliensis* in Rio de Janeiro (Brazil) revealed by a PCR multiplex non-isotopic hybridisation assay. *Trans R Soc Trop Med Hyg* 99: 905-913.

de Queiroz Balbino V, Coutinho-Abreu IV, Sonoda IV, Melo MA, de Andrade PP, de Castro JA, Rebelo JM, Carvalho SM, Ramalho-Ortigao M 2006.Genetic structure of natural populations of the sand fly *Lutzomyia longipalpis (Diptera: Psychodidae)* from the Brazilian northeastern region. *Acta Trop* 98: 15-24.

de Souza CM, Fortes-Dias CL, Linardi PM, Dias ES 2004.Phenetic studies on randomly amplified polymorphic DNA-polymerase chain reaction-variability of four geographical populations of *Lutzomyia whitmani (Diptera: Psychodidae)* in Brazil. *Rev Soc Bras Med Trop* 37: 148-153.

de Souza Rocha L, Falqueto A, dos Santos CB, Grimaldi G Jr, Cupolillo E 2007. Genetic structure of *Lutzomyia (Nyssomyia) intermedia* populations from two ecologic regions in Brazil where transmission of *Leishmania (Viannia) braziliensis* reflects distinct eco-epidemiologic features. *Am J Trop Med Hyg* 76: 559-565.

Dias ES, Franca-Silva JC, da Silva JC, Monteiro EM, de Paula KM, Goncalves CM, Barata RA 2007.Flebotomíneos *(Diptera: Psychodidae)* de um foco de leishmaniose tegumentar no Estado de Minas Gerais. *Rev Soc Bras Med Trop* 40: 49-52.

Dillon RJ, Ivens AC, Churcher C, Holroyd N, Quail MA, Rogers ME, Soares MB, Bonaldo MF, Casavant TL, Lehane MJ, Bates PA.Analysis of ESTs from Lutzomyia longipalpis sand flies and their contribution toward understanding the insect-parasite relationship. *Genomics* 88: 831-840.

do Nascimento JC, de Paiva BR, dos Santos Malafronte R, Fernandes WD, Galati EA 2007.Natural infection of phlebotomines *(Diptera: Psychodidae)* in a visceral-leishmaniasis focus in Mato Grosso do Sul, Brazil. *Rev Inst Med Trop S Paulo* 49: 119-122.

dos Reis DC, Gazzinelli A, Silva CA, Gazzinelli MF 2006. Health education and social representation: an experience with the control of tegumentary leishmaniasis in an endemic area in Minas Gerais, Brazil. *Cad Saude Publica* 22: 2301-2310.

Dujardin JP, Le Pont F, Baylac M 2003. Geographical versus interspecific differentiation of sand flies *(Diptera: Psychodidae):* a landmark data analysis. *Bull Entomol Res* 93: 87-90.

Dujardin JP, Le Pont F 2004.Geographic variation of metric properties within the neotropical sandflies. *Infect Genet Evol* 4: 353-359.

Escovar J, Ferro C, Cardenas E, Bello F 2002. Comparacion cariotipica de cinco especies de *Lutzomyia (Diptera: Psychodidae)* de la serie townsendi grupo verrucarum en Colombia. *Biomédica* 2002 Dec, 22 (4): 499-509.

Fazito do Vale V, Pereira MH, Gontijo NF 2007. Midgut pH profile and protein digestion in the larvae of *Lutzomyia longipalpis (Diptera: Psychodidae). J Insect Physiol,* Jun 17 [E-pub ahead of print]

Feliciangeli MD 2004.Natural breeding places of phlebotomine sandflies. *Med Vet Entomol* 18: 71-80.

Feliciangeli MD, Mazzarri MB, Campbell-Lendrum D, Maroli M, Maingon R 2003a. Cutaneous leishmaniasis vector control perspectives using lambdacyhalothrin residual house spraying in El Ingenio, Miranda State, Venezuela. *Trans R Soc Trop Med Hyg* 97: 641-646.

Feliciangeli MD, Mazzarri MB, Blas SS, Zerpa O 2003b. Control trial of *Lutzomyia longipalpis* s.l. in the Island of Margarita, Venezuela. *Trop Med Int Health* 8: 1131-1136.

Feliciangeli MD, Delgado O, Suarez B, Bravo A 2006. *Leishmania* and sand flies: proximity to woodland as a risk factor for infection in a rural focus of visceral leishmaniasis in west central Venezuela. *Trop Med Int Health* 11: 1785-1791.

Fouque F, Gaborit P, Issaly J, Carinci R, Gantier JC, Ravel C, Dedet JP 2007.Phlebotomine sand flies *(Diptera: Psychodidae)* associated with changing patterns in the transmission of the human cutaneous leishmaniasis in French Guiana. *Mem Inst Oswaldo Cruz* 102: 35-40.

Franca-Silva JC, Barata RA, Costa RT, Monteiro EM, Machado-Coelho GL, Vieira EP, Prata A, Mayrink W, Nascimento E, Fortes-Dias CL, da Silva JC, Dias ES 2005.Importance of *Lutzomyia longipalpis* in the dynamics of transmission of canine visceral leishmaniasis in the endemic area of - Porteirinha Municipality, Minas Gerais, Brazil. *Vet Parasitol* 131: 213-220.

Galati EAB 2003. Classificação de Phlebotominae. in Rangel EF and Lainson R, Flebotomíneos do Brasil. Fiocruz, RJ, Brazil. *Chapt* 2.1: 23-52.

Garcia Guevara B 2007. Aporte de la etnografía en el conocimiento de los códigos socioculturales de la leishmaniasis cutánea localizada en un programa de educación para la salud, en Venezuela. *Cad Saude Publica* 23 Suppl 1: S75-83.

Gouvêa MV, Werneck GL, Costa CH, de Amorim Carvalho FA. Factors associated to Montenegro skin test positivity in Teresina, Brazil. *Acta Trop* 2007 Aug 2 [E-pub ahead of print].

Hamilton JG, Maingon RD, Alexander B, Ward RD, Brazil RP 2005.Analysis of the sex pheromone extract of individual male *Lutzomyia longipalpis* sandflies from six regions in Brazil. *Med Vet Entomol* 19: 480-488.

Jorquera A, Gonzalez R, Marchan-Marcano E, Oviedo M, Matos M 2005. Multiplex-PCR for detection of natural *Leishmania* infection in *Lutzomyia spp.* captured in an endemic region for cutaneous leishmaniasis in state of Sucre, Venezuela. *Mem Inst Oswaldo Cruz* 100: 45-48.

Kasap OE, Alten B 2005.Laboratory estimation of degree-day developmental requirements of *Phlebotomus papatasi (Diptera: Psychodidae). J Vector Ecol* 30: 328-333.

Kato H, Uezato H, Katakura K, Calvopina M, Marco JD, Barroso PA, Gomez EA, Mimori T, Korenaga M, Iwata H, Nonaka S, Hashiguchi Y 2005. Detection and identification of *Leishmania* species within naturally infected sand flies in the Andean areas of Ecuador by a polymerase chain reaction. *Am J Trop Med Hyg* 72: 87-93.

Kato H, Uezato H, Gomez EA, Terayama Y, Calvopina M, Iwata H, Hashiguchi Y 2007. Establishment of a mass screening method of sand fly vectors for *Leishmania* infection by molecular biological methods. *Am J Trop Med Hyg* 77: 324-329.

Lainson R, Rangel EF 2005. *Lutzomyia longipalpis* and the eco-epidemiology of American visceral leishmaniasis, with particular reference to Brazil: a review. *Mem Inst Oswaldo Cruz* 100: 811-827.

Malone JB, Nieto P, Tadesse A 2006. Biology-based mapping of vector-borne parasites by geographic information systems and remote sensing. *Parasitologia* 48: 77-79.

Marassá AM, Consales CA, Galati EA, Nunes VL 2006.Identificação do sangue ingerido por *Lutzomyia (Lutzomyia*) *longipalpis* (Lutz & Neiva, 1912) e *Lutzomyia* (*Lutzomyia*) *almerioi* (Galati & Nunes, 1999) pela técnica imunoenzimática do ELISA de captura, no sistema avidina-biotina. *Rev Soc Bras Med Trop* 2006 Mar-Apr, 39 (2): 183-186.

Margonari CS, Fortes-Dias CL, Dias ES 2004.Genetic variability in geographical populations of *Lutzomyia whitmani* elucidated by RAPD-PCR. *J Med Entomol* 41: 187-192

Margonari C, Freitas CR, Ribeiro RC, Moura AC, Timbo M, Gripp AH, Pessanha JE, Dias ES 2006. Epidemiology of visceral leishmaniasis through spatial analysis, in Belo Horizonte municipality, state of Minas Gerais, Brazil. *Mem Inst Oswaldo Cruz* 101: 31-38.

Matos E, Mendonca I, Azevedo C 2006. *Vavraia lutzomyiae* n. sp. *(Phylum Microspora)* infecting the sandfly *Lutzomyia longipalpis (Psychodidae, Phlebotominae),* a vector of human visceral leishmaniasis. *Eur J Protistol* 42: 21-28.

Mazzoni CJ, Souza NA, Andrade-Coelho C, Kyriacou CP, Peixoto AA 2006 .Molecular polymorphism, differentiation and introgression in the period gene between *Lutzomyia intermedia* and *Lutzomyia whitmani*. *BMC Evol Biol* 2006 Oct 27, 6: 85.

Meireles-Filho AC, Amoretty PR, Souza NA, Kyriacou CP, Peixoto AA 2006. Rhythmic expression of the cycle gene in a hematophagous insect vector. *BMC Mol Biol* 2006 Oct 27; 7: 38.

Meneses CR, Cupolillo E, Monteiro F, Rangel EF 2005. Micro-geographical variation among male populations of the sandfly, *Lutzomyia (Nyssomyia) intermedia*, from an endemic area of American cutaneous leishmaniasis in the state of Rio de Janeiro, Brazil. *Med Vet Entomol* 19: 38-47.

Monteiro MC, Lima HC, Souza AA, Titus RG, Romao PR, Cunha FQ 2007. Effect of *Lutzomyia longipalpis* salivary gland extracts on leukocyte migration induced by Leishmania major. *Am J Trop Med Hyg* 76: 88-94.

Montoya L, Gallego M, Gavignet B, Piarroux R, Rioux JA, Portus M, Fisa R 2007 .Application of microsatellite genotyping to the study of a restricted *Leishmania infantum* focus: different genotype compositions in isolates from dogs and sand flies. *Am J Trop Med Hyg* 76: 888-895.

Myskova J, Svobodova M, Beverley SM, Volf P 2007.A lipophosphoglycan-independent development of *Leishmania* in permissive sand flies. *Microbes Infect* 9: 317-324.

Nieves E, Pimenta PF 2002. Influence of vertebrate blood meals on the development of *Leishmania (Viannia) braziliensis* and *Leishmania (Leishmania) amazonensis* in the sand fly *Lutzomyia migonei (Diptera: Psychodidae). Am J Trop Med Hyg* 67: 640-647.

Noguera P, Rondon M, Nieves E 2006. Effect of blood source on the survival and fecundity of the sandfly *Lutzomyia ovallesi* Ortiz *(Diptera: Psychodidae),* vector of *Leishmania*. *Biomedica* 26 Suppl 1: 57-63.

Oliveira CD, Diez-Roux A, Cesar CC, Proietti FA 2006. A case-control study of microenvironmental risk factors for urban visceral leishmaniasis in a large city in Brazil, 1999-2000. *Rev Panam Salud Publica* 20: 369-376.

Oliveira F, Kamhawi S, Seitz AE, Pham VM, Guigal PM, Fischer L, Ward J, Valenzuela JG 2005.From transcriptome to immunome: identification of DTH inducing proteins from a *Phlebotomus ariasi* salivary gland cDNA library. *Vaccine* 24: 374-390.

Oliveira-Pereira YN, Rebelo JM, Moraes JL, Pereira SR 2006.Diagnóstico molecular da taxa de infecção natural de flebotomíneos (Psychodidae, *Lutzomyia*) por *Leishmania* sp na Amazônia maranhense. *Rev Soc Bras Med Trop* 39: 540-543.

Ochsenreither S, Kuhls K, Schaar M, Presber W, Schonian G 2006.Multilocus microsatellite typing as a new tool for discrimination of *Leishmania infantum* MON-1 strains. *J Clin Microbiol* 44: 495-503.

Rohousova I, Volf P 2006. Sand fly saliva: effects on host immune response and *Leishmania* transmission. *Folia Parasitol* (Praha) 53: 161-171.

PANAFTOSA 2006. *Consulta de Expertos OPS/OMS sobre Leishmaniasis Visceral en las Américas. Brasilia 23-25 noviembre 2005. Informe Final.* www.panaftosa.org.br-inst-zoonosis-leish.HTM

Paiva BR, Secundino NF, Nascimento JC, Pimenta PF, Galati EA, Junior HF, Malafronte RS 2006. Detection and identification of Leishmania species in field-captured phlebotomine sandflies based on mini-exon gene PCR. *Acta Trop* 99: 252-259.

Paiva BR, Secundino NF, Pimenta PF, Galati EA, Andrade Junior HF, Malafronte Rdos S 2007. Padronização de condições para detecção de DNA de *Leishmania* spp. em flebotomíneos *(Diptera, Psychodidae)* pela reação em cadeia da polimerase. *Cad Saude Publica* 23: 87-94.

Pardo RH, Carvajal A, Ferro C, Davies CR 2006. Effect of knowledge and economic status on sandfly control activities by householders at risk of cutaneous leishmaniasis in the subandean region of Huila department, Colombia. *Biomedica* 26 Suppl 1: 167-179.

Pasotti L, Maroli M, Giannetto S, Brianti E 2006.Agrometeorology and models for the parasite cycle forecast. *Parasitologia* 48: 81-83.

Pérez JE, Veland N, Espinosa D, Torres K, Ogusuku E, Llanos-Cuentas A, Gamboa D, Arevalo J 2007.Isolation and molecular identification of *Leishmania (Viannia) peruviana* from naturally infected *Lutzomyia peruensis (Diptera: Psychodidae)* in the Peruvian Andes. *Mem Inst Oswaldo Cruz* 102: 655-658.

Perruolo G, Noris Rodriguez N, Feliciangeli MD 2006.Isolation of *Leishmania (Viannia) braziliensis* from *Lutzomyia spinicrassa* (species group Verrucarum) Morales Osorno Mesa, Osorno and Hoyos 1969, in the Venezuelan Andean region. *Parasite* 13: 17-22.

Pessoa FA, Medeiros JF, Barrett TV 2007. Effects of timber harvest on phlebotomine sand flies *(Diptera: Psychodidae)* in a production forest: abundance of species on tree trunks and prevalence of trypanosomatids. *Mem Inst Oswaldo Cruz* 102: 593-599.

Peterson AT, Shaw J 2003. *Lutzomyia* vectors for cutaneous leishmaniasis in Southern Brazil: ecological niche models, predicted geographic distributions, and climate change effects. *Int J Parasitol* 33: 919-931.

Pimenta DN, Leandro A, Schall VT 2007. A estética do grotesco e a produção em saúde: segregação ou empatía? O caso das leishmanioses no Brasil. *Cad Saude Publica* 23: 1161-1171.

Pinto-da-Silva LH, Fampa P, Soares DC, Oliveira SM, Souto-Padron T, Saraiva EM 2005.The 3A1-La monoclonal antibody reveals key features of *Leishmania (L) amazonensis* metacyclic promastigotes and inhibits procyclics attachment to the sand fly midgut. *Int J Parasitol* 35: 757-764.

Rodriguez NM, De Guglielmo Z, Barrios MA, Barrios RM, Zerpa O, Feliciangeli MD 2005. Genetic homogeneity within *Leishmania (L.) infantum* isolated from human and dogs: the relationship with the sandfly fauna distribution in endemic areas of Nueva Esparta State, Venezuela. *Parasitology* 130: 611-619.

Rogers ME, Sizova OV, Ferguson MA, Nikolaev AV, Bates PA 2006. Synthetic glycovaccine protects against the bite of *Leishmania*-infected sand flies. *J Infect Dis* 194:512-518.

Rojas CA, Weigle KA, Tovar R, Morales AL, Alexander B 2006. A multifaceted intervention to prevent American cutaneous leishmaniasis in Colombia: results of a group-randomized trial. Biomedica 26 Suppl 1:152-66.

Rotureau B, Ravel C, Nacher M, Couppie P, Curtet I, Dedet JP, Carme B 2006a.Molecular epidemiology of *Leishmania (Viannia) guyanensis* in French Guiana. *J Clin Microbiol* 44: 468-473.

Rotureau B, Ravel C, Couppie P, Pratlong F, Nacher M, Dedet JP, Carme B 2006b. Use of PCR-restriction fragment length polymorphism analysis to identify the main new world *Leishmania* species and analyze their taxonomic properties and polymorphism by application of the assay to clinical samples. *J Clin Microbiol* 44: 459-467.

Salomón OD, Orellano PW 2005. *Lutzomyia longipalpis* in Clorinda, Formosa province, an área of potential visceral leishmaniasis transmission in Argentina. *Mem Inst Oswaldo Cruz* 100: 475-476.

Salomon OD, Orellano PW, Quintana MG, Perez S, Sosa Estani S, Acardi S, Lamfri M 2006. Transmision de leishmaniasis tegumentaria en Argentina. Medicina (B Aires) 66:211-9.

Salomon OD, Wilson ML, Munstermann LE, Travi BL 2004. Spatial and temporal patterns of phlebotomine sand flies *(Diptera: Psychodidae)* in a cutaneous leishmaniasis focus in northern Argentina. *J Med Entomol* 41: 33-39.

Santamaria E, Ponce N, Zipa Y, Ferro C 2006. Presencia en el peridomicilio de vectores infectados con *Leishmania (Viannia) panamensis* en dos focos endémicos en el occidente de Boyacá, piedemonte del valle del Magdalena medio, Colombia. *Biomedica* 26 Suppl 1:82-94.

Secundino NF, Eger-Mangrich I, Braga EM, Santoro MM, Pimenta PF 2005. *Lutzomyia longipalpis* peritrophic matrix: formation, structure, and chemical composition. *J Med Entomol* 42: 928-938.

Shaw J 2007.The leishmaniases--survival and expansion in a changing world. A mini-review. *Mem Inst Oswaldo Cruz* 102: 541-547.

Silva ES, Gontijo CM, Melo MN 2005.Contribution of molecular techniques to the epidemiology of neotropical *Leishmania* species. *Trends Parasitol* 21: 550-552.

Silva JG, Werneck GL, Cruz Mdo S, Costa CH, de Mendonca IL 2007. Infecção natural de *Lutzomyia longipalpis* por *Leishmania* sp. em Teresina, Piauí, Brasil. *Cad Saude Publica* 23: 1715-1720.

Souza NA, Andrade-Coelho CA, Peixoto AA, Rangel EF 2005. Nocturnal activity rhythms of *Lutzomyia intermedia* and *Lutzomyia whitmani (Diptera: Psychodidae)* in a transmission area of American cutaneous leishmaniasis in Rio de Janeiro State, Brazil. *J Med Entomol* 42: 986-992.

Spiegel CN, Jeanbourquin P, Guerin PM, Hooper AM, Claude S, Tabacchi R, Sano S, Mori K 2005. (1S,3S,7R)-3-methyl-alpha-himachalene from the male sandfly *Lutzomyia longipalpis (Diptera: Psychodidae)* induces neurophysiological responses and attracts both males and females. *J Insect Physiol* 51: 1366-1375.

Sucen. Superintendencia de Controle de Endemias 2005. Encontro de *Lutzomyia edwardsi* infectada na região da Grande de São Paulo. *Rev Saude Publica* 39: 137-138.

Teodoro U, dos Santos DR, dos Santos AR, Oliveira O, dos Santos ES, Neitzke HC, Monteiro WM, Rossi RM, Lonardoni MV, Silveira TG 2006.Avaliação de medidas de controle de flebotomíneos no Município de Lobato, Estado do Paraná, Sul do Brasil. *Cad Saude Publica* 22: 451-455.

Vilela ML, Souza NA, Oliveira SM, Costa-Pinto D, Cabello PH, Rangel EF, Traub-Cseko YM 2006. Considerations on the effect of anti-sandfly antibodies on biological parameters of *Lutzomyia longipalpis* (Lutz & Neiva, 1912) *(Diptera: Psychodidae: Phlebotominae). Braz J Biol* 66: 175-1783.

Volf P, Myskova J 2007.Sand flies and *Leishmania*: specific versus permissive vectors. *Trends Parasitol* 23: 91-92.

Volf P, Hajmova M, Sadlova J, Votypka J 2004.Blocked stomodeal valve of the insect vector: similar mechanism of transmission in two trypanosomatid models. *Int J Parasitol* 34: 1221-1227.

Ximenes M de F, Castellon EG, De Souza Mde F, Menezes AA, Queiroz JW, Macedo e Silva VP, Jeronimo SM 2006. Effect of abiotic factors on seasonal population dynamics of *Lutzomyia longipalpis (Diptera: Psychodidae)* in northeastern Brazil. *J Med Entomol* 43: 990-995.

Watts PC, Hamilton JG, Ward RD, Noyes HA, Souza NA, Kemp SJ, Feliciangeli MD, Brazil R, Maingon RD 2005. Male sex pheromones and the phylogeographic structure of the *Lutzomyia longipalpis* species complex *(Diptera: Psychodidae)* from Brazil and Venezuela. *Am J Trop Med Hyg* 73: 734-743.

Werneck GL, Costa CH, Walker AM, David JR, Wand M, Maguire JH 2007. Multilevel modelling of the incidence of visceral leishmaniasis in Teresina, Brazil. *Epidemiol Infect* 135: 195-201.

WHO 2003. *Report of the Scientific Working Group meeting on Insect Vectors and Human Health, Geneva, 12–16 August, 2002.* UNDP/WB/WHO-TDR Geneva (TDR/SWG/VEC/03.1).

WHO 2004. *Report of the Scientific Working Group meeting on Leishmaniasis, Geneva, 2–4 February, 2004.* UNDP/WB/WHO-TDR Geneva (TDR/SWG/04).

Ecoepidemiology of American Tegumentary Leishmaniasis due to *Leishmania braziliensis*

Rolando Oddone
Departmento de Producción Bioquímica
Instituto de Investigaciones en Ciencias de la Salud
Universidad Nacional de Asunción, Asunción, Paraguay

Among the public health problems in Latin-American countries, special attention should be paid to tegumentary leishmaniasis (TL). TL has been registered in all the American countries, except in Canada, Chile, Uruguay and in most of the Caribbean islands (1).

Initially, the occurrence of cases were the consequence of the intrusion of humans in the sylvatic transmission cycle, between sylvatic reservoirs of different species of *Leishmania* and the sandflies vectors in activities like deforestation, exploitation of wood and minerals, tracing of roads and highways and construction of damps (2). This association between woods, sylvatic reservoirs and sandflies led previously to the idea that the deforestation could cause the eradication of the species of *Leishmania* (3).

However, in the last decades, another epidemiologic pattern of the disease has been observed in the outskirts of the cities (4, 5). In the new epidemiologic situation coming from the deforestation, some mammal hosts of the parasite invade areas colonized by humans, where certain sandflies are in process of adaptation to the new environment. Keeping their feeding habits, such sandflies manage to transmit the parasite between humans and domestic mammals (6). Campbell-Lendrum (3), for instance, showed convincing examples of the domestication phenomena of TL, widely shown in Latin America, although previously reported in some areas of deforestation in Brazil (7, 8).

In the past, during the decades of 1930 and 1940, transmission was associated to the vectors *Lutzomyia whitmani*, *Lu. pessoai* and *Lu. migonei* these species showing wild behaviour (9). Nowadays, *L. (V.) braziliensis* is found associated to a variety of sandflies (*Lu. wellcomei*, *Lu.complexa, Lu.migonei, Lu.whitmani s.l.*, and *Lu.intermedia*) which lead to different transmission cycles in many geographic areas, where the vector is found inside and around the houses and in domestic animal shelters (4, 5, 10). The species of the *Lu. intermedia* complex *(Lu. intermedia* o *Lu. neivai)* and *Lu. migonei* have been incriminated as suspected vectors of *L. braziliensis* in domestic foci of Brazil, Paraguay and Argentina (11, 12). On the other hand, *Lu. migonei* was found in peridomestic habitats shared with dogs and horses (13, 14).

It has been suggested that *Lu.whitmani s.l.* could represent a notable example of high tolerance to drastic ecological changes. Based in ecological niche models, Peterson and Shaw predicted that *Lu.whitmani s.l.* would be able to tolerate the effects of global climatic alterations, considering its high capacity of survival with climatic changes (15).

Obviously, the climate is a critical factor affecting the distribution of species of *Lutzomyia*. While the global rising temperatures could lead to an increase of *Leishmania* and sandlies development, there is a notable impact of the climate variability in the TL casuistic, as it has been observed in the effect of El Niño Southern Oscillation in some localities of Columbia (16). This and other studies suggest that the variations of the incidence of vector-transmitted diseases are associated to annual changes in weather conditions (17, 18).

Besides the trouble that domestication process of *Leishmania* represent, Campbell-Lendrum believes that the cost-effectiveness campaigns against the domiciliary transmission of TL have real achievable goals (3).

References

1. Arias J., Beltrán F, Desjeux P and Walton B. 1996. *Epidemiología y control de la leishmaniasis en las Américas, por país o territorio. Cuaderno Técnico* No. 44. OPS, Washington.
2. Lainson R 1988. Ecological interactions in the transmission of the leishmaniases. *Phil Trans R Soc B 321*: 389-404.
3. Campbell-Lendrum D, Dujardin JP, Martinez E, FeliciangeliMD, Enrique Perez J, et al. 2001. Domestic and peridomestic transmission of American cutaneous leishmaniasis: changing epidemiological patterns present new control opportunities. *Mem Inst OswaldoCruz 96*: 159-162.
4. Rangel EF. 1995. *Epidemiology of American Cutaneous Leishmaniasis in Brazil. Tropical Diseases, Society and the Environment. Procedings from a Research Seminar, TDR/ SAREC*, p. 103-110.
5. Rangel EF, Lainson R 2003. Ecologia das leishmanioses: transmissores de leishmaniose tegumentar americana. In: EF Rangel, R Lainson (eds.), *Flebotomíneos do Brasil.* Fiocruz, Rio de Janeiro, pp. 291-310.
6. da Costa SM, Cechinel M, Bandeira V, Zannuncio JC, Lainson R, et al. 2007. *Lutzomyia (Nyssomyia) whitmani s.l.* (Antunes & Coutinho, 1939) *(Diptera: Psychodidae: Phlebotominae):* geographical distribution and the epidemiology of American cutaneous leishmaniasis in Brazil—Mini-review. *Mem Inst Oswaldo Cruz*, 102 (2): 149-153.
7. Tolezano JE. 1994. Ecoepidemiological aspects of American cutaneous leishmaniasis in the State of São Paulo, Brazil. *Mem Inst Oswaldo Cruz 89*: 427-434.
8. Gomes AC. 1994. Sand fly vectorial ecology in the State of São Paulo. *Mem Inst Oswaldo Cruz 89*: 457-460.
9. Ministério da Saúde, Fundação Nacional da Saúde. 2000. *Manual da controle da Leishmaniose Tegumentar Americana*. FUNASA, Brasilia/DF, p. 14.
10. Lainson R, Shaw JJ. 2005. New World Leishmaniases. In: FEG Cox, D Wakelin, SH Gillespie, DD Despommier (editors), *Topley & Wilson's Microbiology & Microbial Infections,Parasitology*, 10th ed., ASM Press, London, p. 313-349.
11. Rangel EF, Azevedo ACR, Andrade CA, Souza NA, Wermelinger ED. 1990. Studies on sandfly fauna *(Diptera: Psychodidae)* in a focus of cutaneous leishmaniasis in Mesquita, Rio de Janeiro State, Brazil. *Mem Inst Oswaldo Cruz 85*: 39-45.
12. Salomón OD, Wilson ML, Munstermann LE, Travi BL. 2004. Spatial and temporal patterns of phlebotominae sand flies *(Diptera: Psychodidae)* in a cutaneous leishmaniasis focus in Northern Argentina. *J Med Entomol 41*: 33-39.
13. Aguiar GM, Vilela ML, Lima RB. 1987. Ecology of the sandflies of Itagauí, an area of cutaneous leishmaniasis in the state of Rio de Janeiro, food preferences *(Diptera, Psychodidae, Phlebotominae). Mem Inst Oswaldo Cruz 81*: 477-479.
14. Azevedo ACR, Rangel EF, Queiroz RG. 1990. *Lutzomyia migonei* (França, 1920) naturally infected with perypylarian flagellates in Baturité, a focus of cutaneous leishmaniasis in Ceará State, Brazil. *Mem Inst Oswaldo Cruz 85*: 479.
15. Peterson AT, Shaw J. 2003. *Lutzomyia* vectors for cutaneous leishmaniasis in Southern Brazil: ecological niche models, predicted geographic distributions, and climate change effects. *Int J Parasitol 33*: 919-931.
16. Cárdenas R, Sandoval CM, Rodríguez-Morales A and Franco-Paredes C. 2006. Impact of climate variability in the occurrence of Leishmaniasis in northeastern Colombia. *Am. J. Trop. Med. Hyg.,* 75(2): 273–277
17. Cabaniel G, Rada L, Blanco JJ, Rodriguez-Morales AJ, Escalera JP. 2005. Impacto de Los Eventos de El Niño Southern Oscillation (ENSO) sobre la Leishmaniosis Cutánea en Sucre, Venezuela, a través del Uso de Información Satelital, 1994-2003.*Rev Peru Med Exp Salud Publica 22:* 32–38.
18. Rodriguez-Morales AJ, Rada L, Cabaniel G, Benítez J, Blanco JJ, Escalera JP. 2005. Comparación del impacto de la variabilidad climática sobre la Leishmaniasis cutánea americana en dos estados de Venezuela: Sucre y Trujillo. *Parasitología Latinoamericana 60* (Num Extraord) T°2: 222.

Leishmaniasis Vaccine Development

Ivan Dario Velez, MD, PhD
Programa de Estudio y Control de Enfermedades Tropicales (PECET)
Universidad de Antioquia, Medellin, Colombia
idvelez@udea.edu.co

Leishmaniasis represents an important health and socioeconomic problem in 88 countries around world where this disease is endemic. Current control measures rely on early diagnosis and chemotherapy to people suffering this disease. No prophylactic drugs are available and in most countries prevention programs of the disease are not carried out. Considering the higher increase of information on genetics and biology of the parasite and knowledge on clinical and experimental immunology of leishmaniasis is reasonable to think that a vaccine is feasible and that in the near future, a vaccine will become an important tool in the control of leishmaniasis. However, to date, there are no vaccines against human leishmaniasis in routine use anywhere in the world, but several vaccine preparations are in more or less advanced stages of testing. The elaboration of an effective vaccine faces some difficulties such as the significant *Leishmania* antigenic diversity, number of *Leishmania* species affecting humans (around 20), diversity of reservoirs and vectors, and different clinical forms of the disease, and the fact that the parasites have a digenetic life cycle in to hosts: sandfly vector and mammalian reservoir. All together these factors demonstrate the complexity of this problem. Currently several problems remain to be solved before get a *Leishmania* vaccine. These problems include:

1. Selection of the antigen (it must be able to induces protective immune response and must be safe (must not exacerbate the disease or cause pathological reactions in vaccinated individuals).
2. Delivery route.
3. Adjuvant types.
4. Characterization of immunostimulatory effects.
5. Characterization of immune response markers in order to establish that the vaccine candidate induce protective immunity; this information is pivotal to select vaccine candidate, which can be evaluated in phase II and later in Phase III.
6. Specific features of the population to be tested in Phase III.

The search of a vaccine against human cutaneous leishmaniasis has been practiced for decades. Pioneering studies were carried out by Pessoa et al. in 1941. In these studies, 18 isolated of American cutaneous leishmaniasis were used in three doses. They observed 80% prophylactic efficacy and no adverse effects in 527 vaccinated and 600 control individuals (1, 2). These studies continued in the 1960s and 1970s led by professor Mayrink from Brazil, who performed several trials. As vaccines candidates he used a mixture of merthiolate-killed promastigotes from 5 patients isolates with *C. parvum* or no adjuvant using three doses in 614 vaccinated and 974 control subjects, though he could not determine the vaccine efficacy because cases were not found in the vaccine group but there was 78% Montenegro Skin Test (MST) conversion in the control group (3). In 1981, Dr. Carlos Antunes started a new clinical trial with Dr. Mayrink's vaccine using 2 IM doses, 7 days interval and saline as placebo. In total, three assays were performed and 33% to 68% of the vaccinated subjects had a converted MST. In the vaccinated people that did not convert the MST the vaccine did not protect but in those that converted the MST an overall efficacy of about 49% was observed. (4)

In Venezuela, Professor Jacinto Convit made several clinical trials using dead promastigotes plus BCG as adjuvant. In 1991, he started a clinical trial in Lara State with 15,500 volunteers and 5 arms that received 3 doses of L *braziliensis*+BCG, *L amazonensis*+BCG, only *L amazonensis*, only BCG and a control that received saline. However, conclusive results were not obtained. In 1992, he initiated a new assay again in Lara State but this time with 3 arms and 3 doses of *L amazonensis* +BCG, only BCG and a control with saline. This time, 8,740 MST negative volunteers were included that lived in endemic area but at the end of the study cases were not observed in the different groups so it was not possible to conclude about the vaccine efficacy (5)

In Ecuador, Dr. Rodrigo Armijos made a vaccine with heat killed promastigotes from three *Leishmania* isolates from patients and used BCG as adjuvant. The control group received only BCG. He vaccinated 844 children and they were followed up for one year. Dr. Armijos reported 85% MST conversion and 2.1% CL incidence in the vaccine group compared with 20% MST conversion and 7.6% CL incidence in the control group for a protective efficacy of 72.9% (6)

In the Old World, Soviet and Israeli researchers carried out several clinical assays in the 70's using live and attenuated *L major* promastigotes in procedures known as leishmanization, an ancestral common practice in foci of the so called "Oriental sore" (7). However, the use of live vaccines has had many problems, including the development of large uncontrolled skin lesions. Therefore, the use of live virulent organisms for vaccination was discontinued.

In February 1995, a Workshop about vaccine efficacy trials, organized by TDR/WHO, PAHO and the Universidad Federal de Bahia was held in Salvador, Bahia, Brazil. During the workshop it was recommended to start clinical assays of first generation vaccines, manufactured according to GMP standards and following the GCP procedures with the sponsorship of TDR/WHO.

Two vaccine candidates were selected: killed *L. major* plus BCG with or without alum for the Old World and autoclaved *L amazonensis* vaccine with or without BCG as adjuvant for the New World.

None of the killed parasites has been shown to be sufficiently efficacious as a prophylactic vaccine to be used in control programmes. Some of the vaccines tested in this way include:

1. A single injection of killed *L. major* plus BCG versus BCG alone in a *L. major* endemic area in Iran. Volunteers included 2,453. MST conversions in vaccine group: 36%. **No significant difference in overall incidence in 2 groups. The same vaccine scheme in a *L tropica* focus in Iran provided the same results. The conclusion was that a single dose of ALM+BCG is not sufficiently immunogenic (8,9).**

2. **Two doses of killed *L. major* vaccine plus BCG versus BCG alone against kala-azar in Sudan included 2,306 volunteers; 18% converted the MST. The protection rate was 9.9%.** Although side effects were minimal and the incidence of visceral leishmaniasis was significantly lower in those individuals whose LST became positive following vaccination compared to non responders, the vaccine was not sufficiently immunogenic to be used in control programs (10).

3. **A new clinical trial using 3 injections of the same vaccine against *L major* and *L tropica* was not sufficient protective (11).**

For the clinical trials in America, WHO asked Biobras the manufacture of a vaccine against *L amazonensis* (Leishvacin) following the GMP. Clinical trials were carried out in three countries. In Venezuela, Dr. Convit performed a clinical trial with Leishvacin plus BCG versus only BCG, applied in three doses and 6,941 volunteers were included in 1996. Using a similar scheme, Dr. R. Armijos started an assay in Ecuador. One year later, cases were not found in the vaccine and placebo groups in none of the countries so no conclusions about the vaccine efficacy could be made.

Leishvacin was also evaluated in Colombia. In a phase II study, the safety and immunogenicity of the vaccine with and without BCG as adjuvant was determined in 296 volunteers. In a randomized, placebo-controlled, double-blind vaccine trials, two vaccination schemes were compared: an intradermal Leishvacin plus BCG versus only BCG and an intramuscular Leishvacin intramuscular versus placebo (saline) was evaluated. The volunteers were MST-negative healthy adults, without leishmaniasis records, living in non endemic zones. They received 3 doses with 20 days interval between doses. Local and systemic toxicity was evaluated according to WHO scales.

The intradermal group presented nodular and ulcerous lesions as reactions to BCG that were unacceptable for volunteers and consequently the group was cancelled. The volunteers that received the intradermal vaccine were evaluated at 80 days and one year post-vaccination. MST conversions of 83% and 95% were found in the vaccine group versus 17% and 5% in the placebo group, without antibodies production a specific proliferation against *Leishmania* in the vaccine group but not in the placebo group. High levels of gamma interferon but not of IL10 were found only in the vaccine group, evidencing the induction of a Th1 immune response by the vaccine (12).

These results led to the performance of phase III to determine the vaccine efficacy but previous experiences in other countries made necessary a good selection of the volunteer population, suitable to make a phase III clinical trial.

Due to the requirements of including MST-negative volunteers so that the protective immune response could be attributed to the vaccine and not to a natural infection, it was proposed to WHO to perform a trial in a sentinel population, i.e. people exposed to a high risk of acquiring the disease during some time. Thus, a study was carried out in Colombian soldiers that were starting their military service; 538 soldiers were included and evaluated. Initially, they were examined physically and asked about having suffered CL, then MST was applied to them and 79% of them were MST-negative.

The soldiers were periodically evaluated during one year searching for CL lesions. MST was repeated one year later and 28% of the initially MST-negative soldiers converted to positive and 10.1% of this group suffered CL while the 7.1% of the initially MST-positive soldiers had CL. The difference between the two groups was not statistically significant demonstrating that the military population has a high risk of acquiring CL, that is a suitable population for the evaluation of a vaccine efficacy and that for New World leishmaniasis having suffered the disease previously or being MST+ are not protection indicators if a parasite reinfection occurs.

Due to the demonstration that this was a suitable population for the vaccine evaluation in phase III, the trial started with 2,600 volunteers, 1,300 received Leishvacin in 3 IM doses and the other half placebo**.** The volunteers were followed up during one year. The vaccine was found to be safe and one year post-vaccination, 84.3% of the volunteers that received the vaccine had converted MST versus 16.8% in the placebo group. However, the case incidence in vaccine group was 7.8% and in the placebo group 6.8% with a difference that is not statistically significant and that showed that no protection was conferred by the vaccine (13).

More recently, the scientific research has been oriente to the search of second generation vaccines. The newer vaccines comprise recombinant DNA-derived antigens, peptides and non protein antigens. These second generation vaccines may involve target antigens that are species and life cycle stage specific, but also antigens that are shared by promastigotes and amastigotes. Some are conserved among *Leishmania* species, while others are not. Several antigens have been identified and characterized that might be potential vaccine candidates. Antigens with vaccine potential include the GP63, LACK, PSA-2, P-8, gp46/M-2, cysteine proteases, glucose regulated protein (GRP78), HASPB1 (K26), and the fucose-mannose ligand (FML). All of these antigens have shown potential in animal models of one or more forms of leishmaniasis. However, a systematic approach to determine which of these is/are most appropriate for clinical development is needed. The first second generation vaccine candidate tested in humans is Leish 111f vaccine (IDRI) (14). Leish111f is a combination of three different antigens, LeIF (*Leishmania* elongation initiation factor), LmSTI1 (*Leishmania major* stress-inducible protein 1) and TSA (Thiol-specific antioxidant from *L. major* promastigotes) using MPL (Monophosphoryl lipid A from *S. minnesota*) as adjuvant. A phase I study carried out in MST+ individuals in Colombia showed that 10 µg Leish111f in 25 µg MPL-SE induced IgG antibodies, and both IFN-□ and IL-5 at days 28 and 56 after vaccination. A second study in phase II in MST- Colombians individuals using 10 µg Leish111f in 25 µg MPL-SE or 10 µg Leish111f alone or placebo showed that IgG antibodies were produced at 84 and 168 days after vaccination in Leish111f vaccinated groups with or without adjuvant but not in the placebo group. Production of IFN-□ was higher in individuals vaccinated with Leish111f and adjuvant while IL-5 levels were detected in both vaccine groups but not in the placebo group.

In conclusion, several, double-blind, randomized trials have been conducted using various preparations of killed *Leishmania* (whole parasite) with or without BCG as adjuvant but also with second generation vaccines. It should be noted, however, that first generation vaccines are crude antigens and it is difficult to standardize them. BCG, used as adjuvant, is not standardized and various strains have different activities. However, some success has been obtained from these vaccine clinical trials: training in GCP, GMP, building infrastructure, set up national/institutional ethical committees, discrimination between

immunotherapy vs. immunochemoterapy. How not to run a field vaccine trial? What is the best population (endemic *vs.* non-endemic) and knowledge on natural history of zoonotic vs. anthroponotic cutaneous leishmaniasis?

A vaccine against leishmaniasis is not available yet and much basic information is needed specially referred to the clarification of the immune response markers that would allow to proceed from phase II clinical assay (immunogenicity) to phase III (efficacy).

References

1. Pessoa, S. B., and B. R. Pestana. 1941. Enseaio sobre vacinacao preventiva na leishmaniose tegumentar americana com germenes mortos. *Arq. Hig. Saude Publ* 6: 141-147.
2. Pessoa, S. B. 1941. Segunda nota sobre a vacinacao preventiva na leishmaniose tegumentar americana com leptomones mortas. *Rev. Paul. Med.* 19: 106.
3. Genaro O, de Toledo VP, da Costa CA, Hermeto MV, Afonso LC, Mayrink W. Vaccine for prophylaxis and immunotherapy, Brazil. *Clin Dermatol* 1996 Sep-Oct, 14 (5): 503-512.
4. Antunes CM, Mayrink W, Magalhaes PA, Costa CA, Melo MN, Dias M, Michalick MS, Williams P, Lima AO, Vieira JB, et al. Controlled field trials of a vaccine against New World cutaneous leishmaniasis. *Int J Epidemiol* 1986 Dec, 15 (4): 572-580.
5. Convit J. Leishmaniasis: Immunological and clinical aspects and vaccines in Venezuela. *Clin Dermatol* 1996 Sep-Oct, 14 (5): 479-487.
6. Armijos et al. 1998. Field trial of a vaccine against New World cutaneous leishmaniasis in an at-risk child population: safety, immunogenicity, and efficacy during the first 12 months of follow-up. *J. Infect. Dis* 177: 1352-1357
7. Khamesipour A, Dowlati Y, Asilian A, Hashemi-Fesharki R, Javadi A, Noazin S, Modabber F. Leishmanization: use of an old method for evaluation of candidate vaccines against leishmaniasis. *Vaccine*. 2005 May 25, 23 (28): 3642-3648.
8. Sharifi I, FeKri AR, Aflatonian MR, Khamesipour A, Nadim A, Mousavi MR, Momeni AZ, Dowlati Y, Godal T, Zicker F, Smith PG, Modabber F. Randomised vaccine trial of single dose of killed *Leishmania major* plus BCG against anthroponotic cutaneous leishmaniasis in Bam, Iran. *Lancet* 1998 May 23, 351 (9115): 1540-1543.
9. Momeni AZ, Jalayer T, Emamjomeh M, Khamesipour A, Zicker F, Ghassemi RL, Dowlati Y, Sharifi I, Aminjavaheri M, Shafiei A, Alimohammadian MH, Hashemi-Fesharki R, Nasseri K, Godal T, Smith PG, Modabber F. A randomised, double-blind, controlled trial of a killed *L. major* vaccine plus BCG against zoonotic cutaneous leishmaniasis in Iran. *Vaccine* 1999 Feb 5, 17 (5): 466-472.
10. Bahar K, Dowlati Y, Shidani B, Alimohammadian MH, Khamesipour A, Ehsasi S, Hashemi-Fesharki R, Ale-Agha S, Modabber F. Comparative safety and immunogenicity trial of two killed *Leishmania major* vaccines with or without BCG in human volunteers. *Clin Dermatol* 1996 Sep-Oct, 14 (5): 489-495.
11. Khamesipour A, Rafati S, Davoudi N, Maboudi F, Modabber F. Leishmaniasis vaccine candidates for development: a global overview. *Indian J Med Res* 2006 Mar, 123 (3): 423-438.
12. Velez ID, Agudelo S, Arbelaez MP, Gilchrist K, Robledo SM, Puerta JA, Zicker F, Berman J, Modabber F. Safety and immunogenicity of a killed *Leishmania (L.) amazonensis* vaccine against cutaneous leishmaniasis in Colombia: a randomized controlled trial. *Trans R Soc Trop Med Hyg* 2000 Nov-Dec, 94 (6): 698-703.
13. Velez ID, Gilchrist K, Arbelaez MP, Rojas CA, Puerta JA, Antunes CM, Zicker F, Modabber F. Failure of a killed *Leishmania amazonensis* vaccine against American cutaneous leishmaniasis in Colombia. *Trans R Soc Trop Med Hyg* 2005 Aug, 99 (8): 593-598.
14. Coler RN, Goto Y, Bogatzki L, Raman V, Reed SG. Leish-111f, a recombinant polyprotein vaccine that protects against visceral Leishmaniasis by elicitation of CD4+ T cells. *Infect Immun* 2007 Sep, 75 (9): 4648-4654.

Treatment of Visceral Leishmaniasis in Europe

Eric Rosenthal, MD, PhD
Department of Internal Medicine, Archet Hospital,
University of Nice Sophia Antipolis, France
rosenthal.e@chu-nice.fr

Visceral leishmaniasis causes estimated 500,000 new cases of disease and more than 50,000 deaths a year; 90% of cases occur in just 5 countries: India, Bangladesh, Nepal, Sudan, and Brazil [1]. In South Asia and the Horn of Africa the predominant mode of transmission is anthroponotic (AVL) [2]. In these areas, humans with kala-azar or post-kala-azar dermal leishmaniasis (PKDL) provide the principal reservoir for ongoing transmission [3, 4], and incomplete or irregular treatment of human VL leads to drug pressure and rapid development of resistant parasites [5]. In the Mediterranean, the Middle East and Brazil, the disease is zoonotic (ZVL): the domestic dog is the principal reservoir host sustaining transmission to humans [2]. ZVL disease burdens are lower than in Asia and Africa [6]. In these regions most human VL disease occurs in children or immunocompromised adults [7]. However, the incidence of VL as an opportunistic infection in HIV-infected patients has fallen substantially owing to the widespread introduction of highly active anti-retroviral therapy (HAART) [8, 9]. Access to treatment is generally much better in Europe than in Asia and Africa. For HIV patients therapeutic problems persist, particularly in patients with an incomplete immune reconstitution. In these patients data are insufficient to make firm recommendations on the best regimens for primary treatment and secondary prophylaxis of VL [10, 11].

Emergence of Liposomal Amphotericin B in the Treatment of VL

After their introduction in the therapy of VL in the early part of the 20th century, pentavalent antimonials have been considered the standard treatment for VL for more than 60 years. They have been extensively used and have been demonstrated to be generally safe and effective. However, during the last decades, the emergence in certain geographical areas of *Leishmania* strains resistant to pentavalent antimonials, coupled with some drug toxicity and prolonged administration, has prompted the evaluation of alternative drugs, including lipid formulations of amphotericin B. Liposomal amphotericin B has the highest therapeutic index of existing antileishmanial drugs, a moderately long

serum half-life of 7 hours, and sustained presence in the tissues for several weeks post-treatment. Over the past decade, liposomal amphotericin B has been used increasingly to treat visceral leishmaniasis (VL), as first-line treatment in some endemic regions such as northern cost of the Mediterranean Basin, and in others as second-line treatment for patients who fail to benefit from conventional therapy.

Clinical Trials with Liposomal Amphotericin B

Immunocompetent Patients

Thirteen clinical trials of liposomal amphotericin B for treatment of VL have been published; most were open label dose-finding studies or randomized open label comparisons with other antileishmanial drugs. Most of them have been performed in India with at least 10 different regimens tested including single shots. Indian experience demonstrated that total doses of 10 to 20 mg/kg in various dosing schedules gave cure rates > 95%. Conversely, liposomal amphotericin B was demonstrated to be safe, causing substantially less toxicity than conventional amphotericin B desoxycholate or amphotericin B lipid complex (ABLC, Abelcet®) [12, 13]. Three randomized comparative trials for treatment of fungal infections in neutropenic patients also confirmed significantly lower renal toxicity for liposomal amphotericin B than for conventional amphotericin B desoxycholate or ABLC [14]. In Europe, clinical trials demonstrated 90-98% efficacy with a total dose of 18-21 mg/kg in immunocompetent patients. A variety of regimens are currently in use. For imported cases in the USA, the FDA recommends 3 mg/kg days 1-5, 14 and 21 for a total dose of 21 mg/kg [15]. Published case series and current pediatric practice in southern Europe suggest good efficacy for a total dose of 20 mg/kg. In Italy, the standard regimen consists of 3 mg/kg days 1-5 and 10, for a total dose of 18 mg/kg [6]. Cascio *et al* evaluated in a retrospective analysis the efficacy and safety of this regimen in infantile cases of Mediterranean VL diagnosed over a 10 year period [16]. All the 164 HIV-negative children enrolled were initially cured and did not present adverse events due to drug infusion. Seven patients who relapsed were successfully retreated with the same regimen. Short courses of liposomal amphotericin B have been recently evaluated in Europe. Syriopoulou *et al* conducted an open prospective study evaluating a schedule consisting in 10 mg/kg/day on 2 consecutive days in Mediterranean VL [17]. Forty-one children received this regimen and were compared to 30 children who, in a previous study were treated with 4 mg/kg daily for 5 days, and 52 who were treated with meglumine antimoniate. Treatment success was noted for 40 of 41 children treated with the 2 dose regimen. Abatement of fever, reduction of spleen size, and correction of laboratory parameters occurred more quickly among the children who received 2 doses of liposomal amphotericin B than among comparison groups. Many pediatricians currently use this regimen [18]. In adults, this

regimen needs to be validated in Mediterranean VL. Our preliminary experience in a short series showed similar results than in children [19].

Patients Coinfected with HIV

In HIV-VL coinfected patients, there have been no formal randomized clinical trials of liposomal amphotericin B treatment or secondary prophylaxis regimens, and only two open label dose-finding studies. In patients with severe immunosuppression, relapse rates after antileishmanial treatment are extremely high [20]. A randomized trial of ABLC vs Sb^V showed comparable efficacy but lower toxicity for ABLC [21]. The efficacy of Sb^V and liposomal amphotericin B were comparable in most case series, but the lower rate of toxicity for liposomal amphotericin B has caused most clinicians to consider it as the antileishmanial drug of choice in HIV-coinfected patients. Secondary prophylaxis with doses of liposomal amphotericin B or other antileishmanials every 2-4 weeks after initial clinical cure of VL is now the standard of care in Europe [10, 11, 22], but data are insufficient to recommend a specific regimen. For some authors, clinical experience to date suggests that discontinuation of secondary antileishmanial prophylaxis can be considered in patients whose CD4+ lymphocyte count rises above 200-350 cells/µl in response to HAART, but that prophylaxis should be continued in those with counts below 200 cells/µl [11]. However, other authors observe that HAART is not sufficient to control the disease, despite increases in CD4+ lymphocyte counts and undetectable viral loads, suggesting that secondary prophylaxis should be maintained indefinitely [23, 24].

WHO Recommendations for the Use of Liposomal Amphotericin B in the Treatment of VL

Recently, the WHO convened a consultative meeting to discuss current expert knowledge of, and experience with, liposomal amphotericin B in the treatment of VL, and to produce a consensus document with clear guidelines for dosage and clinical use of liposomal amphotericin B for VL. Participants were experts with specialties ranging from basic research to clinical medicine and access to drugs, and representing a wide variety of VL-endemic regions. Recommendations on use of amphotericin B alone or in combination have been given for different forms such as zoonotic and anthroponotic visceral leishmaniasis, and for HIV-*Leishmania* co-infected patients [25]. In zoonotic visceral leishmaniasis (the Mediterranean Basin, Middle East and Brazil) the consensus recommendations were as follows:

1. A total liposomal amphotericin B dose of 20 mg/kg is adequate to treat immunocompetent children and adults in these regions.
2. The exact dosing schedule can be flexible (divided into doses of 10 mg/kg on 2 consecutive

days or in smaller divided doses) but liposomal amphotericin B pharmacokinetics suggest that the initial dose will provide better tissue levels if at least 5 mg/kg is given.

3. The schedule of 10 mg/kg/day on 2 consecutive days needs to be validated in adults with ZVL.
4. Veterinary use of liposomal amphotericin B, and other new antileishmanial drugs (miltefosine, paromomycin), should be avoided in order to prevent the development of resistance.

Liposomal Amphotericin B in "Real Life" in 2007

In Europe, liposomal amphotericin B is approved as a first-line therapy of visceral leishmaniasis including immunocompromised hosts in a dozen of countries (data provided by Gilead France). Among these countries, Portugal is the only endemic area for the disease (Austria, Ireland, Finland, Germany, Lithuania, Norway, Portugal, Russia, Slovenia, Sweden, the Netherlands, United Kingdom). In three other countries endemic for VL, liposomal amphotericin B is approved for the treatment of VL, but only as a second-line therapy, in case of intolerance or resistance to antimonials (Greece, France and Spain). Italy is another country from the Mediterranean Basin endemic for VL. Although liposomal amphotericin B is not approved for the treatment of the disease, Italy is the only country in which information about the use of liposomal amphotericin B in "real life" is available [6]. A retrospective analysis was performed on data collected at the main reference center for VL surveillance in Italy. First-line drug treatments were recorded in 573 immunocompetent patients with VL. In the past 12 years, the proportion of antimonial treatments decreased from 100% to 2.8%, while the proportion of amphotericin B treatments increased from 0% to 97.2%. Of those amphotericin B drugs, liposomal amphotericin B accounted for most regimen (92.8%). Liposomal amphotericin B was administered to both children and adults at a standard dose of 3 mg/kg/day for 5 consecutive days plus an additional 3 mg/kg/day on day 10. Clearly, results of this study showed a countrywide change in therapy over the period considered. Even though the change was relatively gradual over a 16-year period, meglumine antimoniate, the traditionally effective drug has been almost fully replaced by liposomal amphotericin B in the treatment of VL. In France, in Spain and probably in Greece, such a change in first-line treatment on a nationwide scale is highly plausible. However, to date, no data is available.

Many things must be taken in account in the therapeutic management of VL, including the safety/efficacy ratio of the drug, socioeconomical and cultural factors, the endemicity of *Leishmania* strains, the immune and nutritional status of the population, the parasite chemoresistance level. Obviously the cost of the drugs is a crucial factor. The total cost of the treatment depends both on the price of the drug and the price of the hospitalisation. In Western Europe hospital charges are high. Despite liposomal amphotericin B is an expensive compound, we evaluated that in France, the classical liposomal amphotericin B regimen compared to meglumine antimoniate may reduce from US$13, 000 to 9, 000 for one patient (drug and hospitalization) [26, 27]. In other parts of the world, although hospital costs can be appreciably reduced by short courses and especially single-doses regimens, the savings did not offset the cost of the drug in countries such as India, where hospital charges are low.

Conclusion

Today, treatment of VL in Europe is neither an acute individual nor a public health problem, with the exception of severely immunocompromised patients. In comparison to classical compounds, liposomal amphotericin B has favorable efficacy/safety and cost/efficacy profiles. Liposomal amphotericin B is recommended as the first-line therapy for visceral leishmaniasis in Europe, and it constitutes nowadays the standard treatment in clinical practice.

References

1. World Health Organization. *Leishmaniasis disease burden (web page)*. 2005 [cited 2005 04/27/2005]; Available from: http://www.who.int/leishmaniasis/burden/en/.
2. Pearson, R.D., S.M.B. Jeronimo, and A. de Queiroz Sousa, *Leishmaniasis*, in *Tropical infectious diseases: principles, pathogens and practice*, R.L. Guerrant, D.H. Walker, and P.F. Weller, eds. 1999, Churchill Livingstone: Philadelphia. 797-813.
3. Bern, C., et al., *Risk factors for kala-azar in Bangladesh. Emerging Infectious Diseases* 2005, 11: 655-662.
4. Addy, M. and A. Nandy, Ten years of kala-azar in west Bengal, Part I. Did post-kala-azar dermal leishmaniasis initiate the outbreak in 24-Parganas? B*ull World Health Organ* 1992. 70 (3): 341-346.
5. Sundar, S., et al., Failure of pentavalent antimony in visceral leishmaniasis in India: report from the center of the Indian epidemic. *Clin Infect Dis* 2000, 31 (4): 1104-1107.
6. Gradoni, L., M. Gramiccia, and A. Scalone, Visceral leishmaniasis treatment, Italy. *Emerg Infect Dis* 2003, 9 (12): 1617-1620.
7. Rosenthal, E., P. Marty, and J.P. Cassuto, Visceral leishmaniasis and human immunodeficiency virus (HIV) infection in the south of France. *Presse Med*, 1995. 24 (35): 1666.
8. Rosenthal, E., et al., Declining incidence of visceral leishmaniasis in HIV-infected individuals in the era of highly active antiretroviral therapy. *AIDS*, 2001, 15 (9): 1184-5.
9. del Giudice, P., et al., Impact of highly active antiretroviral therapy on the incidence of visceral leishmaniasis in a French cohort of patients infected with human immunodeficiency virus. *J Infect Dis* 2002, 186 (9): 1366-1370.
10. Laguna, F., Treatment of leishmaniasis in HIV-positive patients. *Ann Trop Med Parasitol* 2003, 97 (Suppl 1): 135-142.
11. Berenguer, J., et al., Discontinuation of secondary anti-leishmania prophylaxis in HIV-infected patients who have responded to highly active antiretroviral therapy. *AIDS* 2000, 14 (18): 2946-2948.
12. Thakur, C.P., A single high dose treatment of kala-azar with Ambisome (amphotericin B lipid complex): a pilot study. *Int J Antimicrob Agents* 2001, 17 (1): 67-70.
13. Sundar, S., et al., Amphotericin B treatment for Indian visceral leishmaniasis: conventional versus lipid formulations. *Clin Infect Dis* 2004, 38 (3): 377-383.
14. Deray, G., Amphotericin B nephrotoxicity. *J Antimicrob Chemoth*er 2002. 49 (Suppl 1): 37-41.
15. Meyerhoff, A., U.S. *F*ood and Drug Administration approval of AmBisome (liposomal amphotericin B) for treatment of visceral leishmaniasis. *Clin Infect Dis* 1999. 28 (1): 42-48; discussion 49-51.
16. Cascio, A., et al., A 6-day course of liposomal amphotericin B in the treatment of infantile visceral leishmaniasis: the Italian experience. *J Antimicrob Chemother* 2004. 54 (1): 217-220.
17. Syriopoulou, V., et al., Two doses of a lipid formulation of amphotericin B for the treatment of Mediterranean visceral leishmaniasis. *Clin Infect Dis* 2003. 36 (5): 560-566.
18. Kafetzis, D.A., et al., Treatment of paediatric visceral leishmaniasis: amphotericin B or pentavalent antimony compounds? *Int J Antimicrob Agents* 2005, 25 (1): 26-30.
19. Jeandel, P.-Y., et al., Amphotéricine B liposomale en cure courte dans le traitement de la leishmaniose viscérale méditerranéenne de l'adulte non immunodéprimé. *Rev Med Interne* 2007. 28 (Suppl 1): 44-45.
20. Davidson, R.N., et al., Liposomal amphotericin B (AmBisome) in Mediterranean visceral leishmaniasis: a multi-centre trial. Q *J Med* 1994, 87 (2): 75-81.
21. Laguna, F., et al., Amphotericin B lipid complex versus meglumine antimoniate in the treatment of visceral leishmaniasis in patients infected with HIV: a randomized pilot study. *J Antimicrob Chemother* 2003, 52 (3): 464-468.

22. López-Velez, R., et al., Amphotericin B lipid complex versus no treatment in the secondary prophylaxis of visceral leishmaniasis in HIV-infected patients. *J Antimicrob Chemother* 2004, 53 (3): 540-543.

23. Casado, J.L., et al., Relapsing visceral leishmaniasis in HIV-infected patients undergoing successful protease inhibitor therapy. *Eur J Clin Microbiol Infect Dis* 2001, 20 (3): 202-205.

24. Mira, J.A., et al., Frequency of visceral leishmaniasis relapses in human immunodeficiency virus-infected patients receiving highly active antiretroviral therapy. *Am J Trop Med Hyg* 2004, 70 (3): 298-301.

25. Bern, C., et al., *Liposomal amphotericin B for the treatment of visceral leishmaniasis. Clin Infect Dis* 2006, 43 (7): 917-924.

26. Marty, P. and E. Rosenthal, Treatment of visceral leishmaniasis: a review of current treatment practices. *Expert Opin Pharmacother* 2002, 3 (8): 1101-1108.

27. Rosenthal, E. and P. Marty, Recent understanding in the treatment of visceral leishmaniasis. *J Postgrad Med* 2003, 49 (1): 61-8.

Vector Control of the Leishmaniasis Program in Brazil: Advances and Challenges

Sena JM, Elkhoury ANSM, Alves WA, Gomes MLS

Secretaria de Vigilância em Saúde, Ministério da Saúde, Brasília, DF, Brazil

The measures recommended by the Leishmaniasis Programs are surveillance actions of human cases, vectors and reservoirs. An analysis of the epidemiological situation indicates the prevention and control actions to be adopted that are different for tegumentary and visceral leishmanioses.

VL surveillance has as an objective the reduction of mortality and morbidity rates through early diagnosis and treatment of human cases and decrease of transmission through control of infected dogs and transmitter agents, i.e. *L. longipalpis* and *L. cruzi.* The objectives of TL surveillance are early diagnosis and treatment of cases in order to reduce the deformities caused by the disease and monitor the epidemiological and environmental indicators that, in this case, refer to the entomological surveillance of the species involved in transmission.

The municipalities with VL transmission are stratified according to the transmission profile obtained from cases averages in the last five years as follows: sporadic (<2.4 cases), moderate (≥2.4 and <4.4) and intense (≥4.4). The strategies are different for each situation according to the epidemiological risk and, in municipalities with register of the first case, actions are defined after an entomological research.

For the TL Program, a surveillance model based in the circuits of disease production was developed making possible the identification of priority areas for surveillance and monitoring in the territorial units of the country.

The entomological surveillance is an instrument that collects qualitative and quantitative information about phlebotomines aimed at identifying vulnerable or receptive areas for leishmaniasis transmission, supporting the definition of autochthonous cases and knowing the presence and distribution of vectors as well as monitoring of the behavior of epidemiologically relevant species. The collected information subsidized prevention and control actions of leishmaniases and also contributes to evaluate their impact.

For the entomological surveillance of VL, the performance of collection, research and entomological monitoring methodologies are recommended and indicated according to risk situations. For municipalities with TL, it is recommended that research actions of focus and monitoring are addressed to the areas of disease circuits or disease production poles, considering climate and different physiogeographical aspects.

In the last years, the Ministry of Health invested in the acquisition of equipment and vehicles to support the actions of phlebotomines entomology and professionals training. Different institutions and research groups collaborated in the qualification of human reosurces and technicians training for decentralized vector identification. In this sense, the advance achieved in relation to the improvement of collection, identification and analysis systematization of entomological information opportunously and integrately with the technicians of entomological surveillance.

The distribution of phlebotomine vector species has been evidenced by the systematized research carried out by the entomology service so that an extension of areas with register information of species not identified so far or which presence has not been recorded previously is observed. Since the incorporation of the entomological surveillance activities to the Leishmaniasis Program in an extended surveillance context, it was possible to operationalize control actions more rationally especially in reservoirs as well as chemical control actions with a perspective of control focused in risk areas for leishmanioses transmission. Meanwhile, there are many challenges to be overcome standing out among them: the physical structure of entomological laboratories in the states and municipalities with an extension of entomological equipment, coverage of areas still without surveillance, technical qualification for the interpretation of entomological indicators.

In relation to vector control actions for VL, it is recommended that the chemical control is performed only in municipalities with register of first autochthonous case immediately after an entomological survey, municipalities or areas with moderate and intense transmission, in the most adequate period, municipalities or areas with VL outbreaks, after evaluation and delimitation of the area. For TL, chemical control is recommended only in situations of intradomiciliary transmission with proved vector adaptation.

The efficacy, impact duration and resources required for the different endemic areas of insecticide use, though its extended use in VL actions, have varied. The chemical treatment of dwellings, associated to environmental management, has shown reduction of vector infestation. Some studies that evaluated control strategies in urban areas demonstrated that the results referring to the individual use of insecticide

are lower in comparison to the elimination of infected dogs. The association of these strategies during a period of at least three years has showed positive results in the control of this endemic.

Due to the epidemiological characteristics and still insufficient knowledge about several elements that are part of the theoretical model of leishmaniasis transmission, the control strategies of this endemic are still little effective and therefore, actions related to the diagnosis and treatment of human cases are priorities. Meanwhile, the educational and environmental management activities should be integrally implemented with the rest to be more effective in all situations.

Conclusions Related to Leishmaniasis Control and Research

Dr. Oscar Salomón

During the *Update of American Trypanosomiasis and Leishmaniasis Control and Research* meeting held in Rio de Janeiro, Brazil, 6–7 November 2007, the following gaps of knowledge in the topics related with leishmaniases were addressed:

Eric Rosenthal discussed the current human treatment of VL in Europe by country, reviewing also the clinical trials performed to prove the relative advantages (therapeutic index, tissue persistence, toxicity, efficacy/safety, cost/efficacy) of liposomal amphotericin B schedules over pentavalent antimonials both for immunocompetent children and adults, and HIV-infected patients. WHO SWG recommendations for the use of liposomal amphotericin B for the Mediterranean basin, Middle East and Brazil VL were revised. The short series recommended for children for immunocompetent adults needs validation and also the regime for HIV-infected patients as the first-line therapy, and secondary prophylaxis every 2–4 weeks after initial cure. The socioeconomical and cultural factors (the cost analysis for Europe include higher hospitalisation charges), the endemicity of *Leishmania* strains, the immune and nutritional status of the population, and the parasite chemoresistance level must be evaluated in the therapeutic management of VL.

Ivan Dario Velez updated the knowledge about trials on leishmaniases vaccine candidates in different testing stages, from live virulent organisms to whole killed or live-attenuated parasites to DNA-derived antigens, peptides and non protein antigens with and without adjuvant. A prophylactic vaccine for leishmaniasis is feasible but several problems remain to be solved both on the design of vaccine development-trials, and on structural-ethical issue:

1. selection of the antigen (protective and safe, immunotherapy vs immunochemotherapy),
2. delivery route,
3. adjuvant types (e.g. BCG strain,
4. characterization of immunostimulatory effects,
5. characterization of immune response (natural/induced),
6. specific features of the population to be tested in phase III (endemic vs epidemic),
7. lack of specific training (field vaccine trials),
8. infrastructure,
9. national ethical committees,

10. basic field-based knowledge about zoonotic vs anthroponotic coetaneous leishmaniasis,
11. *Leishmania* antigens-life cycle-host diversity.

Roberto Badaro reviewed the problem of enviromental (climate, geographic features, status of developmentnt, vector distribution, reservoir) and human leishmaniasis (genetic susceptibility, acquired deficiencies) risk factors, global importance of leishmaniasis, and clinical spectrum of the disease host-parasite investigation, mainly VL. The risk factors of infection/disease discussed were those associated a) with parasite factors: *Leishmania* diversity (phenotipical, genetical, immunomodulators); and b) with host factors: human behavior-domestic animals, immune cytokine-driven response or clinical severity, genetic susceptibility-resistance, nutritional and micronutritional level and age to focuse control measures, immune-competence of the population (HIV-AIDS overlap). Urban epidemic VL emergence in Latin America is attributed to changes in ecology, migration and epidemiologic patterns. The need of a more accurate diagnosis and notification system, more investigation in the immune mechanism of disease, host genetic factors of susceptibility/markers to clinical outcomes were underlined, and vector-related ecological research.

Rolando Oddone reviewed some aspects of the eco-epidemiology of ACL, focused in *L. braziliensis*, from the original sylvatic zoonotic cycle to the recent peri-urban pattern, with reservoir dispersion in human settlements and vector domestication, vector species shift, and indoor behavior. The results of the ongoing multinacional project were also presented. Gaps in research: Physiopathogenic factors regarding development of ML, and high incidence of ML in Paraguay (25% of the whole notified ACL cases). Enviromental factors for domestic transmisssion of ACL, and predictive tools for urban VL. New oral drugs for ACL and VL. *Leishmania* genetic polymorphism (transcriptional, post-trascriptional and proteomic) causing ACL, and parasite antigen expression-immunological response relationship.

Elci Villegas Avila showed the successful results of lambdacyhalothrin 12,5/m^2 impregnated curtains on human ACL incidence in a randomized paired control trial performed during a year in an urban area of Venezuela with indoor ACL transmission all the year round and an incidence rate of 4%. From the Villegas and Oddone presentations it is inferred again the need of basic field vector-related data currently updated for each eco-epidemiological scenario, integrated with data from social sciences (place and time of human risk behaviors), standardized comparable protocols (including variables and outcomes), and scaling-up protocols from experimental pilot designs to program actual interventions.

Joana Martins de Sena showed the achievements and challenges on vector control of the Program of Leishmaniasis of Brazil. The improvements obtained from the definition of operational measures based

on risk stratification (AVL), and epidemiological circuits-environmental variables (ACL), as the investment in human and physical resources that result in a descentralized sandfly collection, identification and an integrated surveillance strategy. The remaining challenges are:

General

1. Leishmaniases should be priorized as a health public problem.
2. Support to surveillance and control actions.
3. Support to research and development of alternatives for diagnosis, treatment, prevention and control.

Scientific-Technical

1. Research oriented to surveillance and control.
2. Development of diagnosis tests with better sensitivity and specificity.
3. Chemotherapy developments with improved cost-effectivity, safety, easier administration and sostenibility.
4. Assessment of new tools for prevention and control of LV.

Intitutional-Programmatic

1. Broader the network health for an adequate diagnosis and treatment of human cases.
2. Integrate LV surveillance and control measures to primary health.
3. Priorizate the surveillance of leishmanioses.
4. Use of new tools for surveillance and surveillance monitoring.

Managerial

1. Focus interventions in priorized areas.
2. Integrate procedures of intervention.
3. Define control strategies in order to obtain the expected impact.
4. Intensify health education – KAP.

Vector Surveillance and control

1. Strengthen the vector surveillance in Brazil.
2. Public policies to implement activities at municipality level (human and physical resources) to intensify and assure the quality of chemical control and surveillance activities.
3. Stimulate and priorizate enviromental managment, health education and community participation.
4. ACL vector control depends on the transmission cycle and the probed presence of vector species in the domicile, while the impact of any proposal should be rigurously evaluated according to the new normative of PVLTA.

Oscar Daniel Salomon addresses the trend and questions in the research on vector of leishmaniasis. The specific gaps discussed are:

1. **Species identification-vector incrimination:** Standard protocols adequate to field conditions for vector and parasite taxonomic identification and infections rates; regular monitoring and evaluation of epidemiological role of each species/subspecies/clades-variants.
2. **Parasite-vector-host interactions:** Molecular and eco-epidemiological factors that modulates the species-specific competent-permissive-refractoriness; vector saliva infection enhancement/host protection/vaccine.
3. **Ecoepidemiology from focus to regional levels:** Vector population-parasite transmission structure and dynamics; vector biology and behavior; atypical transmission and developmental projects monitoring; dispersion studies (AVL continental - microfoci); vector and parasite markers/techniques appropriate to study molecular epidemiology at focus level; minimum protocols cultural-sensitive for different scenarios; descriptive-predictive models oriented to bring actual operational answers.
4. **Surveillance and control:** Standard protocols including cost-effective and acceptability evaluations, diversion effect up to the border of the intervention, and risky behavior human schedule; progressive scale-up of the most promissory strategies; vector surveillance community-based strategies.

Common needs for institutional strengthening were noted in many presentations:

- ✓ **Lack of adequate priorization of leishmaniasis** in public health policies in many countries.
- ✓ **Minimum standard protocols** for each topic defined by consensus al least with experts from all the countries involved, with periodical updates
- ✓ **Research networks** to capacitate and synergize different capabilities.
- ✓ **Multicentric networks** to replicate the protocols to validate/frame conclusions in different eco-epidemiological scenarios.
- ✓ **Integrated inter/multi/transdisciplinaries approaches**.
- ✓ **Pilot projects** that scales up research-based proposals with standard protocols.
- ✓ **Cheap and reliable infection tests** for different parasites/hosts/ eco-epidemiological scenarios.
- ✓ For VL control, the **current available VL canine treatments and vaccines are not recommended**, and the **canine use of drugs for human leishmaniasis treatment should be avoided**.

Annex: Conference Photos


Eradication: a perspectives for
Chagas Disease ?
Rio de Janeiro 2007
João Carlos Pinto Dias, MD
Oswaldo Cruz Foundation, Brazil