

ACTIVITY REPORT

2007-2008



CENTRE DE RECERCA EN
SALUT INTERNACIONAL
DE BARCELONA

CENTRO DE INVESTIGACIÓN
EN SALUD INTERNACIONAL
DE BARCELONA

BARCELONA CENTRE
FOR INTERNATIONAL
HEALTH RESEARCH

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CRESIB

INTRODUCTION

The Barcelona Centre for International Health Research (CRESIB) is a new research centre created by several academic and biomedical research institutions in Barcelona: *Hospital Clínic de Barcelona [HC]*, *Universitat de Barcelona [UB]*, *Institut d'Investigacions Biomèdiques August Pi i Sunyer [IDIBAPS]* and the *Generalitat de Catalunya* (Government of Catalonia). The goal of the CRESIB is to contribute to the global response to the international health challenges of an increasingly globalised world in which vast inequalities persist.

The CRESIB centers its research activities around two key areas; (i) diseases that affect the poorest people on the planet and (ii) the health challenges that arise due to migration and new emerging diseases. The overall goal of the CRESIB is to contribute to the international effort to readdress the imbalance that exists between the causes and the distribution of the global burden of disease and the direction of the human and financial investments in the R&D effort: the so-called 10/90 gap. This gap reflects the fact that today still 90% of the R&D resources are directed to health problems that account for only 10% of the global burden of disease. Conversely only 10% of the global R&D effort is directed towards diseases that account for 90% of the global burden of disease, those which affect particularly the poorest communities and countries of this globalised world.

Generating knowledge and developing new tools and strategies in the fight against diseases that affect the poor is not only an act of justice, but also a key strategy to break the vicious cycle of disease and poverty in which individuals, communities and countries are trapped. Research constitutes a key tool to break this cycle of disease and poverty and thus contribute to the economical and social development of numerous countries.

The creation of the CRESIB in mid-2006, within the framework of the *Generalitat de Catalunya* policy to create competitive research centres of international excellence (Programme of Research Centres of the *Generalitat de Catalunya* – CERCA), capitalises on the long history of medical care, teaching and research at HC and UB, both leaders in global health in Spain for more than 30 years. The Economical and Financial Management Office of CRESIB is ran by the *Fundació Clínic per a la Recerca Biomèdica* (FCRB).

The CRESIB is founded on the three inseparable conceptual axes of all medical and academic institutions: medical care, research and training, which given the specificity of the centre, are complemented with international cooperation.

The primary mission of the CRESIB is research, following a strategy to address health problems from a translational perspective. The centre's research activities cover aspects such as understanding of the genetic basis and the molecular mechanisms of host-pathogen interactions, as well as immunological, clinical and epidemiological research and public health. Strengthening collaborations with research centres in endemic countries is at the core of all research activity design.



Training and capacity building constitute a fundamental pillar of the CRESIB culture. The centre participates in courses and programmes of international health at the UB at the undergraduate, graduate and doctoral levels in collaboration with other universities including the *Universitat Pompeu Fabra* (UPF) and *Universitat Autònoma de Barcelona* (UAB). The CRESIB collaborates with research centres and institutions in Mozambique and Morocco, giving support to the “Training Fellows” programmes, which frequently leads to the completion of doctoral theses at the UB.

From the perspective of international cooperation, the CRESIB collaborates in the development and strengthening of institutions and research groups in developing countries. These activities are carried out through the FCRB, which has been fundamental in the creation and development of the *Centro de Investigação em Saúde de Manhiça* (CISM) in Mozambique (www.manhica.org) and the *Fundação Manhiça*, recently founded to manage the CISM. FCRB also played a key role in the “Programme of strengthening the national strategies of maternal and child health, research and specialist training” in Morocco (www.fundacioclinic.ma). The CRESIB also provides technical and medical assistance in Morocco and Mozambique, always closely related to research and training.

The origins of the CRESIB in Barcelona date back to the activities developed by the former Tropical Medicine Unit, later the Tropical Medicine and International Health Services (<http://www.hospitalclinic.org/Secciones/Asistencia/SaludInternacional/tabid/137/Default.aspx>) as well as to the preventive activities at the Assistance to the International Traveller Service at the HC (<http://www.hospitalclinic.org/Secciones/Asistencia/ActividadPrivada/tabid/133/Default.aspx>).

The CRESIB is part of the *Instituto de Investigaciones Sanitarias Hospital Clínic-IDIBAPS* and the *Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública* (www.ciberesp.es), which integrate 54 groups from 35 Spanish research centres with the objective to create knowledge relevant to the elaboration of programmes and health policies.

FOREWORD



PEDRO L. ALONSO
Director



NÚRIA CASAMITJANA
Deputy Director

The *Centre de Recerca en Salut Internacional de Barcelona* (CRESIB) was created with the ambition to become a biomedical research institute of excellence, to help improve health and to promote the development of the disadvantaged populations.

We live in an increasingly interconnected and globalised world where unacceptable imbalances exist in the health and development of populations and countries. As a further offence to the dignity of individuals, these inequalities question the very viability of a world in which the place of birth dramatically determines one's possibility to live and to develop their potential. The CRESIB, the result of a wager of the Catalan Health and Science System, aspires to become a global player and a focal point in the research in international health. This centre aims to contribute to overcoming the imbalances in the world by articulating and leading innovative responses. These challenges are undertaken with the conviction that research and the generation of knowledge are formidable tools and offer great potential for change.

This report summarises the activities carried out at the CRESIB since its creation, a period of time during which we combined the work needed to shape the administrative and scientific structure of the centre with the development of research activities. The CRESIB was not started from scratch, but was born from the historical core –a lively and dynamic one– of international health research within our country: the *Hospital Clinic de Barcelona* (the first tertiary hospital with tropical medicine and international health services, started in the early 1980s), the *Universitat de Barcelona* (with nearly half a century of continuous academic activity in this area, especially through the Master of Tropical Medicine programme that will soon reach its 40th year, making it the oldest postgraduate course offered by this university) and

the technical, health and scientific cooperation programmes operated abroad, (especially those in Mozambique and Morocco that were developed through the *Fundació Clinic per a la Recerca Biomèdica* (FCRB) and are more than 10 years old).

The CRESIB has very solid foundations and complementary components that have allowed it to be established as a new research centre with a scientific strategy based on translational research. The CRESIB is relatively small and has adopted a matrix structure that brings together different researchers and multiple disciplines that focus on the same problem; the objective is to improve the efficiency and to maximise the impact of the results.

Throughout 2007–2008, the research lines at the centre have been consolidated and have expanded to new and innovative areas. The research on malaria continues to be the backbone of the CRESIB. During this period, important contributions have been made in the development of new control measures against malaria caused by *Plasmodium falciparum*, including the clinical development and conceptual testing of the currently most advanced vaccine – RTS,S –, new drugs for the treatment of this disease and the research on the intermittent preventive treatment with sulphadoxine-pyrimethamine (SP), whose international consortium (IPTi Consortium) is based and coordinated from Barcelona. In addition, the implementation of malERA (Malaria Eradication Research Agenda), constituted under the umbrella of the WHO to develop a global research agenda focused on the eradication of malaria, consolidates the CRESIB as a global actor in the research of this disease.

The arrival of Professor Hernando del Portillo with his team from the *Universidade de São Paulo*, in Brazil, has

complemented our malaria research group with one of the strongest teams in the study of the other important human malarial parasite: *Plasmodium vivax*. Another new incorporation, Dr. Ned Hayes from the Centers for Disease Control and Prevention (CDC), U.S.A., has enriched our commitment to public health, epidemiological surveillance and investigation of vector-borne diseases.

Other important lines of work within the centre have been the consolidation of our research in acute respiratory and diarrhoeal diseases, both in Mozambique and Morocco. In addition, the team of Dr. Joaquim Gascon has developed pioneering work in its effort to describe the hidden burden of Chagas disease in the immigrant South American population in Catalonia. This has allowed the creation of a research agenda that focuses on epidemiological, clinical and pathophysiological aspects of this disease and seeks to improve the health policies aimed at the immigrant population, to safeguard the quality and safety of blood banks, to prevent the vertical transmission of the disease and to improve health care for those affected. This group has also promoted a very successful collaboration with Bolivia and the research teams at the *Universidad Mayor de San Simón* in Cochabamba.

Finally, the significant increase in multicentre projects coordinated by the CRESIB should be highlighted, the consequence of which has been a large increase in international collaborations. Presently, the centre maintains joint projects with over 60 centres in 30 countries all over the five continents.

Since its inception, the CRESIB has maintained its commitment to developing and strengthening the research capacities of the low-income countries; in some cases through partnerships that have allowed the development of strategies and joint research projects and long-term

training. A clear example is the *Centro de Investigação em Saúde de Manhiça* (CISM) in Mozambique, with which the CRESIB maintains a close and ongoing strategic alliance in terms of research and training.

Research centres are their individuals and their visions. Beyond the necessary physical infrastructure, the real treasure lies in the research, technical and administrative staff. In this respect, the CRESIB has adopted a clear strategy of internationalisation and currently brings together individuals of more than 20 nationalities.

The support and encouragement of our sponsors has been a key to our success during these first years and will remain so in the future. We face an uncertain financial landscape, but we have the talent and ambition for further growth in the pursuit of excellence. We have a strong team of researchers and will continue to incorporate others that are able to contribute to strengthening the international competitiveness of the centre. We will also adapt and reformulate our strategic plan, gathering the experiences of these years and responding to new challenges and opportunities. The CRESIB has an excellent external Scientific and Technical Advisory Committee (STAC), which has assumed a role of critical appraisal and support for the development of the centre. In short, starting from its historical core, the first years of the CRESIB have involved a lot of work, and have yielded many results. There is much to do, but we have the elements to consolidate, in Barcelona, a nucleus of excellence for research and global health action, and we look to the future with optimism.

PEDRO L. ALONSO
Director

NÚRIA CASAMITJANA
Deputy Director

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Department of Health
Generalitat de Catalunya

3. SCIENTIFIC AND TECHNICAL ADVISORY COMMITTEE

The CRESIB board has a Scientific and Technical Advisory Committee (STAC), which is comprised of reputable researchers and experts in the field of international health, all of whom are from outside the centre.

The STAC's functions consist of advising on the scientific activities of the CRESIB and the policies for the recruitment of scientific staff, as well as evaluating the researchers and the research projects that are carried out within the centre.

Members of the Scientific and Technical Advisory Committee

DR. JOSÉ ALCAMI
Head of AIDS Immunopathology Unit
National Microbiology Centre
Instituto de Salud Carlos III
Madrid (Spain)

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Diseases of the Developing World Drug Discovery
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Initiative for Vaccine Research
World Health Organization
Geneva (Switzerland)

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Deputy Director
PROF. DR. NÚRIA CASAMITJANA BADIA

Economic and Financial Director
MR. JOAN VIVES TOMÁS

MANAGEMENT REPORT



JOAN VIVES
Economic and Financial Director

On 12 July 2006, the *Generalitat de Catalunya*, *Universitat de Barcelona* (UB), *Institut d'Investigacions Biomèdiques August Pi i Sunyer* (IDIBAPS), and the *Hospital Clínic de Barcelona* (HC) agreed to establish a private foundation: the *Centre de Recerca en Salut Internacional de Barcelona* (CRESIB).

The CRESIB was legally set-up as a private corporate body, as detailed in the Constitutional Deed, document number 2.090, granted before the notary Pedro-Àngel Casado Martín.

The centre is governed by the Catalan Law 5/2001 of 2 May, of private foundations and other applicable regulations, and especially by the statutes governing their operation.

The CRESIB is located at c/Villarroel, number 170 (registered address, in the premises of the *Hospital Clínic*), 08036 Barcelona, and has the fiscal identification number (N.I.F.) G-64334048.

Since its registration with the number 2314 on 20 April 2007, in the Register of Foundations of the *Generalitat de Catalunya*, the CRESIB has full legal capacity to work as a non-profit institution.

The activity of the CRESIB during 2007–2008 can be divided into two distinct, but interrelated, parts. One side involves the operation and structure of the centre itself, whereas the other side is directly related with the research projects that have been developed over time.

Since its inception, the CRESIB has received, for its proper functioning, €2,800,000 in contributions from the *Generalitat de Catalunya*, through the Department of Innovation, Universities and Enterprise (DIUE) and the Department of Health. The other founding entities have contributed with the assignment of working space and by facilitating the participation of personnel.

The centre has recruited, among others, an ICREA Professor and two researchers with financial support from the *Ramón y Cajal* and *Beatriu de Pinós* programmes.

The consolidation of the scientific structure of the centre has been accompanied with the parallel organization of administrative and project management infrastructure. In addition, the CRESIB has established an agreement with the *Fundació Clínic per a la Recerca Biomèdica* (FCRB), which is responsible for providing a support service to the Economic and Financial Management Office of the CRESIB.

The CRESIB accounts have been audited favourably by Faura Cases Auditors Consultors.

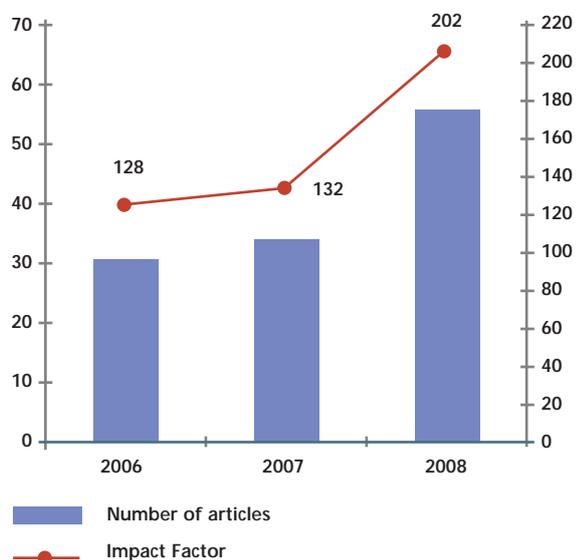
Over the next four years, the CRESIB faces the challenge to consolidate its leadership position in biomedical research in international health. The CRESIB will continue working to ensure a balanced growth through the development of skills, procedures and management tools that are critical to promote research and ensure a setting of organisational excellence.

JOAN VIVES
Economic and Financial Director



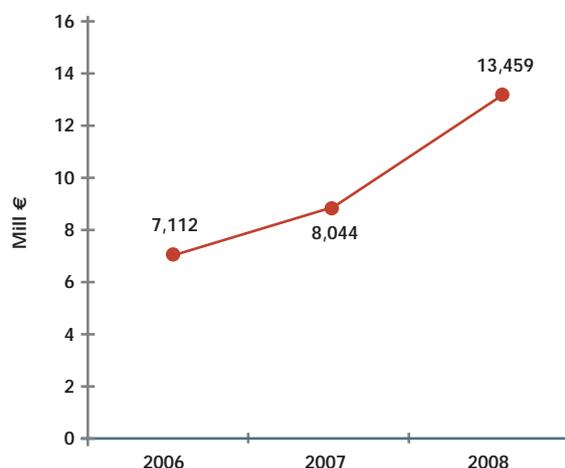
FACTS AND FIGURES

Publications by the CRESIB



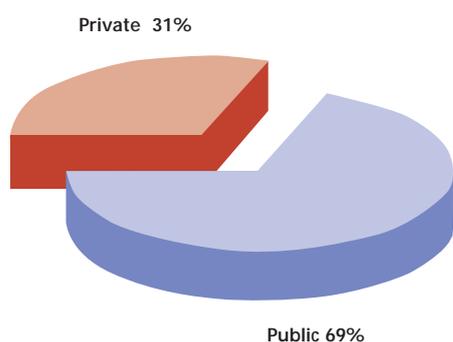
Number of articles published by researchers from the CRESIB and their impact factors, 2006–2008.

Research funding



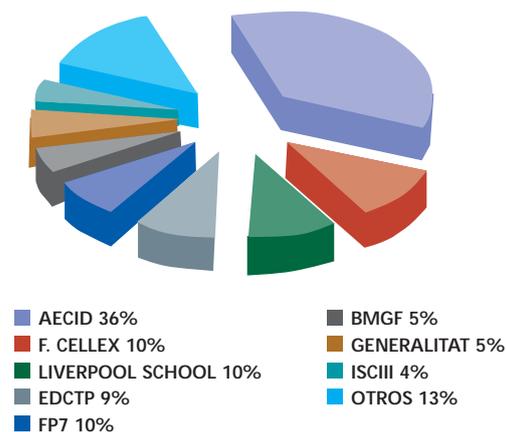
CRESIB funding, 2006–2008. The centre is financed through funds for research in international health (competitive grants and structures) that are awarded to the CRESIB and its founding entities (HC, IDIBAPS and UB), as well as the FCRB, which acts as manager of these funds.

Public and private funding



A comparison of public and private funding received by the CRESIB in 2007–2008.

Financers



The principal funding sources of the CRESIB in 2007–2008. The range of financers that fund the activities of the centre is broad and represents both public and private institutions. The major public contributor is the *Agencia Española de Cooperación Internacional para el Desarrollo* (AECID) and the major private contributor is the Bill & Melinda Gates Foundation.

CRESIB IN THE NEWS

2007

THE FIRST JOSEPH MASDEVALL CONFERENCE "NEW FRONTIERS IN GLOBAL HEALTH" ORGANISED BY THE CRESIB



(L to R) Regina Rabinovich, Pedro L. Alonso, Marius Rubiralta, Antoni Plasència and Marcel Tanner during the closing ceremony of the conference.

In January 2007, the CRESIB held, in Barcelona, their first conference "New Frontiers in Global Health" named after Joseph Masdevall in honour of the great Catalan epidemiologist of the 18th century whose activities led him to successfully resolve several epidemics of malaria in Spain.

This meeting had a large number of delegates and was attended by academics and people from national institutions, as well as international scientific experts. The speakers included: Marcel Tanner from the Swiss Tropical Institute (Switzerland), Maria Freire from the Global Alliance for TB Drug Development (U.S.A.), Myron Levine from the Center for Vaccine Development at the University of Maryland School of Medicine (U.S.A), Regina Rabinovich from the Bill & Melinda Gates Foundation (U.S.A) and David Mabey from the London School of Hygiene & Tropical Medicine (U.K.). The closing ceremony included, in addition to Dr. Pedro L. Alonso, Marius Rubiralta (UB) and Antoni Plasència (*Generalitat de Catalunya*).

CRESIB ORGANISES THE THIRD WORKSHOP ON IMPORTED CHAGAS DISEASE

In February 2007, within the framework of the training programme driven by the CRESIB, the researcher and specialist in Chagas disease, Dr. Joaquim Gascon (CRESIB, HC, UB) organised the third workshop on imported Chagas disease entitled "Vertical Transmission, Paediatric and Chronic Digestive Chagas". The workshop was attended by international research experts, which inclu-

ded, amongst others, Jean Jannin (WHO), Faustino Torrico (*Universidad Mayor de San Simón de Bolivia*) and Joffre Rezende (Goiania Institute of Gastroenterology, *Universidad Federal de Goiás*).



Faustino Torrico, a la izquierda, con Joaquim Gascon, al término del III Taller de Chagas .

The central themes of this workshop were particularly relevant since the main mode of transmission of this disease in non-endemic countries, in which there is a high proportion of immigrants from high risk areas, is the transmission from mother to child during pregnancy or at the time of delivery. With this workshop the CRESIB aimed to contribute to the training of health professionals for the correct diagnosis and treatment of this disease, which until recently was alien in such environments.

CRESIB PUSHES THE RESEARCH INTO *PLASMODIUM VIVAX* MALARIA WITH THE CREATION OF AN INTERNATIONAL CONSORTIUM



(L to R) Hernando A. del Portillo (ICREA Research Professor, CRESIB), Pedro L. Alonso (Director, CRESIB) and Núria Casamitjana (Deputy Director, CRESIB) during the press conference held on 17 July 2007.



The support of the CRESIB to research on *Plasmodium vivax* malaria, a parasite causing more than 70 million cases of malaria in the world each year, was reflected in the creation of an international consortium on *P. vivax* malaria. This consortium was developed in collaboration with the relevant centres and international researchers in this field. It has the purpose to contribute to the better understanding and characterisation of *P. vivax* malaria, as well as to encourage and accelerate the development of new control tools, with particular emphasis on vaccines. With these objectives, the CRESIB developed a line of research on *P. vivax* malaria under the direction of Hernando A. del Portillo (ICREA Research Professor, CRESIB), one of the few experts in molecular biology and vaccine development against this parasite.

THE RESULTS ON THE EFFICACY AND SAFETY OF THE PHASE II CLINICAL TRIAL OF A VACCINE CANDIDATE AGAINST MALARIA IN AFRICAN CHILDREN UNDER ONE YEAR ARE PRESENTED BY THE CRESIB



(L to R) John Aponte (CRESIB), Esperanza Sevene (*Universidade Eduardo Mondlane*, Mozambique) and Eusebio Macete (CISM), participants in the study, presented the results obtained during a press conference to celebrate this occasion.

On 27 July 2007, the CRESIB presented for the first time the findings of the good safety profile and tolerability of the vaccine candidate (RTS,S) against malaria in 214 African babies aged between 10 and 18 weeks. These findings were confirmed three months later with the publication of the results of this clinical trial in *The Lancet*.

The RTS,S vaccine reduced, by up to 65%, new malarial infections in babies in the study (three months after

receiving the treatment of three doses) and reduced episodes of clinical malaria by 35% (six months after the first dose), while demonstrating a safety profile similar to routine vaccines. As stated by Pedro L. Alonso, Director of the CRESIB, "these unprecedented results strengthen the view that this vaccine could help reduce the intolerable burden of the disease and death from malaria".

THE ANNOUNCEMENT OF THE IMPLEMENTATION OF PHASE III CLINICAL TRIALS WITH THE MALARIA VACCINE CANDIDATE RTS,S



(L to R) The researchers Inacio Mandomando (CISM), Pedro L. Alonso (CRESIB), Betuel Sigauque (CISM), Maria Nélia Mañaca (CISM), Clara Menéndez (CRESIB) and Esperanza Sevene (*Universidade Eduardo Mondlane*, Mozambique) at the *Hospital Clinic de Barcelona* when announcing the launch of Phase III clinical trial of the RTS,S vaccine.

Following the positive results obtained with the malaria vaccine candidate in the phase II clinical trial, the CRESIB, through its director Pedro L. Alonso, together with researchers from the *Centro de Investigação em Saúde de Manhica* (CISM, Mozambique), confirmed in October 2007, the launching of the phase III clinical trial of this vaccine, developed by the GlaxoSmithKline laboratories. This is a large study that will commence in 2008 and will involve 16,000 African children and 11 research centres from seven African countries. If this study confirms the results previously obtained by the team of Dr. Alonso, the vaccine will be ready for registration and subsequent marketing.

2008

THE CRESIB ORGANISES THE FOURTH WORKSHOP ON CHAGAS DISEASE AND THE INTERNATIONAL MEETING NETWORK ON CHAGAS OF THE WORLD HEALTH ORGANISATION (WHO)



Joaquim Gascon (L) talks with Elisa Sicuri, researchers from the CRESIB.

From 4–6 February 2008, headed by Dr. Joaquim Gascon (CRESIB, HC-UB) and Dr. Jean Jennin (WHO), the fourth workshop on Chagas disease entitled "Treatment and Vertical Transmission" was held in Barcelona. This workshop was followed by a consensus meeting based on the exchange of information derived from the workshop. A meeting of the WHO network on Chagas "Second meeting for a Chagas disease initiative in non-endemic countries" followed to raise the awareness and inform on the correct management of Chagas disease in countries where this disease is not endemic.

The above meetings resulted in the development of a consensus document about the management of digestive Chagas that will be published in 2009 in "*Enfermedades Infecciosas y Microbiología Clínica*", the official journal of the Spanish Society of the same name, and in the journal "Gastroenterology and Hepatology".

CRESIB ORGANISES, TOGETHER WITH GAVI ALLIANCE AND LA CAIXA, THE INTERNATIONAL SYMPOSIUM "ADVANCING IMMUNISATION IN DEVELOPING COUNTRIES: NEW HORIZONS IN CHILDREN'S HEALTH"

The CRESIB, together with funding from the Global Alliance of Vaccines and Immunisations (GAVI Alliance) and the social project "la Caixa", organised on 24 April 2008, in Barcelona, the symposium "Advancing in immunisation in developing countries: New horizons in Children's Health", which brought together national and

international scientists, researchers, politicians and national and international funders of the highest level.



(L to R) Graça Machel (GAVI Alliance), Her Royal Highness Princess Cristina, Bernat Soria (Health Minister, Spain), Marina Geli (Regional Health Minister, *Generalitat de Catalunya*) and Pedro L. Alonso (Director, CRESIB) in the closing ceremony of the symposium.

The forum outlined the progress to date in childhood immunisation, as well as advances in the cutting edge research to fight diseases causing death in these populations. In addition, the financial, logistical and technical barriers that hinder the path to reducing child mortality in developing countries were analysed and the options were discussed.

INVESTITURE OF HONORARY DOCTOR GRAÇA MACHEL BY THE UNIVERSITY OF BARCELONA



(L to R) Eusebio Macete (CISM), Her Royal Highness Princess Cristina, Graça Machel and Magda Robert (GAVI Alliance), Betuel Sigauque (CISM) and Pedro L. Alonso (CRESIB), during the investiture ceremony of Mrs. Machel.

On 4 April 2008, through the impetus of the CRESIB (HC-UB), Mrs. Graça Machel, president of the GAVI Alliance, defender of the rights of women and children, and the recipient of the Prince of Asturias Award for International

Cooperation in 1998, was awarded an Honorary Doctorate by the *Universitat de Barcelona* (UB). In this way, the CRESIB also contributed in recognising the extensive work of a figure well known for her dedication to education in her country, Mozambique, for her leadership in attracting international attention to the problems of women and children in war-torn countries, and for her participation in building a strong civil society in Mozambique. Mrs. Machel is an activist for the education and rights of women and children, not only in Mozambique and Africa, but also in the rest of the world.

CONSTITUTION OF THE FUNDAÇÃO MANHIÇA, MOZAMBIQUE, WITH THE ENCOURAGEMENT OF THE CRESIB

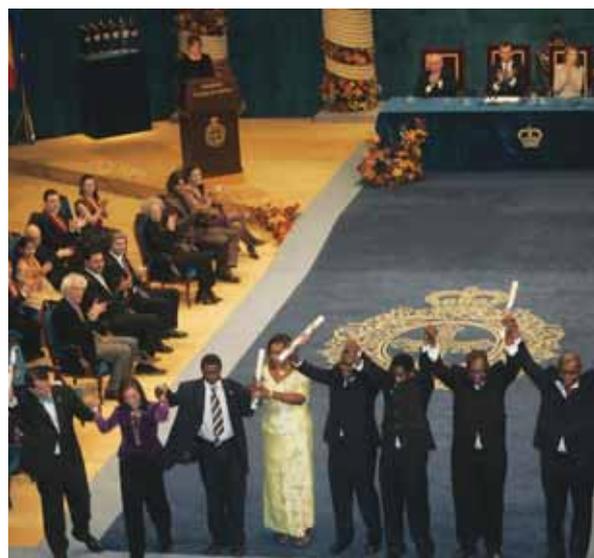


(Foreground) Graça Machel, (table: L to R) Marius Rubiralta (Rector, UB), Juan Manuel Molina (Spanish Ambassador to Mozambique), Aída Libombo (Vice-minister of Health, Mozambique), Pascoal Mocumbi (President, *Fundação Manhiça*) and Joao de Carvalho (Director, *Instituto Nacional de Saúde de Mozambique*), during the signing of the constitutional public deed of the *Fundação Manhiça* in Maputo, Mozambique.

On 25 February 2008, the *Fundação Manhiça* was established in Mozambique by the founding members: The state of Mozambique, represented by the Ministry of Health; The Kingdom of Spain, represented by the Ministry of Foreign Affairs – *Agencia Española de Cooperación Internacional para el Desarrollo* (AECID); the *Instituto Nacional de Saúde de Mozambique*; the CRESIB (HC-UB); and Dr. Pascoal Mocumbi, an honorary founding member.

The creation of this foundation represents a further step in the on-going collaboration between the governments of Spain and Mozambique in the field of health research. This collaboration began in 1980 and was consolidated in 1996 with the creation of the *Centro de Investigação em Saúde de Manhiça* (CISM), as an essential tool to help solve the most important problems of public health in Mozambique.

THE CENTRO DE INVESTIGAÇÃO EM SAÚDE DE MANHIÇA (CISM) RECEIVES THE PRINCE OF ASTURIAS AWARD FOR INTERNATIONAL COOPERATION



Ceremony for the Prince of Asturias Award, the representatives from the centres (L to R in pairs): *Centro de Investigação em Saúde de Manhiça* (Mozambique), Ikafara Health Institute (Tanzania), Malaria Research & Training Centre (Mali) and Kintampo Health Research Centre (Ghana).

On 24 October 2008, the *Centro de Investigação em Saúde de Manhiça* (CISM) in Mozambique, linked to the CRESIB and co-directed by Dr. Pedro L. Alonso and Dr. Clara Menéndez, was one of the four African centres of research that received the award from the Prince of Asturias Foundation, in the category of International Cooperation. With this prize, the Foundation wanted to recognise the important work of these centres and their researchers in the fight against malaria through research, health care and education.

PROJECT IN COLLABORATION WITH THE MINISTRY OF HEALTH OF THE KINGDOM OF MOROCCO FOR THE IMPLEMENTATION OF THE SPECIALISATION IN PUBLIC HEALTH EPIDEMIOLOGY

This project, promoted by the *Fundació Clínic per a la Recerca Biomèdica* (FCRB) and the *Institute National d'Administration de Santé* (INAS) in Morocco, and financed by the social project "la Caixa" has as its priority objective the consolidation of a high quality, sustainable academic offer within the INAS in the field of epidemiology.

gy and public health, while strengthening the epidemiological surveillance capacities of the Ministry of Health. The institutions guiding this training are the *Universitat de Barcelona* (UB), the *Universitat Pompeu Fabra* (UPF), the CRESIB and the INAS.



(L to R) Enric Grau (FCRB), Luis Planas (Spanish Ambassador to Rabat), Yasmina Baddou (Health Minister, Kingdom of Morocco), Marina Geli (Regional Health Minister, *Generalitat de Catalunya*), Núria Casamitjana (Depuy Director, CRESIB) and Raimon Belenes (Director, *Hospital Clínic de Barcelona*), at the presentation ceremony of the FCRB/AECID/Ministry of Health of Morocco agreement.

The implementation of this training programme in public health epidemiology within the INAS allowed, in the first installation, the specialised training of 27 professionals from the national health system who exercise functions of epidemiological vigilance in the Regional Health Observatories in different zones of Morocco. The second installation of this programme was inaugurated in October 2008.

CRESIB RESEARCHERS PARTICIPATE IN THE FULL GENOME SEQUENCING OF *PLASMODIUM VIVAX*, PUBLISHED IN NATURE



Carmen Fernández-Becerra (L) and Hernando A. del Portillo (R), during the press conference on the publication of their research.

On 9 October 2008, Dr. Hernando del Portillo and Dr. Carmen Fernández-Becerra, authors from the CRESIB, the only Spanish centre participating in the study, announced the publication of the complete genome sequence of

Plasmodium vivax in the prestigious journal *Nature*. These researchers have led the efforts to understand and combat *P. vivax* over many years, first from Brazil and now from the CRESIB, and are pioneers in this field for their discovery of the first family of virulence genes of this parasite (*Nature* 2001). Their experience in the study of the virulence of *P. vivax* has allowed them to validate and analyse the data obtained in the sequencing conducted by The Institute for Genomic Research (TIGR, U.S.A.).

The analysis of the *P. vivax* genome, more similar to that of *P. falciparum* than expected, has shown that this parasite may have alternative mechanisms for infecting the erythrocytes, human blood cells in which they inhabit and reproduce during its complex life cycle. These alternative routes of infection were not observed in previous research and now the genetics particularities that cause this to happen are known.

THE FUNDACIÓN RAMÓN ARECES GIVES 1 MILLION EUROS TO RESEARCH ON THE IMMUNOPATHOLOGY OF MALARIA



(L to R) Raimundo Pérez-Hernández y Torra (Director, *Fundación Ramón Areces*), Marina Geli (Regional Health Minister, *Generalitat de Catalunya*) and Pedro L. Alonso (Director, CRESIB), during the agreement signing in Barcelona.

The *Fundación Ramón Areces* wished to boost, with the agreement signed on 21 October 2008, in Barcelona, the Malaria Immunopathology Programme led by the CRESIB Director, Dr. Pedro L. Alonso. This programme will include funding of 1 million euros and will last for four years. Part of the investigation will be developed at the *Centro de Investigação em Saúde de Manhiça* (CISM, Mozambique),



with which the CRESIB maintains a strategic collaboration. This proposal has an important component of training that includes a pre-doctoral fellowship for a young Mozambican researcher, which will be integrated into the CISM training programme. The development of the Malaria Immunopathology Programme will allow researchers of the CRESIB to better understand the disease and advance the development of control measures and treatment.

Dr. Pedro L. Alonso, CRESIB Director, is the head of the MalERA Steering Committee, whose secretariat is based at the CRESIB. MalERA has been presented to the board of the Roll Back Malaria Partnership (RBM) and has its endorsement.

THE SETTING-UP AND STARTING OF malERA (MALARIA ERADICATION RESEARCH AGENDA)



Information on the establishment of malERA in the journal Nature (09/10/2008).

In October 2008, the malERA project was launched (<http://malera.tropika.net>), a consultative process between the academic and research communities, to identify knowledge gaps and new tools necessary for the eradication of malaria. The overall purpose of this initiative is to develop an agenda for multidisciplinary research and development (R&D) focused on the eradication of malaria. MalERA does not aim to dictate the activities of the particular organisations, but to reach a consensus among the research institutions and sponsors about the direction that should be taken with respect to R&D and future relations with malaria, with the ultimate goal of eradication.

RESEARCH PROGRAMMES

- MALARIA
- HIV/AIDS
- ACUTE RESPIRATORY INFECTIONS
- DIARRHOEAL DISEASES
- CHAGAS DISEASE
- OTHER RESEARCH PROJECTS





MALARIA

1. MALARIA

Malaria is a communicable disease that negatively impacts the health and economy of the suffering populations, so much so that it is considered as one of the principal obstacles to the development and economic growth in countries where it is endemic.

Currently, more than 80 countries are exposed to this disease, which accounts for more than 40% of the world's population. The elevated demographic growth means that there are now more people vulnerable to sickness and death from malaria than ever before. Most of the exposed countries are developing and each year encounter between 300 and 500 million cases of clinical malaria, which claim more than a million lives, mainly those of children under five years and pregnant women in sub-Saharan Africa.

The basic research in malaria developed by the CRESIB encompasses the study of the causative agents of the disease, namely, parasites of the genus *Plasmodium* and mainly of the species *P. falciparum* and *P. vivax*. The studies include, among other aspects, the pathological and immunological mechanisms of the agents and the discovery of antigens for vaccine development.

Results from epidemiological studies conducted by the centre include a description of the clinical presentation of malaria epidemiology and the evaluation of new malaria treatments and other control measures such as vaccines, insecticide-impregnated mosquito nets and intermittent preventive treatment in infants and pregnant women. Work is also being conducted on anthropological and economic aspects related to health interventions, including acceptability and cost effectiveness.

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Project Assistants

Montserrat Pi, Yolanda Antín, Patricia García



1.1. THE *PLASMODIUM VIVAX* CONSORTIUM

For a long time research into *Plasmodium vivax* has been relegated to second place, being largely neglected, although this species is responsible for 80–300 million cases of clinical malaria each year, severe manifestations of the disease (including death) is a major socio-economic burden in countries where it is endemic.

The *Plasmodium vivax* Consortium, created in 2006, is a four-year project funded by the Fundació Cellex and led by the CRESIB researchers Hernando A. del Portillo and Pedro L. Alonso. The consortium combines institutions from Colombia, Brazil, India and Papua New Guinea (PNG), which are representative of the main regions affected by *P. vivax* malaria.

The main objective of the consortium is to deepen the understanding and characterisation of *P. vivax* malaria, and to promote and accelerate the development of new control tools against the disease through the following research projects:

Discovery of antigens for vaccine development:

ROLE OF THE VIR PROTEINS

ROLE OF THE SPLEEN IN
PLASMODIUM VIVAX GENE
EXPRESSION

FUNCTIONAL AND STRUCTURAL
STUDIES OF THE SPLEEN IN
RETICULOCYTE-PRONE NON-
LETHAL MALARIA

IMMUNE RESPONSES CORRELATED WITH CLINICAL PROTECTION IN PAPUA NEW GUINEA

Characterisation of severe *Plasmodium vivax* malaria:

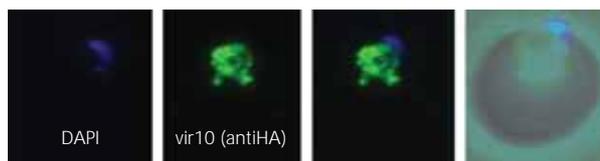
CLINICAL CHARACTERISATION OF SEVERE
PLASMODIUM VIVAX MALARIA IN BIKANER (INDIA)
AND MANAUS (BRAZIL)

MOLECULAR STUDIES OF SEVERE MALARIA
BY *PLASMODIUM VIVAX*

DESCRIPTION OF THE EPIDEMIOLOGY OF MALARIA IN CAREIRO (AMAZON, BRAZIL)

ROLE OF THE VIR PROTEIN

Seven years ago, the research group led by Dr. Fernández-Becerra and Dr. del Portillo identified and characterised the principal multigenic subtelomeric family in *Plasmodium vivax*, denoted *vir* ("*P. vivax* variant genes"). Through many different methods they have studied, in depth, this multigenic family, including the recently identified complete genetic repertoire of *vir* genes in the genome of the Salvador I strain of *P. vivax*.



The fluorescence microscopic analysis of the transgenic line pARL1-3HA-vir10.

This family is the largest multigenic family of human malaria ever described and it corresponds to approximately 5% of the coding genome of *P. vivax*. Initially, it was thought that the function of this super family was antigenic variation, however, recent studies indicate that this is not the main function and the actual biological function is still unknown.

Malarial parasites have developed sophisticated mechanisms to export proteins inside the red blood cells and in this way they are able to reach different subcellular compartments. From sequences of the exported proteins of *P. falciparum*, through two different computational analyses, the existence of a *motif* (a recurring pattern common in a series of related sequences) called PEXEL/HT (*Plasmodium* export element/host targeting signal) has been detected. This *motif* appears to be a signal for the transportation of malarial proteins to the surface of the infected red blood cells. The analysis of the complete sequence of *vir* genes from the Salvador I strain showed that only 171 *vir* genes contain the anticipated transmembrane domains (TM) and that only four *vir* genes, all of which belong to the subfamily D, possess the exact *motif* PEXEL/HT, whereas 160 Vir proteins possess the PEXEL *motif*.

From the blood of patients infected with *P. vivax*, the sequences of the aminoacids obtained by amplifying the different subfamilies of Vir from the genetic material of the parasite were analysed *in silico*. In this manner, it was realised that the subfamily A is related to the multigenic subtelomeric family SURFIN, of *P. falciparum*, and

that the subfamily Vir D contains 2TM domains similar to those of the multigenic family PfmC-2TM. The SURFIN proteins are located on the surface of the merozoites and the infected red blood cells, whereas the PfmC-2TM proteins are located in the Maurer fissures.

The data as a whole show that the *Vir* proteins can have different subcellular locations on the surface of the membrane of the infected reticulocytes, which would indicate that they possess different functions. Unfortunately, there is still no system for the continuous *in vitro* cultivation of *P. vivax* that allows the material to be obtained in sufficient quantities for experimentation, therefore the data continue to be speculative.

The proposal that is currently being developed as an alternative system consists in exploring the heterologous transfection of *vir* genes in *P. falciparum*, that is, to introduce the genes of *P. vivax* into *P. falciparum*. According to the results obtained, the expression of the *pvcr-t-o* gene of *P. vivax*, orthologous to *pfcr-t* in *P. falciparum* (two genes from different species that have similar sequences and locations within the genome), showed that the Vir protein is located, together with *pfcr-t*, in the digestive vacuole and that the transgenic line increased the half maximal inhibitory concentration (IC₅₀) to chloroquine (CQ), thus indicating its role in the resistance towards this antimalarial treatment.

The main objective of this project is to determine the function of the Vir proteins through their expression in *P. falciparum*. The specific objectives include: (i) the construction of transgenic lines of *P. falciparum* expressing, *in trans*, different Vir proteins, (ii) the determination of their subcellular location with confocal laser microscopy, (iii) the establishment of an *in vitro* cultivation of *P. vivax* within a short period of time and (iv) the validation of the discoveries obtained from the heterologous transfection studies.

Principal Investigators

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Co-investigators

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Jaume Ordi

Laboratory Technician

Pep Astola

In collaboration with:

- Peter H. David. L'Institut Pasteur, Paris (France).
- Tobias Spielmann. Tropical Medicine Institute of Hamburg, Hamburg (Germany).

Funding agencies

Fundació Cellex, Barcelona (Spain).

Duration of the project

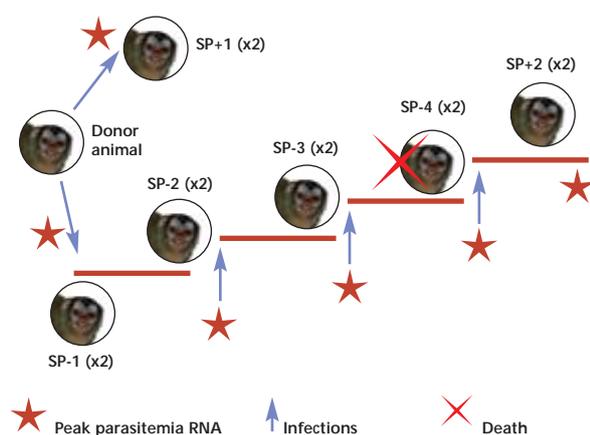
2008 – 2011.



Discovery of antigens for vaccine development

ROLE OF THE SPLEEN IN *PLASMODIUM VIVAX* GENE EXPRESSION

The pathology of malaria is associated with the capacity of the infected red blood cells (iRBC) to cytoadhere to the capillaries of internal organs and escape destruction by the spleen. Since it is widely accepted that *P. vivax* does not cytoadhere, the key issue in the pathology of this type of human malaria is to discover how *P. vivax* escapes destruction by the spleen and establishes chronic infections.



The experimental design for the infection with the Sal I strain of *P. vivax* in *Aotus lemurinus griseimebra* monkeys with (sp+) and without (sp-) a spleen.

Experimental malarial infections in splenectomised animals (from which the spleens have been removed) have demonstrated the key role of the spleen in the expression of variant antigens implicated in cytoadhesion and sequestration. Thus, the parasites of *P. knowlesi* that express variant proteins on the surface of the iRBC rapidly lost this expression and the serum agglutination capacities of the iRBC were diminished when transferred into splenectomised *Rhesus* monkeys. These properties were, however, recovered when the parasites were transferred back to monkeys with their spleens intact. Similarly, the infection of splenectomised monkeys with *P. fragile* showed that the expression of the variant proteins on the surface of the iRBC depends on the presence of this organ.

Studies of splenectomised *Saimiri* monkeys infected with *P. falciparum* showed a slightly different result; the iRBCs lost their previous capacity to cytoadhere, although they appeared to express a new group of variant antigens. In rodent models it was also shown that the ability of the iRBC to cytoadhere was associated with variant antigens and was lost when carrying out experimental infections on splenectomised mice. When these parasites were trans-

ferred back to normal mice, they recovered their capacity to sequester.

As a whole, these data demonstrate the importance of the spleen in the expression of variant antigens associated with cytoadherence. Since it is assumed that *P. vivax* does not cytoadhere, it is particularly important to understand the role of the spleen in the expression of the proteins. Furthermore, since the complete genome sequence of *P. vivax* is publicly available (www.tigr.org/tdb/e2k1/pva1/pva1.shtml) this gene expression analysis can be conducted globally.

To date, there have been several visits to the *Centro Internacional de Vacunas* in Cali (Columbia) where experimental infections were performed with the Salvador I strain of *P. vivax* on 15 *Aotus* monkeys, with and without the spleen. In each of these visits, parasites from the peripheral blood of all infections were obtained. The samples were then taken to the *P. vivax* malaria laboratory at the CRESIB in Barcelona, where the total RNA was extracted from these parasites, its integrity verified and aliquots were used to label the RNA with fluorophores. The labelled RNA was used for microarray hybridisations that represent the entire genome of the Salvador I strain. Work has begun on the identification of these genes whose expression depends on the presence of the spleen and, in addition, a microarray of proteins is being constructed to validate these results.

The identification of *P. vivax* genes whose expression depends on the presence of the spleen will contribute to the understanding of the biology of this parasite and the identification of new vaccine candidates. The main goal of this project is to identify genes of *P. vivax* for which the expression is dependent on the spleen in the experimental model of the *Aotus lemurinus griseimebra* monkey infected with the Salvador I strain of *P. vivax*.

Principal Investigators

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In collaboration with:

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- Ariane Machado Lima and Ricardo Venzio. Universidade de São Paulo, São Paulo (Brazil).

Funding agencies

Fundació Cellex, Barcelona (Spain).

Duration of the project

2007 – 2009.

Discovery of antigens for vaccine development

FUNCTIONAL AND STRUCTURAL STUDIES OF THE SPLEEN IN RETICULOCYTE-PRONE NON-LETHAL MALARIA

The spleen is a complex organ adapted for the selective destruction of old erythrocytes (with reduced deformability), infectious microorganisms (that escape the epithelial barriers) and *Plasmodium*-harbouring red blood cells.



Nuclear magnetic resonance image of a Balb/c mouse. Arrow indicates the spleen.

In rodent models with non-lethal malaria and an affinity for reticulocytes (a situation closely resembling malaria caused by *P. vivax*), it was found that the “open” circulation of the spleen was suddenly and temporarily changed to a “closed” circulation. This is due to the formation of syncytial layers of fibroblasts that form physical barriers, termed barrier cells. This observation indicates that the barrier cells of the spleen play an essential role in how, during reticulocyte-prone non-lethal malaria, the destruction of infected reticulocytes is limited, thereby allowing the parasite to establish chronic infections. In rodent models with normocyte-prone lethal malaria, the barrier cell-dependent remodelling does not occur, a situation closely resembling malaria caused by *P. falciparum*.

Based on the above data, a working hypothesis has been advanced that stipulates that the spleens in mammals experiencing the development of non-lethal reticulocyte-prone *Plasmodium* species are structurally remodelled; this implicates the formation of barrier cells. The remodelled spleen shows new functions that are usually performed by the bone marrow in non-pathological conditions, such as erythropoiesis. When this occurs, the infected reticulocytes cytoadhere to the luminal domain of the barrier cells, thus avoiding destruction by the macrophages. Furthermore, the *P. vivax* merozoites released from the spleen during this process encounter new reticulocytes for invasion.

Based on the above assumptions, Balb/c mice were infected with the non-lethal *P. yoelii* strain 17XNL, which has an affinity for the reticulocyte (similar to *P. vivax*), and with

the lethal *P. yoelii* strain 17XL (similar to *P. falciparum*). The histopathological analysis of the infected spleens revealed the retention of *P. yoelii* 17XNL was about three times higher than that of *P. yoelii* 17XL. In addition, real-time imaging of transgenic parasites of the *P. yoelii* strains 17XNL and 17XL, which express a fluorescent green protein as they pass through the spleen, showed a reduced mortality and a lack of directionality in the non-lethal strain.

The visualisation of the infected spleens by electron microscopy indicated that these results are due to cytoadherence. Furthermore, the global analysis of the infected spleens identified potential molecular markers of barrier cells of fibroblastic origin. One of these markers permitted the visualisation, by confocal laser microscopy, of a tissue barrier in the spleen. Finally, a reduced phagocytosis of the spleen macrophages in infections with non-lethal strains, compared with lethal strains, was observed.

All these data suggest the existence of a new mechanism for the cleaning or clearance by the spleen in non-lethal malaria with an affinity for the reticulocyte. This occurs through the formation of a tissue barrier in this organ that specifically adheres infected reticulocytes. Thus, the identification of molecules related to this interaction may reveal new targets for vaccine development.

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Funding agencies

Fundació Cellex, Barcelona (Spain).

Duration of the project

2008 – 2011.



IMMUNE RESPONSES CORRELATED WITH CLINICAL PROTECTION IN PAPUA NEW GUINEA

The complete sequencing of the *P. vivax* genome has marked a new milestone in research aimed at finding new targets for the development of vaccines and drugs against this parasite. In fact, there currently exists the possibility to use high-performance methodologies to find new targets among the approximately 5400 genes of this organism.



(Left) Location of the Ilhaita zone, close to Maprik, in Papua New Guinea, where a prospective clinical-epidemiological longitudinal study of the *P. vivax* consortium is being developed.
(Right) One of the sample collection points.

The existence of clinical protection for *P. vivax* malaria was initially reported in Brazil, more specifically, in a community on the banks of the Amazon. Subsequent to this finding, two recombinant proteins that represent the N-terminus and C-terminus region of the major surface antigen of the merozoites of *P. vivax* (PvMSP1) were used to determine whether any association exists between this clinical protection and the response to this antigen. Later studies showed that indeed there is an association between IgG antibodies of the subclass 3 and the amino-terminus of the molecule, and a reduced risk of clinical malaria.

More recently, a study with serum samples of children from Papua New Guinea also showed an association between reduced risk of infection and antibodies against region II of the “Duffy binding protein”, a region that requires a specific folding of the molecule.

The above work, the only to date to have shown an association between a reduced risk of *P. vivax* malaria and naturally acquired immune responses against specific molecules of the parasite, reinforces the importance of longitudinal prospective studies in endemic areas and the use of correctly folded recombinant proteins.

Until recently, all studies were carried out using the ELISA technique. This technique, although considered the “gold-standard”, has a large limitation in that it requires

large quantities of serum or plasma; this is particularly relevant when testing samples from infants. For this reason, high-performance techniques have been developed that do not require large quantities of antigens or serum. One such technique, Bioplex, allows the analysis of up to 100 different antigens with only 1–2 microlitres of serum or plasma.

During 2008, a project was initiated to search for new vaccine candidates against *P. vivax*. This involved exploring the correlation between immune responses to hundreds of antigens and the risk of clinical malaria. In this research, a series of specific objectives were drawn including the longitudinal study of a population of children in Papua New Guinea and another population of children in Brazil, as well as the collection of clinical data, serum samples and material from the parasite of molecular studies using Bioplex. Initially, antigens that are today considered as strong candidates against *P. vivax*, such as MSP1-N, MSP1-19, CSP, AMA1, DBP and MSP5, will be produced, although the final objective is to use a panel of approximately 300 new proteins of *P. vivax*.

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Funding agencies

Fundació Cellex, Barcelona (Spain).

Duration of the project

2007 – 2011.

CHARACTERISATION OF SEVERE *PLASMODIUM VIVAX* MALARIA

Principal Investigators

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Lung X-ray of a patient with severe *P. vivax* malaria in Hospital Clinic de Barcelona. This is the first case of severe *P. vivax* malaria reported in Spain.

Characterisation of severe *Plasmodium vivax* malaria

CLINICAL CHARACTERISATION OF SEVERE *PLASMODIUM VIVAX* MALARIA IN BIKANER (INDIA) AND MANAUS (BRAZIL)

In recent years, the paradigm that viewed *P. vivax* as a clinically benign parasite is no longer considered true. Although the majority of clinical episodes caused by this parasite are not severe, there is growing evidence that demonstrates that this parasite may be responsible for serious medical conditions or death.

Despite of the above, the WHO has not established criteria for severe *P. vivax* malaria, nor has it studied the pathogenesis of the clinical complications of the severe form of this disease. Given this scenario, a prospective descriptive study to characterise the clinical presentation of severe *P. vivax* malaria in patients admitted to two tertiary reference hospitals in Bikaner (India) and Manaus (Brazil) will be conducted. Both hospitals will estimate the frequency of hospitalisations of patients with *P. vivax* malaria, the frequency of patients with exclusively confirmed infections of *P. vivax* that fulfil the criteria of severe malaria and the

risk factors for patients admitted with *P. vivax* malaria. In addition, the clinical presentation of this disease in two distinct continents using a single protocol will be compared.

To date, the protocol of this study has been completed and submitted to the relevant ethical committees.

Principal Investigators

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Funding agencies

Fundació Cellex, Barcelona (Spain).

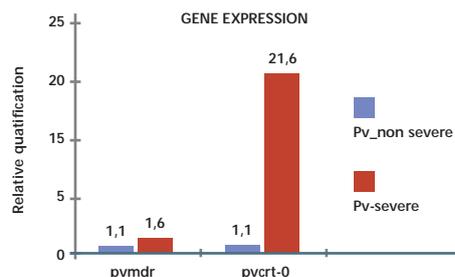
Duration of the project

2007 – 2010.



MOLECULAR STUDIES OF SEVERE MALARIA BY *PLASMODIUM VIVAX*

Clinical infections caused by *P. vivax* are always associated with non-severe symptoms such as fever, headaches, fatigue, chills, muscle pain and, in particular, paroxysms. Recently, however, and each time in greater proportion, severe symptoms associated exclusively with infections of this parasite are being reported. These include renal failure, jaundice, acute respiratory distress, anaemia, hyperparasitemia, thrombocytopenia, pulmonary oedema and splenic rupture.



Expression levels of the genes related with the pvcr-t-0 and pvmdr1 chloroquine resistance in two patients, one with severe and one with moderate *P. vivax* malaria.

Although for the moment the molecular basis of the severe manifestations of the *P. vivax* infection are not known, it is known that cases of severe malaria have begun to appear in parallel to the emergence of strains resistant to chloroquine. In fact, a recent study in Papua New Guinea confirmed that in this region, where chloroquine is not used as the first line of treatment, there exists hundreds of cases of severe *P. vivax* malaria. Although this study showed no direct association between resistance to chloroquine and severe *P. vivax* malaria, the data suggest this is the case.

There are two proteins related with chloroquine resistance in *P. falciparum*, namely, PfCRT and PfMDR1. These two proteins are transported from the membrane of the digestive vacuole. The present research group has characterised the genes that code the orthologous proteins in *P. vivax* (PvCRT-o and PvMDR1) and has shown that it is the level of expression, and not the mutations, that must be associated with the resistance phenotype.

The specific objective of this project is to develop an interdisciplinary programme of clinical research to decipher the molecular basis of severe malaria. In particular, the aim is to use advanced techniques of gene expression to obtain molecular markers for severe *P. vivax* malaria. These mar-

kers have been developed with two patients admitted to Hospital Clínic de Barcelona (HC) who presented episodes of *P. vivax* malaria, one with severe symptoms and the other with moderate symptoms. Both had travelled to the Brazilian Amazon (Manaus) in 2007. In 2000, two other patients with moderate symptoms were treated at the Centro de Pesquisa em Medicina Tropical, in the Brazilian Amazon (Rondonia).

To exclude the possibility that the symptoms of the severely ill Spanish patient were due to *P. falciparum*, PCR techniques were used. By real-time quantitative PCR methods, the levels of transcription of the two principal transporters that are theoretically related with the resistance of *P. vivax* to chloroquine were compared; the transporter of chloroquine resistance of *P. vivax* (PvCRT-o) and the transporter of multidrug resistance of *P. vivax* (PvMDR1).

The results showed that some of the severe clinical symptoms were exclusively due to *P. vivax*. The patient presented acute respiratory conditions and required admission to the intensive care unit. The magnetic method showed highly purified infected reticulocytes in mature stages. In addition, it was observed that the parasites obtained from the severely ill patient had levels of PvMDR1 up to 2.9 times higher and levels of PvCRT-o up to 21.9 times higher than the levels expressed in patients with moderate symptoms.

This is the first case of severe malaria exclusively associated with *P. vivax* malaria in Spain. The results suggest that the clinical severity may be associated with increased levels in the expression of the parasitic genes that appear to be involved in chloroquine resistance. A more profound exploration of the potential expression levels of PvMDR1 and, particularly of PvCRT-o, as molecular markers for severe *P. vivax* disease is necessary.

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In collaboration with:

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Funding agencies

Fundació Cellex, Barcelona (Spain).

Duration of the project

2007 – 2011.

DESCRIPTION OF THE EPIDEMIOLOGY OF MALARIA IN CAREIRO (AMAZON, BRAZIL)

A prospective study is being conducted to determine the epidemiology of malaria in Careiro, an endemic area close to Manaus (Brazil).



Health centre in Careiro, Brazil.

Before the start of this study, a census of the population of the area was conducted (ca. 800 inhabitants). Currently, the participants are being monitored for 18 months, through passive case detection and cross-sectional visits every six months (two during the dry season and two during the rainy season). The visits include the clinical history, a physical examination and blood sampling.

Age-specific prevalences of infection: anaemia, splenomegaly and Duffy antigen deficiency will be estimated, as well as incidencies of clinical malaria. In addition, a description of the presentation of clinical malaria, the seasonality and the use of malaria control tools will be conducted. An entomological study will be carried out to describe the main local vectors associated with the transmission of malaria and the rates of inoculation.

Principal Investigator

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Funding agencies

Fundació Cellex, Barcelona (Spain).

Duration of the project

2008 – 2010.

1.2. SEVMAL: ADHESIVE PHENOTYPES OF *PLASMODIUM FALCIPARUM* INFECTED ERYTHROCYTES AND THEIR CONTRIBUTION TO SEVERE MALARIA IN CHILDREN

Severe malaria is a major cause of child mortality in malaria endemic zones. The clinical manifestations are very diverse and include cerebral malaria, severe anaemia, prostration, respiratory distress, hypoglycaemia and multiple seizures.



A researcher from the *Centro de Investigação em Saúde de Manhica* (CISM, Mozambique) archiving filter papers and slides from the malaria studies.

The mechanisms involved in the pathogenesis of severe malaria are not fully known. There is, however, evidence to suggest that the severe form of the disease is related to the adhesion of parasite-infected erythrocytes to endothelial cells, non-infected erythrocytes (rosetting) and other infected erythrocytes (auto-agglutination). The massive accumulation of infected erythrocytes appears to be responsible for the dysfunction in organs and tissues that lead to severe forms of the disease. Variable proteins of the parasite mediate the mechanisms of adhesion and it is postulated that they contribute to the sequestration

of the parasite in capillaries of the brain and other vital organs. Other human receptors have been identified that could also mediate this interaction, including CD36, ICAM1 (molecules of intercellular adhesion) and CSA (chondroitin sulphate A).

This project aims to study the role of various human receptors in severe malaria. Among the receptors that will be tested is the recently identified C1q (gC1qR) receptor. To this end, the phenotypes for the adhesion of parasites isolated from children with severe malaria (n=75) and with uncomplicated malaria (n=75), recruited for the study in the *Hospital Distrital de Manhica* (Mozambique), have been analysed. Moreover, the immunological responses of these children against *P. falciparum* are being characterised.

The preliminary results have shown that prostration (one of the most common clinical manifestations among the subjects studied) is associated with the capacity of the infected erythrocytes to bind to gC1qR ($p=0.044$) and that severe anaemia is associated with the frequency of rosette formation ($p=0.010$). Furthermore, a negative association between severe malaria and binding to CD36 receptor was observed ($p=0.040$). The identification of the molecular mechanisms implicated in the cytoadhesion of the infected erythrocytes will allow the development of therapeutic strategies that inhibit or reverse the sequestration and the microcirculatory obstruction, and may therefore prevent and/or treat severe malaria.

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Funding agencies

Fondo de Investigación Sanitaria (FIS), Instituto de Salud Carlos III (ISCIII), Madrid (Spain).

Duration of the project

2007 – 2009.

1.3 AGEMAL: EXPOSURE TO *PLASMODIUM FALCIPARUM* AND DEVELOPMENT OF IMMUNITY AGAINST MALARIA IN CHILDREN UNDER ONE YEAR

AgeMal is a four-year research project (2005–2009) with the main objective to understand the role of the age of exposure to *P. falciparum* during the first year of life on the development of naturally acquired immunity (NAI) against malaria. The results obtained from this research will provide relevant information for the design of clinical trials and the development of malaria vaccine candidates and other tools for control, including the optimum period of inoculation during infancy.



AgeMal team: (L to R, back row) Alfredo Mayor, David Roberts, Denise Doolan, Pedro L. Alonso, Peter Le Souef, Chetan Chitnis, Louis Schofield, (front row) Evelin Schwarzer, Carlota Dobaño, Caterina Guinovart and Leanne Robinson.

Currently, the development of NAI against *P. falciparum* malaria is poorly understood. Previous studies of continuous and intermittent chemoprophylaxis in infants have provided evidence that the age of first exposure to *P. falciparum* during infancy may be important in the

development of NAI, as measured by the incidence of clinical malaria during the second year of life. These studies suggest that exposure to *P. falciparum* prior to five months of age does not lead to the development of NAI, whereas exposure to *P. falciparum* after five months of age does.

The main objective of this project is to evaluate the effect of exposure to erythrocytic-stage antigens of *P. falciparum* during certain periods of the first year of life on the development of the NAI.

AgeMal has four components:

- Field study
- Immune responses
- Oxidative stress
- Genetic factors of the host

AgeMal is coordinated by the CRESIB, and has the participations of other institutions.

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- Louis Schofield. The Walter and Eliza Hall Institute of Medical Research, Melbourne (Australia).
- Chetan Chitnis. International Center for Genetic Engineering and Biotechnology, New Delhi (India).
- Denise Doolan. Queensland Institute of Medical Research, Brisbane (Australia).

Funding agencies

FP6 programme, European Union.

Duration of the project

2005 – 2009.



FIELD STUDY

To explore the effects of the age of exposure on the development of the NAI, a randomised, double blind and placebo controlled three-arm clinical trial was designed in an endemic area of southern Mozambique.

In this study, the exposure to *P. falciparum* was selectively controlled in different periods of infancy (2.5–5 months, 5.5–10.5 months or without any prophylaxis) with monthly chemoprophylaxis with sulphadoxine-pyrimethamine (SP) and artesunate. For this, 350 infants from HIV-negative mothers were recruited and allocated to one of three cohorts.

The participants of the study were monitored for the detection of active and passive cases, as well as being submitted to cross-sectional visits up to 24 months of age. This makes it possible to compare the risk of clinical malaria and anaemia during the second year of life between the cohorts, as well as to correlate the type and quality of the

immune responses (antibodies against several *P. falciparum* antigens, cytokines), oxidative stress markers and genetic factors of the host.

The results obtained in this study will contribute to a better understanding of the determinants for the development of responses against *P. falciparum* early in life and the potential limitations of early life immunisation.

The monitoring of the participants of the study will be completed in March 2009 and will be followed by the analysis of the data.

IMMUNE RESPONSES

Blood samples of the participants in the AgeMal field study, collected at 0, 5, 10, 12, 15, 20 and 24 months of age are being used to study the development of immune responses to malaria.

The immune responses under study are:

- Antibodies to haemozoin and GPI in plasma samples by the ELISA method.
- Antibodies to blood-stage antigens (VSA, DBL- α , MPS-1, EBA-175, AMA-1) in plasma samples (cord and peripheral blood) with the FACS and ELISA techniques.
- Cytokines, specific for *P. falciparum*, produced by lymphocytes in culture supernatants by a combined technique of immunoassay and flow cytometry (multiplexed bead assays).

Antibody and cytokine measurements will be compared between the cohorts to analyse the differences in the development of immune responses to malaria.

These results will be correlated with the risk of clinical malaria during the second year of life.

This study aims:

- To look for associations between specific immune responses and the risk of clinical malaria during the second year of life.
- To describe, prospectively, the acquisition of antibodies and cytokines produced in response to toxins and antigens of *P. falciparum* during the first two years of life.
- To determine the contribution of the maternal antibodies to the overall antibody pool during the first five months of life.

The data from this study are currently under analysis and once results are obtained they should shed light on the determinants of the development of anti-*P. falciparum* responses in infancy.

OXIDATIVE STRESS

This component of the AgeMal study investigates the impact of the age of exposure to *P. falciparum* on the oxidative stress markers, and their influence on the development of the NAI.

During a *Plasmodium* infection, both host and parasite are under oxidative stress. The oxidative state of immune system cells has been shown to influence the quality of the immune response, for example, the redox state of the macrophages influences the pattern of the released cytokines (modulating their glutathione content, GSH). Another example is the maturation of the dendritic cells, which are inhibited by the pro-oxidant effects of haemozoin. In addition, malnutrition, very common in malaria endemic areas, negatively affects the antioxidant potential of the host.

The anti-oxidant/pro-oxidant balance therefore appears to influence the quality and quantity of the immune response against *P. falciparum*, although clinical research to date has not yet established the role of these phenomena in the development of protective immunity.

In this context, a series of experiments have been carried out, including the characterisation of oxidant and antioxidant markers in plasma and erythrocyte samples of Manhiçan children who participated in the AgeMal field study. A number of parameters have also been measured among which are the membrane surface markers (by flow cytometry) and the glutathione compartments. Isolated membranes (ghosts) were also analysed to study the lipid peroxidation of the membrane lipids, and the binding to denatured haemoglobin membranes (haemachromes) and free heme.

The data obtained in this component of the AgeMal study are currently under analysis.

GENETIC FACTORS OF THE HOST

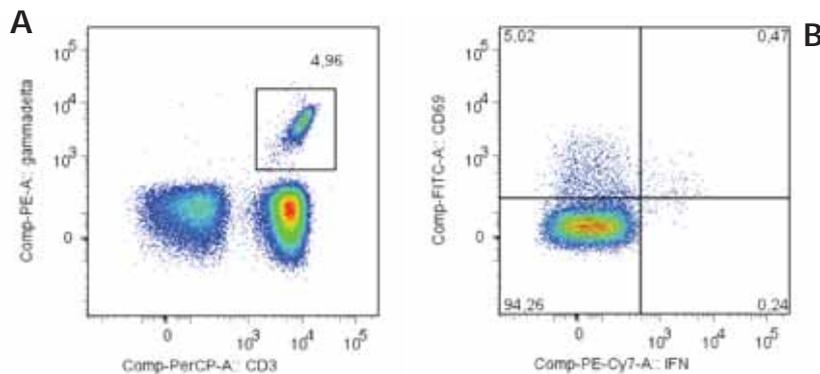
The component of the AgeMal project researching the genetic factors of the host is being implemented through collaborators at the Immunogenetic Research Group, led by Prof. Peter Le Souef, at the University of Western Australia.

A study has been conducted on immunogenetic factors to determine genetic polymorphisms that affect the

development of the immune responses against malaria. This component is currently under analysis.

1.4. THE STUDY OF IMMUNITY AND SUSCEPTIBILITY MARKERS TO MALARIA IN INDIVIDUALS EXPOSED TO *P. FALCIPARUM* INFECTION

With the continuous exposure to *P. falciparum*, individuals acquire with age an effective natural immunity against this disease. The underlying mechanisms of this protection are, however, still unknown.



Activation of $\gamma\delta$ cells induced by *P. falciparum*-infected erythrocytes. White cells were cultivated with *P. falciparum*-infected erythrocytes over 72 hours, stained and analysed by flow cytometry to study the immune response. From the plot of live lymphocytes, the $\gamma\delta^+$ CD3⁺ cells are selected (A) and the early activation markers CD69 and the intracellular IFN γ (B) are detected.

To date, a comprehensive analysis of the role of the cellular immune response has not been possible due to the large blood volumes required for such a study and the limited number of parameters that can be studied by classical methodologies.

Recent advances in multiplex and high-throughput methods support the simultaneous characterisation of a variety of mediators and cellular phenotypes, therefore allowing the qualitative and quantitative analysis of malarial immunity. Using these techniques, a study has been designed with the overall objective to perform a comprehensive analysis of cellular immunity to identify responses against *P. falciparum*, which could be used as immunity and/or susceptibility markers against malaria.

The specific objects of this study, initiated in December 2008, are:

1. To identify and standardise sensitive and specific immunological techniques that allow the simultaneous measurement of multiple cytokine responses with small sample volumes.

2. To characterise the markers of clinical immunity in children.
3. To characterise the immunopathological markers of children with severe malaria.
4. To characterise the immunopathological markers of placental malaria in pregnant women.
5. To develop tools for the statistical analysis and correlation of immunological and clinical data.

To achieve this, a collection of cryopreserved blood samples from six studies conducted in Mozambique and/or Barcelona, which were designed to investigate the immunity and pathogenesis against malaria from different perspectives, are being analysed.

The characterisation of the immune responses correlated with protection against *P. falciparum* will greatly facilitate the development and evaluation of new vaccine candidates, as well as the deployment of effective interventions against malaria.

Principal Investigator

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Funding agencies

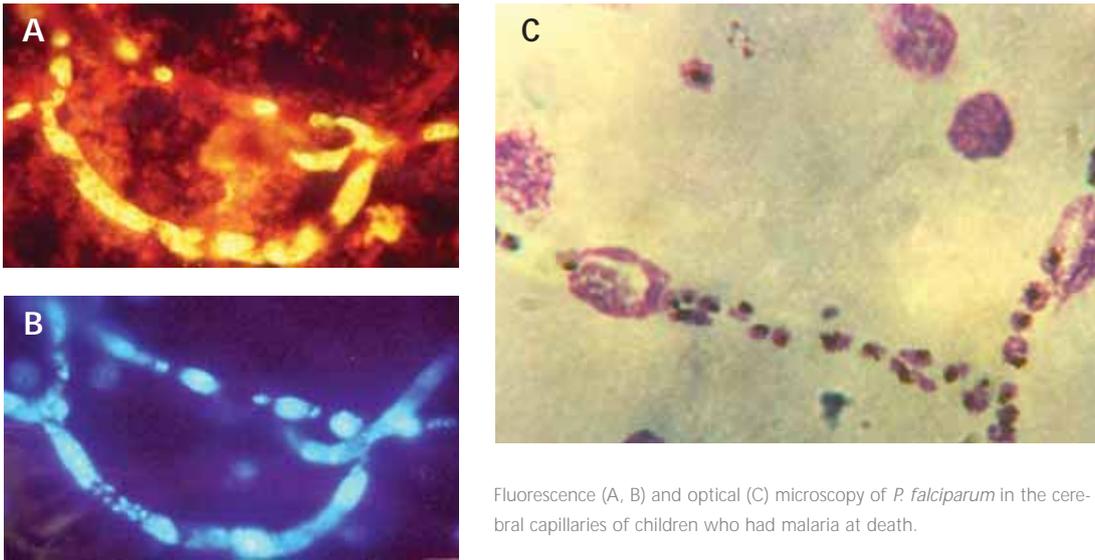
Plan Nacional de I+D, Ministerio de Ciencia e Innovación, Gobierno de España (Spain).

Duration of the project

2008 – 2011.

1.5. PROGRAMME OF THE IMMUNOPATHOLOGY OF MALARIA

The present programme aims to foster research on the immunopathology of malaria by developing two principal lines of research: (i) the characterisation of immunity against *P. falciparum* and (ii) the development of trials for the study of immune responses against *P. falciparum* induced by experimental vaccines in children.



Fluorescence (A, B) and optical (C) microscopy of *P. falciparum* in the cerebral capillaries of children who had malaria at death.

The main objectives for the first line of research are:

1. To characterise the immune mechanisms, antigenic targets, cells and immunological mediators that are determinants in the acquisition of immunity against malaria.
2. To describe the mechanisms, antigenic targets, cells and immunological mediators that are responsible for the protective immunity and induced durability of experimental vaccines.
3. To evaluate the impact that different control measures against malaria may have on the acquisition of immune responses against this parasite.

To investigate the effective immunity mechanisms these studies include both arms of the immune system: the humoral and the cellular responses.

The second objective involves the development of a series of Luminex® assays to simultaneously identify specific antibodies against multiple antigens of *P. falciparum* in reduced volumes of serum/plasma with high performance. In addition, functional tests are being optimised to measure the capacity of the antibodies to inhibit the *in vitro* growth of *P. falciparum*.

To conduct the cellular investigation component (the measurement of cellular responses), techniques for measuring cytokines, interferon gamma (IFN- γ) and interleukin-2 (IL-2), after stimulation with antigens of *P. falciparum*, are being optimised. The measuring of specific and polyfunctional antigenic responses are conducted by flow cytometry.

All the above techniques are being optimised to minimise the volume of blood samples necessary for the tests.

Principal Investigator

Pedro L. Alonso

Co-investigator

Carlota Dobaño

Funding agencies

Fundación Ramón Areces, Madrid (Spain).

Duration of the project

2008 – 2009.

1.6. PHYSIOPATHOLOGICAL STUDIES OF PLACENTAL MALARIA

Currently, the roles of placental sequestration, the placental receptor(s), and the parasitic ligands in the sequestration of parasites, as well the development of protective immunity against malaria during pregnancy are being investigated through the following studies:

- **EPIC**
Physiopathological mechanisms involved in placental malaria infections and their impact on foetal development.
- **PREGMAL**
Characterisation of the *P. falciparum* ligand implicated in adhesion to the placenta and its role in the development of immunity against malaria during pregnancy.

Physiopathological studies of placental malaria

EPIC: PHYSIOPATHOLOGICAL MECHANISMS INVOLVED IN PLACENTAL MALARIA INFECTIONS AND ITS IMPACT ON FOETAL DEVELOPMENT

The aim of this study was to investigate the physiopathological mechanisms involved in placental malaria infections from a multidisciplinary perspective: histological, parasitological, cytometric, immunological and molecular, and to assess the impact of the different alterations on foetal development.



A pregnant Mozambican woman installing a mosquito net in her dormitory.

With the above proposition, an observational, descriptive and transversal study was designed, in which women who gave birth naturally in the maternity ward of the *Centro de Investigação em Saúde de Manhiça* (CISM, Mozambique) participated. The women were assigned to one of the following groups: women with active placental malaria infections (n=50), women with passive placental malaria infections (n=50) and women without placental malaria infections (n=50). A control group of women from a non-endemic area (Barcelona, n=25) was also included in the study.

Samples of the peripheral blood, placental blood from the intervillous space and cord blood were taken, as well as fixed and frozen tissue samples of the placenta.

Various analyses are being conducted on these samples, including a histological and immunohistochemical study of the placenta, a quantification of the number of immune system cells in the peripheral, placental and cord blood (by flow cytometry), and a determination of hormones (estradiol, cortisol), cytokines, chemokines and antibodies against *P. falciparum* in the plasma and the peripheral, placental and cord blood. A parasitic study analysed by thick smear and polymerase chain reaction (PCR) techniques, a study of the infection multiplicity of *P. falciparum*, and a study of gene expression of *P. falciparum* in the peripheral and placental blood by RT-PCR and microarray techniques are also being performed.

This study is currently undergoing statistical analysis of the data and the interpretation of results.

Principal Investigators

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Funding agencies

Fondo de Investigación Sanitaria (FIS), Instituto de Salud Carlos III (ISCIII), Madrid (Spain).

Duration of the project

2003 – 2009.

Physiopathological studies of placental malaria

PREGMAL: CHARACTERISATION OF THE *P. FALCIPARUM* LIGAND IMPLICATED IN ADHESION TO THE PLACENTA AND ITS ROLE IN THE DEVELOPMENT OF IMMUNITY AGAINST MALARIA DURING PREGNANCY

The creation and implementation of the PregMal project responds to the need to establish an area dedicated to investigating the molecular and immunological mechanisms involved in the adhesion of *P. falciparum* to the placenta.

This project aims to characterise, at a functional, antigenic and structural level, the ligand of *P. falciparum* implicated in the adhesion of this parasite to the placenta. The objectives are:

1. To establish the prevalence and specificity of the ligand expression in placental isolates and its association with the phenotype of adhesion.
2. To determine the level of genetic and antigenic conservation of the *P. falciparum* ligand.
3. To characterise the humoral response that is naturally developed against the parasitic ligand in pregnant women in malaria endemic areas.



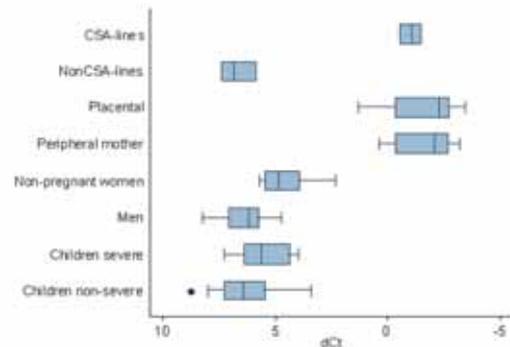


Extraction of blood from a recently expelled placenta infected with *P. falciparum* (CISM laboratory, Mozambique).

To achieve the first two objectives, the phenotypic and transcriptional profiles of 54 placental isolates were compared with the peripheral isolates of pregnant women (n=54), adult males (n=54), non-pregnant women (n=54) and children (n=54), all from Manhica (Mozambique).

For the third objective, the humoral response developed by 408 pregnant women who participated in a clinical trial of intermittent preventive treatment was analysed, retrospectively. The analysis of parasites by adhesion tests and the sequencing of the predominantly transcribed var domains were also carried out. In addition, to characterise the immune responses in pregnant women, the quantity and quality of specific antibodies against the domains of the parasitic ligand, as well as their relationship with the age, parity, exposure and severity of the maternal infection were determined.

The results of this project showed that the parasites isolated from the placenta and the peripheral blood of



Transcription levels of *var2csa* by quantitative PCR in isolates from the Mozambican people (in dCt units, negative values indicate a higher transcription level).

pregnant women transcribed *var2csa* (a variable gene of *P. falciparum* associated with CSA adhesion) at levels greater than those of the parasites isolated from children, men and non-pregnant women. Moreover, it was observed that the placental infection increases the level of antibodies with respect to uninfected men and women, suggesting that the IgG levels reflect exposure to *P. falciparum* during pregnancy.

Finally, it was observed that antibodies against a large range of *P. falciparum* antigens were found to be present in lower levels in women pregnant for the first time than those with previous pregnancies. Therefore, an absence of immunity against placental parasites, together with immunosuppression during the first pregnancy, may explain the increased risk of malaria in women pregnant for the first time. These findings have implications for the evaluation of malaria control strategies during pregnancy and for the development of specific vaccines targeted at pregnant women.

Principal Investigator

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Principal Co-investigator

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Co-investigators

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In collaboration with:

- Chetan Chitnis. International Center for Genetic Engineering and Biotechnology, New Delhi (India).

Funding agencies

Fondo de Investigación Sanitaria (FIS), Instituto de Salud Carlos III (ISCIII), Madrid (Spain).

Duration of the project

2007 – 2009.

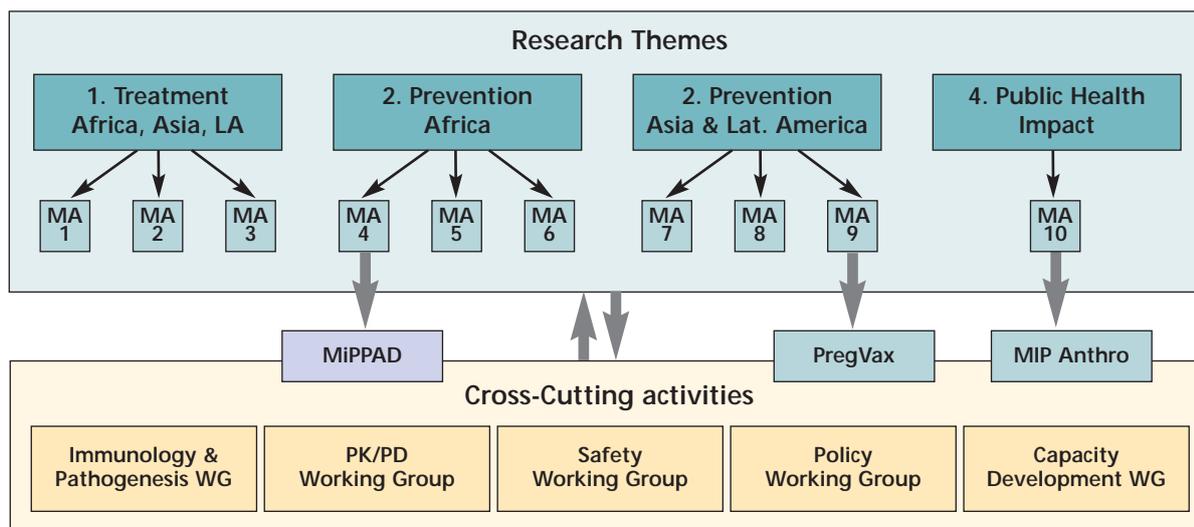
Publications

- Sikora M, Ferrer-Admetlla A, Mayor A, Bertrandpetit J, Casals F. Evolutionary analysis of genes of two pathways involved in placental malaria infection. Hum Genet 2008; 123(4):343-57.

1.7. MALARIA IN PREGNANCY CONSORTIUM (MiPc)

www.mip-consortium.org

The MiP Consortium (MiPc) is a five-year research programme created to evaluate new and improved interventions for the prevention and treatment of malaria during pregnancy, a disease that puts more than 50 million women at risk each year.



As part of this consortium, ten main activities (research studies, denoted “Major Activities” in the schematic) focused on research in three key areas of malaria in pregnancy will be conducted: prevention, treatment and the impact on public health. Renowned institutions from around the world will develop this research and share knowledge and information to provide the evidence needed to improve the control of malaria in pregnancy.

The MiPc is principally funded by the Bill & Melinda Gates Foundation and the European Union. The researchers from CRESIB coordinate three main activities (MiPPAD, PregVax and MiPAnthro) as shown in the diagram.

- MiPPAD: Evaluation of alternatives to the antimalarial drug sulphadoxine-pyrimethamine (SP) for the intermittent preventive treatment in pregnancy (IPTp) in the context of insecticide-treated mosquito nets.
- PregVax: *Plasmodium vivax* infection in pregnancy.
- MiPAnthro: Malaria in Pregnancy Consortium; Impact on public health – anthropological component.

Malaria in Pregnancy Consortium (MiPc)

MIPPAD: EVALUATION OF ALTERNATIVES TO THE ANTIMALARIAL DRUG SULPHADOXINE-PYRIMETHAMINE (SP) FOR THE INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY (IPT_p) IN THE CONTEXT OF INSECTICIDE-TREATED MOSQUITO NETS

Malaria in pregnancy constitutes one of the major preventable causes of low weight births and the leading cause of severe maternal anaemia, which contributes greatly to maternal mortality. For these reasons, it is now a priority to provide endemic countries with effective preventive interventions that reduce the incidence and consequences of malarial infections in pregnant women.

The MiPPAD project (Malaria in Pregnancy Preventive Alternative Drugs) aims to contribute to the development of new clinical interventions to fight malaria in pregnancy by evaluating different alternatives to anti-malarial drugs used for the intermittent preventive treatment in pregnancy (IPT_p) in the context of insecticide-treated mosquito nets (ITNs). For this, the safety and efficacy of sulphadoxine-pyrimethamine (SP), the medication currently recommended for IPT_p, will be compared with other antimalarial drugs. This study will also include HIV-infected pregnant women to gain a better understanding of control tools for malaria during pregnancy in this population.

This project involves institutions from four European countries (Austria, France, Germany and Spain) and five sub-Saharan African countries (Benin, Gabon, Kenya, Mozambique and Tanzania), which will strengthen the coordination and networking between Europe and Africa. The project will also help develop the capacities of African institutions in this area by offering courses, and master and doctoral scholarships.

The CRESIB coordinates the implementation and development of the project, including the preparation and conditioning of the research tools necessary to conduct the research in the participating countries and supporting the organisation of the training activities. The CRESIB is also responsible for ensuring that the

clinical trials are carried out in compliance with the Good Clinical Practice in Research guidelines (GCP), and of the study in Mozambique.

In 2008, the foundations were established to carry out the project and initiate the trials in the centres of the partner countries during the following year.

In collaboration with:

- Michel Cot. Institut de Recherche pour le Développement, Paris (France).
- Achille Massoughbodji. Faculty of Health Sciences, Université d'Abomey Calavi, Cotonou (Benin).
- Peter Ouma. Kenya Medical Research Institute, Kisumu (Kenya).
- Ghyslain Mombo-Ngoma. Medical Research Unit, Albert Schweitzer Hospital, Lambarene (Gabon).
- Meghna Desai. Centers for Disease Control and Prevention, Atlanta (U.S.A.).
- Abdunoor Mulokozi. Ifakara Health Institute, Ifakara (Tanzania).
- Michael Ramharter. Institute of Tropical Medicine, Universität Tübingen, Tübingen (Germany).
- Christa Janko. Vienna School of Clinical Research, Vienna (Austria).

Funding agencies

- European & Developing Countries Clinical Trial Partnership, EDCTP (European Union).
- Malaria in Pregnancy Consortium (MiPc), Liverpool School of Tropical Medicine (LSTM), Liverpool (U.K.).
- Fondo de Investigación Sanitaria (FIS), Instituto de Salud Carlos III (ISCIII), Madrid (Spain).

Co-funders

Member States of the European Union participating in this project.

Duration of the project

2008 – 2013.

Principal Investigator

Clara Menéndez

Co-investigators

Raquel González, John Aponte,

Edgar Ayala

Project Manager

Maria Oziemkowska

Project Assistant

Montse Pi

PREGVAX: *PLASMODIUM VIVAX* INFECTION IN PREGNANCY

Approximately 25 million women live in areas where *P. vivax* infection is endemic. Although the effects of *P. falciparum* malaria in pregnancy have been well characterised, little is known about the impact of the *P. vivax* infection during pregnancy.



Pregnant women from India, participants in the PregVax study.

The Seventh Framework of the European Union has prioritised research in the field of malaria in pregnancy. To fill the knowledge gap that currently exists in this area, an observational cohort study will be carried out, under the PregVax research programme, of pregnant women in Brazil, Columbia, Guatemala, India and Papua New Guinea. These countries, endemic with *P. vivax* malaria, are representative of the majority of infections of this parasite in the world.

In each of the above sites, 2000 pregnant women will be enrolled during routine antenatal visits and monitored until delivery or the end of the pregnancy. Clinical and epidemiological studies and immunological analyses will be performed with the following objectives:

1. To study the prevalence of *P. vivax* infection.
2. To study the impact of *P. vivax* malaria on birth weight, premature births and maternal anaemia.

Principal Investigator

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Co-investigators

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Project Manager

Janifer Quick

Project Assistant

Yolanda Antin

3. To explore the existence of *P. vivax* specific immune responses in pregnancy.

The CRESIB coordinates the development of this project and leads the preparation of research tools necessary to conduct the study in participating countries. The CRESIB is also responsible to ensure that the study is conducted according to the Good Clinical Practice in Research (GCP) guidelines.

A first annual meeting has been held in Barcelona with researchers from all participating institutions. All working tools (protocols, questionnaires and standardised procedures) have been developed, as well as the process of obtaining ethics approval, both in Barcelona and in each of the integrated countries, some of which have already begun the recruitment of study participants.

In collaboration with:

- Mats Wahlgren. Karolinska Institute, Stockholm (Sweden).
- Carlo Severini. Instituto Superiore di Sanità, Rome (Italy).
- Ivo Müller. Papua New Guinea Institute of Medical Research, Goroka (Papua New Guinea).
- Swati Kochar. Medical College, Bikaner (India).
- Chetan Chitnis. International Centre for Genetic Engineering and Biotechnology, New Delhi (India).
- Flor Martínez-Espinosa. Fundação de Medicina Tropical do Amazonas, Manaus (Brazil).
- Norma Padilla. Universidad Del Valle de Guatemala, Guatemala City (Guatemala).
- Miriam Arévalo and Sócrates Herrera. Instituto de Inmunología, Cali (Colombia).
- Meghna Desai. Centers for Disease Control and Prevention, Atlanta (U.S.A.).

Funding agencies

FP7 Programme, European Union.

Co-funders

Malaria in Pregnancy Consortium (MiPc), Ministerio de Ciencia y Tecnología, Madrid (Spain).

Duration of the project

2008 – 2012.

MIPANTHRO: MALARIA IN PREGNANCY CONSORTIUM; IMPACT ON PUBLIC HEALTH – ANTHROPOLOGICAL COMPONENT

The anthropological component of the Malaria in Pregnancy Consortium (MiPc), part of the Public Health Impact research activities has a number of key objectives ranging from the perception of malaria in pregnancy through to the exploration of factors that affect the health policies of the participating countries.



An interview with a pregnant woman in Kenya.

The above objectives can be articulated as follows:

1. To describe how malaria in pregnancy is perceived and prioritised in relation with other health problems in Africa, Latin America and Asia.
2. To study the acceptability of different drugs for the treatment of malaria as well as other integrated interventions for the prevention of malaria in pregnancy (MiP).
3. To identify the broader social, cultural and economic determinants on the demand of MiP interventions.
4. To identify the factors at infrastructure and district levels, which influence the supply of MiP interventions in the context of other reproductive health interventions.

Principal Investigator

Robert Pool

Co-investigators

Lianne Straus, Arantza Meñaca

5. To explore the factors affecting the uptake and implementation of policies in the countries at the level of national policy makers.

Since the start of this study, a systematic review of the anthropological literature on malaria in pregnancy has been conducted and a general research protocol has been designed and was approved by the relevant bodies in September 2008. Contact with different research centres linked to the consortium in Africa, Latin America, Asia and Oceania have been made to determine the possibilities of carrying out this study in these countries.

In collaboration with:

- Jayne Webster, Anne Mills and Kara Hanson. London School of Hygiene and Tropical Medicine, London (U.K.).
- Jenny Hill. Liverpool School of Tropical Medicine, Liverpool (U.K.).
- Abraham Hodgson. Navrongo Health Research Centre, Navrongo (Ghana).
- Harry Tagbor. School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi (Ghana).
- Mary Hamel. Kenya Medical Research Institute/CDC Research Station, Kisumu (Kenya).
- Ivo Müller. Papua New Guinea Institute of Medical Research, Goroka (Papua New Guinea).

Funding agencies

Malaria in Pregnancy Consortium (MiPc).

Duration of the project

2007 – 2010.

1.8. ANEM: AETIOLOGY OF ANAEMIA IN CHILDREN IN A MALARIA ENDEMIC RURAL AREA OF MOZAMBIQUE

The overall objective of this research is to provide guidance for the development and implementation of strategies to prevent anaemia and improve its clinical management. For this, a description of the causes and risk factors of anaemia in children between one month and five years of age in rural areas of Mozambique will be conducted.



Vitamin A supplementation. The Manhica maternal and child health program.

In terms of specific targets, the aims are:

1. To evaluate risk factors potentially related to anaemia and their relative contribution.
2. To explore the validity of available biochemical markers to improve diagnostic tools for iron deficiency.
3. To study the correlation of various parameters of clinical, haematological, biochemical and immunological severity, with the severity of malarial anaemia to identify new pathophysiological mechanisms.

To achieve the proposed targets, a prospective case-control study has been designed for children between one

Principal Investigator

Clara Menéndez

Co-investigators

Montse Renom, Ruth Aguilar,

Mauricio Humberto Rodríguez, Cinta Moraleda

month and five years of age, with pairing frequencies for age (<1 year, 1–2 years and 2–5 years). A total of 1350 children will be recruited: 450 cases, 450 hospital controls and 450 community controls.

The following risk factors will be evaluated: sociodemographic, nutritional, genetic, infections, inflammation, haemolysis, erythrocyte suppression and dyserythropoiesis. In cases of malarial infection, haemozoin in the plasma and bone marrow, and its derived product, 4-hydroxy-2-nonenal (4-HNE), synthesised on the surface of the red blood cells, will be measured. In addition, the IL1, IL4, IL6, IL10, TNF- α , INF γ and MIF cytokines from plasma samples, as well as the TNF- α and INF γ cytokines from 100 bone marrow samples will be determined. The profile of genetic expression of the bone marrow will also be studied to identify the genetically regulated steps that affect anaemia by erythrocyte suppression.

Currently this study is in the phase of recruiting and monitoring participants.

In collaboration with:

- Ariel Achtman, Louis Schofield. Infection and Immunity Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne (Australia).

Funding agencies

Agència de Cooperació Internacional de les Illes Balears, Palma de Mallorca (Spain).

Duration of the project

2008 – 2010.

1.9. INTERMITTENT PREVENTIVE TREATMENT IN INFANTS CONSORTIUM (IPTi)



www.ipti-malaria.org

Intermittent preventive treatment (IPT) consists of administering, at regular intervals, antimalarial drugs regardless of malarial infection or disease.

In contrast to continuous chemoprophylaxis, IPT reduces the number of times it is necessary to administer antimalarial drugs to an individual and circumvents the problem of supply as, in this case, it is given at the time of routine childhood vaccinations.

The IPTi Consortium is a work programme that involves several countries in a coordinated manner to generate rigorous and convincing evidence to help guide IPTi policies. This consortium, established in 2003 and funded by the Bill & Melinda Gates Foundation, involves the participation of 20 institutions, one of which is the CRESIB. The CRESIB manages the secretariat and coordination of the consortium through its project manager, Andrea Egan.

The goal of this consortium is to develop a research and implementation agenda that would rapidly resolve the important scientific questions about this innovative form of malaria control. The questions relate to the efficacy of IPTi with the antimalarial drug sulphadoxine-pyrimethamine (SP) in different epidemiological settings, the safety profile of IPTi, and the possibility of interactions between IPTi and the serological response to the vaccines of the Expanded Programme on Immunisation (EPI). The consortium aims to translate this intervention into health policies and clinical practice.

Using the strength, experience and know-how of each partner, the consortium provides a powerful platform for research and development, evaluation and large-scale use of IPTi in Africa. Furthermore, the consortium also plans to generate information on issues relating to the choice of

antimalarial drugs, relative cost-effectiveness, acceptability, impact on mortality and the effect on the community, as well as to improve the understanding of the immunological response towards *P. falciparum* infection.

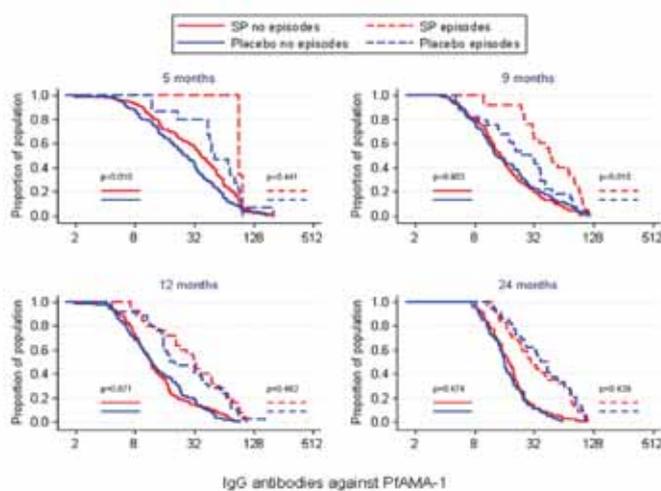
The CRESIB has participated in several projects, among which was a randomised, placebo-controlled trial of IPTi administered at the time of routine EPI visits to Mozambican children. This study showed that IPTi with SP moderately reduced the incidence of clinical malaria in these children during the first year of life, with no evidence of a rebound effect after stopping the intervention, and without interactions with the EPI vaccines (Macete et al. *J Infect Dis.* 2006;194(3):276-85).

The CRESIB has carried out the monitoring of three projects that have improved the understanding of IPTi:

- IPTi Immuno: Impact of intermittent preventive treatment on the development of naturally acquired immunity in Mozambican infants.
- Acceptability of intermittent preventive treatment in infants (IPTi) for the control of malaria and anaemia.
- IPTi Cost-effectiveness working group (CEWG).

IPTi Immuno: IMPACT OF INTERMITTENT PREVENTIVE TREATMENT ON THE DEVELOPMENT OF NATURALLY ACQUIRED IMMUNITY IN MOZAMBICAN INFANTS

This study aims to investigate the impact of IPTi on the development of naturally acquired immunity (NAI). This intervention in children may interfere with the acquisition of immunity to malaria later in life and could result in negative consequences, such as a rebound of clinical malaria, or positive consequences, such as a long-term protection against malaria after termination of treatment.



Antibody levels against *P. falciparum* antigens in Mozambican children following administration of IPTi with SP. Children who have experienced previous episodes of clinical malaria have elevated levels of antibodies. At the ages of five and nine months, the antibody responses are higher in children who have received IPTi treatment with antimalarial drugs (Quelhas et al. *in press*).

To investigate the possible interference with NAI, blood samples were collected from children who had received IPTi with SP or a placebo treatment to measure the pattern of immune responses to *P. falciparum* infection during the first two years of life. The prospective analysis of these immune responses, in parallel with the monitoring of morbidity and mortality, will allow a better understanding of the immunological basis of any potential effect that the IPTi might have on the risk of clinical malaria.

Principal Investigator
Carlota Dobaño
Principal Co-investigator
Clara Menéndez
Co-investigators
Diana Quelhas, Llorenç Quintó, Eusebio Macete

According to preliminary results, the antibody responses do not significantly differ between the treatment groups at any time when measurements were made, with the exception of the responses of IgG and IgG1 to AMA-1 and/or MSP-119, which were significantly higher in the group treated with SP at the ages of 5, 9 and/or 24 months.

The results obtained from this study also indicate that the cytokine responses do not significantly vary in children that receive SP or placebo. Still to be established, however, is the effect that the IPTi may have had on the development of the response to the surface antigens of *P. falciparum* and on the development of functional antibodies that inhibit the *in vitro* invasion of erythrocytes by *P. falciparum*; the analysis of these data is currently being conducted.

According to the results obtained so far, IPTi with SP does not appear to negatively affect the development of the immune responses to the antigens of *P. falciparum*, in fact, in some cases it appears to be associated with higher levels of antibodies.

In collaboration with:

- Chetan Chitnis. International Centre for Genetic Engineering and Biotechnology, New Delhi (India).
- Denise Doolan. Queensland Institute of Medical Research, Brisbane (Australia).
- James Beeson. The Walter & Eliza Hall Institute of Medical Research, Melbourne (Australia).

Funding agencies

Bill & Melinda Gates Foundation, Seattle (U.S.A.).

Duration of the project

2003 – 2009.

Publications

Quelhas D, Puyol L, Quintó L, Serra-Casas E, Nhampossa T, Macete E, Aide P, Mayor A, Mandomando I, Sanz S, Aponte JJ, Chauhan VS, Chitnis CE, Alonso PL, Menéndez C, Dobaño C. Impact of Intermittent Preventive Treatment with Sulfadoxine-Pyrimethamine on Antibody Responses to Erythrocytic-Stage *Plasmodium falciparum* Antigens in Infants in Mozambique. *Clin Vacc Immunol* 2008; 15: 1282-91.

ACCEPTABILITY OF INTERMITTENT PREVENTIVE TREATMENT IN INFANTS (IPTi) FOR THE CONTROL OF MALARIA AND ANAEMIA

The overall objective of the project is to facilitate the implementation and long-term acceptability of intermittent preventive treatment in infants (IPTi), linked to the Expanded Programme on Immunisation (EPI) in Africa. To this end, it is necessary to identify and understand the actual and potential impediments and to make recommendations to overcome these impediments as they arise. In addition, it is also necessary to identify, develop and strengthen the factors that contribute to reducing the burden of malaria and anaemia in infancy.



Medical assistant caring for a baby in Mozambique.

To date, the IPTi acceptability projects have been carried out in seven sites in six countries. Currently in progress are those in Gabon, Ghana, Kenya, Malawi, Tanzania (Kilimanjaro) and Papua New Guinea. The results of the studies in Tanzania (south) and Mozambique have already been published (as part of the same project but with different funding, Pool et al. 2008).

The preliminary results suggest that the IPTi involving different drugs and treatment regimes is generally accepted across a wide range of sites in Africa, with a strong preference for the single-dose paediatric formulation.

IPTi appears to have no negative effect on attitudes towards EPI and is not perceived as immunisation against malaria.

Principal Investigator

Robert Pool

Co-investigators

Marjolein Gysels, Christopher Pell

Project Manager

Marjolein Gysels

In collaboration with:

- Mary Hamel and Frank Odihambo. Centers for Disease Control and Prevention/Kenya Medical Research Institute (CDC/KEMRI), Kisumu (Kenya).
- Rob Newman. Centers for Disease Control and Prevention, Atlanta (U.S.A.).
- Peter Mangesho. National Institute for Medical Research, Tanga (Tanzania).
- Roly Gosling. London School of Hygiene and Tropical Medicine, London (U.K.).
- Don Mathanga. Malaria Alert Centre, College of Medicine, Blantyre (Malawi).
- Ebenezer Ikoom. UNICEF Ghana (Ghana).
- Ivo Müller. Papua New Guinea Institute of Medical Research, Goroka (Papua New Guinea).
- Martin P. Grobusch. Medical Research Unit, Albert Schweitzer. Hospital Lambaréné (Gabon) & Faculty of Health Sciences, University of the Witwatersrand, Johannesburg (South Africa).
- Philip Adongo. Navrongo Health Research Centre, Navrongo (Ghana).

Funding agencies

Bill & Melinda Gates Foundation, Seattle (U.S.A.).

Duration of the project

2007 – 2009.

Publications

Pool R, Mushi A, Armstrong Schellenberg J, Mrisho M, Alonso PL, Montgomery C, Tanner M, Mshinda H, Schellenberg D. The acceptability of intermittent preventive treatment of malaria in infants (IPTi) delivered through the expanded programme of immunization in southern Tanzania. *Malar J* 2008; 7:213.

IPTi COST-EFFECTIVENESS WORKING GROUP (CEWG)

The main objective of the Cost-Effectiveness Working Group (CEWG) is to give guidance to those responsible for making political decisions in choosing efficient and integrated strategies for the control of malaria. The CEWG also helps advocate IPTi based on resources needed and overall effectiveness.

Given the number of centres and countries that are involved in the work of the CEWG, considerable evidence will be available on the economic effectiveness of IPTi, which will positively influence the political decisions and resource allocation for this strategy at sub-national, national and international levels.

The low marginal costs for the administration of IPTi, together with the favourable impact on health that has been shown with SP treatment, constitutes important evidence that the advocates of IPTi can use to favourably influence policy decision makers to assign resources for IPTi intervention. Furthermore, results of the clinical trials that evaluate other treatment options will also allow comparison of the cost-effectiveness of SP treatment as a single therapy or in combination with amodiaquine, artesunate, lapdap and mefloquine.

The CEWG has received funding to conduct cost-effectiveness analyses in four centres: Moshi, Lambaréné, Kisumu and Papua New Guinea. The clinical trials of Manhiça and Southeast Tanzania already have cost-effectiveness components and together with the teams at the Swiss Tropical Institute and CRESIB, they form part of the CEWG.

To date, the CEWG has recruited staff members in each of the six centres, prepared a detailed cost-effectiveness manual, developed tools for collecting data and has carried out, with the teams of each country, the pilot tests of these tools. In addition, they have launched training workshops and completed the data analysis.

Currently, the results of the project are being written for subsequent publication. In the centres where IPTi has had a significant effect in the reduction of malaria, the cost of averted episodes for the IPTi-SP treatment was very low, \$US1.35–4.03 per DALY (disability-adjusted life year) averted, according to specifics of the test, and \$US 0.68–2.27 per DALY averted according to a joint analysis. For IPTi with alternative antimalarial drugs, the lowest cost per case averted was for amodiaquine-artesunate in

Kisumu (\$US 4.62 per DALY averted) and the highest was for mefloquine in Korogwe (\$US 18.56 per DALY averted).

The IPTi was found to be efficient and highly cost-effective at all centres except one (Lambaréné, Gabon), with costs ranging from \$US 2.90 (Ifakara, Tanzania, SP) to \$US 39.63 (Korogwe, Tanzania, mefloquine) per DALY averted. IPTi also reduced the costs of the health system and showed considerable savings in the averted cases. In the only centre where IPTi was not effective in preventing malaria (although it was efficient in preventing cases of anaemia), this intervention was not cost-effective.

Principal Co-investigator
Elisa Sicuri

In collaboration with:

- Benson Obonyo. Kenya Medical Research Institute, Kisumu (Kenya).
- Fred Matovu. Kilimanjaro Christian Medical Centre, Moshi (Tanzania).
- Prosper Biao. Albert Schweitzer Hospital, Lambaréné (Gabón).
- Carol Davy. Papua New Guinea Institute of Medical Research, Goroka (Papua New Guinea).
- Lesong Conteh. Swiss Tropical Institute, Basel (Switzerland).

Funding agencies

Bill & Melinda Gates Foundation, Seattle (U.S.A.).

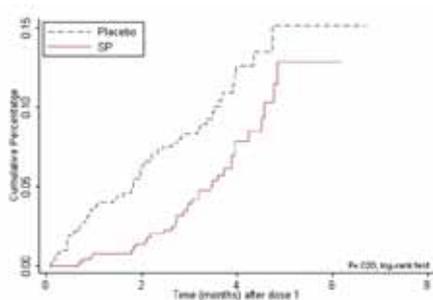
Duration of the project

2006 – 2009.



1.10. TIMNET: INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY IN THE CONTEXT OF INSECTICIDE-TREATED NETS

Prevention of malaria in pregnancy in Africa relies on intermittent preventive treatment (IPTp) and insecticide-treated mosquito nets (ITNs). Although IPTp and ITNs have been shown independently to be effective in reducing the harmful effects of malaria in pregnancy, information about the safety and efficacy of both interventions together is limited.



Protective efficacy (PE 40% [95% CI, 7.40--61.20]; $p=0.020$) of the intermittent preventive treatment with sulphadoxine-pyrimethamine (SP) in the context of insecticide-treated mosquito nets on the reduction of episodes of clinical malaria during pregnancy (Manhiça, Mozambique; Menéndez et al. 2008).

From 2003 to 2006, 1,030 pregnant Mozambican women received long-lasting ITNs during antenatal visits, regardless of HIV status, and were recruited for a randomised, double-blind or a placebo-controlled study to evaluate the safety and efficacy of a two-dose sulphadoxine-pyrimethamine (SP) treatment. The primary objective of this study was to measure the reduction of low birth weights.

The administration of two doses of SP was found to be safe and well tolerated, although it was not associated with a reduction in the prevalence of anaemia at delivery, low birth weight or placental infections. The group of women receiving SP treatment, however, showed a 40% reduction in the incidence of clinical malaria during pregnancy and reductions in the prevalence of peripheral parasitemia and active placental infections. Also, a reduction in severe anaemia at the time of delivery was seen, although it was of borderline statistical significance. These effects were not affected by the month of pregnancy or by the stage of HIV infection. The use of ITNs was greater than 90% in both groups.

The administration of two doses of SP was associated with a reduction in some indicators, although these did not translate into significant improvements in other maternal or birth outcomes, however, the use of ITNs during pregnancy may reduce the necessity of IPTp administration. The distribution of ITNs should be integrated into prenatal visits in

sub-Saharan Africa (Menéndez et al. 2008).

After the above study, the following complementary studies were conducted:

- Intermittent preventive treatment with sulphadoxine-pyrimethamine in pregnant Mozambican women and its effect on the acquisition of immunity in mothers and their children.
- Effect of intermittent preventive treatment on the molecular markers of *P. falciparum* resistance in pregnant women and children in Mozambique.
- Timnetvir: Effect of intermittent preventive treatment with sulphadoxine-pyrimethamine on HIV-positive pregnant women in Mozambique on the prevention of transmission from mother to child (see HIV/AIDS, Section 2.1)

Funding agencies

Banco Bilbao Vizcaya Argentaria, Fundación BBVA, Bilbao (Spain).

Agencia Española de Cooperación Internacional para el Desarrollo (AECID).

Duration of the project

2007 – 2009.

Publications

- Bardaji A, Sigauque B, Bruni L, Romagosa C, Sanz S, Mabunda S, Mandomando I, Aponte J, Sevene E, Alonso PL, Menéndez C. Clinical malaria in African pregnant women. *Malar J.* 2008 Jan 30;7:27.
- Menéndez C, Bardaji A, Sigauque B, Romagosa C, Sanz S, Serra-Casas E, Macete E, Berenguera A, David C, Dobaño C, Naniche D, Mayor A, Ordi J, Mandomando I, Aponte J, Mabunda S, Alonso PL. A randomized placebo-controlled trial of intermittent preventive treatment in pregnant women in the context of insecticide treated nets delivered through the antenatal clinic. *PLoS One.* 2008 April 9;3(4):e1934.

INTERMITTENT PREVENTIVE TREATMENT WITH SULPHADOXINE-PYRIMETHAMINE IN PREGNANT MOZAMBICAN WOMEN AND ITS EFFECT ON THE ACQUISITION OF IMMUNITY IN MOTHERS AND THEIR CHILDREN

Presently, the WHO recommends intermittent preventive treatment (IPT) and insecticide-treated mosquito nets (ITNs) for the control of malaria during pregnancy in Africa. The effect of this intervention on the naturally acquired immunity against *P. falciparum* in women and their children is, however, unknown.

As part of the efficacy clinical trial (TIMNET) to evaluate if IPT in pregnancy provides any additional benefits to the protection afforded by ITNs, the consequences that IPT may have on the health of both mother and child will be fully assessed. In fact, IPT in pregnancy may alter the development of immunity in pregnant women against parasites sequestered in the placenta, and affect the foetus by in utero exposure to the infection. This could affect the development of the foetal immune system and modulate future responses against *P. falciparum* and/or the response to vaccines in the first year of life.

To guide national malaria control programmes in the implementation of IPT during pregnancy, it is necessary to determine the balance between the beneficial effects of maternal interventions (reduction in maternal anaemia and low birth weight) versus harmful effects (impaired immune responses in pregnant women, foetuses and children).

The results of this study showed that HIV-positive women who received IPT treatment with SP (IPT-SP) had lower antibody levels in peripheral and cord blood against parasitic antigens compared with HIV-positive women who received placebo. An association between a high level of antibodies against malaria and a higher risk of suffering a first episode of the disease, in the mother as well as in the child, was observed. The antibody levels

were not associated with a reduction in maternal anaemia, premature births or low birth weight.

According to this study, the association of IPT-SP with the reduction of malarial antibodies in HIV-positive women (not in HIV-negative women) may reflect a higher efficacy of the intervention in the prevention of malaria in HIV-positive women. Furthermore, this reduction of antibodies did not translate to an increased risk of malarial morbidity in either pregnant women or their children.

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Laboratory Technician
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- Antonio Langa and Catarina David. Centro de Investigação em Saúde de Manhiça (CISM), Manhiça (Mozambique).

Funding agencies

Bill & Melinda Gates Foundation, Seattle (U.S.A.).
Banco Bilbao Vizcaya Argentaria, Fundación BBVA, Bilbao (Spain).

Duration of the project

2007 – 2009.

Publications

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EFFECT OF INTERMITTENT PREVENTIVE TREATMENT ON THE MOLECULAR MARKERS OF *P. FALCIPARUM* RESISTANCE IN PREGNANT WOMEN AND CHILDREN IN MOZAMBIQUE

Although intermittent preventive treatment with SP (ITP-SP) is effective in the reduction of malaria in pregnant women and children, there is still a lack of data about the effect of IPTp on the potential selection of resistant parasites.



Genotype of the 108 codon from *dhfr* determined by PCR and restriction enzyme digestion techniques.

To evaluate the effect of the intervention on molecular markers of resistance, a comparison of the frequency of clinical episodes caused by the *P. falciparum* parasite with mutations in the *dhfr* and *dhps* genes (dihydrofolate reductase and dihydropteroate synthetase, respectively) among infected women who received SP or placebo treatment in the context of the TIMNET clinical trial is being conducted.

The *dhfr* and *dhps* genes are of particular relevance, since it was found that mutations in these genes increase the

level of resistance of *P. falciparum* to SP. Currently, the analysis of the mutations 51, 59 and 108 in *dhfr*, and 437 and 540 in *dhps* have been completed using PCR and restriction enzyme digestion techniques. A total of 250 samples from pregnant women, collected during delivery, were analysed; the statistical analysis of the data obtained is in the final stages. A similar study developed in children younger than one year who received SP showed that half of the Manhiçan children were carriers of the parasite with five mutations and that IPT treatment was associated with some changes in the prevalence of genotypes implicated in the resistance to SP (more mutations in *dhfr* and *dhps*). The high prevalence of parasites with five mutations (around 50%), however, did not appear to compromise the efficacy of SP in preventing new episodes of malaria in children under one year old.

The information generated by these studies will be particularly relevant for designing malaria control strategies during pregnancy and to gain a better understanding of the molecular and immunological bases for the susceptibility of pregnant women to malaria.

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Principal Co-investigator

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Funding agencies

Bill & Melinda Gates Foundation, Seattle (U.S.A.).
Fondo de Investigación Sanitaria (FIS), Instituto de Salud Carlos III (ISCIII), Madrid (Spain).

Duration of the project

2008– 2009.

Publications

Mayor A, Serra-Casas E, Sanz S, Aponte JJ, Macete E, Mandomando I, Puyol L, Berzosa P, Dobaño C, Aide P, Sacarlal J, Benito A, Alonso P, Menéndez C. Molecular markers of resistance to sulphadoxine-pyrimethamine during intermittent preventive treatment for malaria in Mozambican infants. *J Infect Dis.* 2008;197:1737-42.

1.11. MALARIA VACCINES

The RTS,S vaccine is based on the circumsporozoite proteins of *P. falciparum*. This vaccine, formulated with adjuvants of the AS0 family, is currently the most advanced vaccine candidate against *P. falciparum* malaria.

The CRESIB, together with the *Centro de Investigação em Saúde de Manhica* (CISM, Mozambique), has been working for several years on the evaluation of RTS,S in collaboration with the PATH Malaria Vaccine Initiative (MVI) and the pharmaceutical company GlaxoSmithKline (GSK) Biologicals.

In 2002, a phase I clinical trial was performed with a group of 60 children between one and four years of age, which was designed to evaluate the safety, reactogenicity and immunogenicity of the RTS,S malaria vaccine candidate, formulated with the adjuvant AS02A.

Phase IIb studies were subsequently designed to evaluate the efficacy, safety and immunogenicity of the RTS,S/AS02A vaccine in 2022 children between one and four years of age. The vaccine was shown to be safe and effective against clinical malaria and *P. falciparum* infection, and immunogenic against the circumsporozoite antigen and the surface antigen of hepatitis B.

After demonstrating the non-inferiority of the new formulation of RTS,S with the adjuvant AS02D (paediatric formulation), compared to the existing formulation in a study with children between two and six years of age, the first phase I/IIb clinical trial, designed with the aim to evaluate the safety, immunogenicity and efficacy of RTS,S/AS02D, began with newborn babies.

The CRESIB and the CISM are preparing to begin the phase III clinical trial of RTS,S/AS01E. This is a multicentre study designed to evaluate the efficacy, safety and immunogenicity of the vaccine in children less than 18 months of age.

The CRESIB is part of the Clinical Trials Partnership Committee (CTPC) currently involved in the design and implementation of this study that will begin in mid-2009.

The ongoing projects in the field of vaccines against malaria, during the 2007–2008 period were as follows:

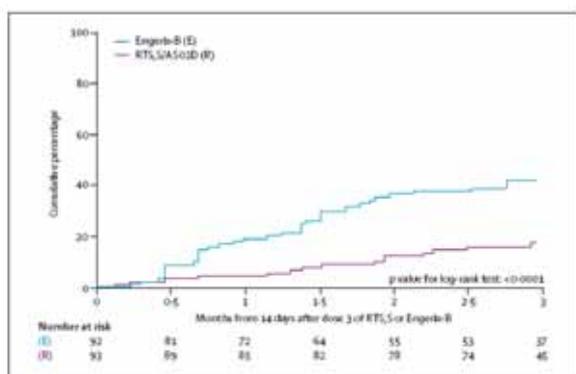
- Phase I/IIb randomised, double-blind and controlled, proof of concept clinical trial to evaluate the safety, immunogenicity and efficacy of the GSK vaccine candidate RTS,S/AS02D in infants living in malaria endemic areas.
- Study of the asexual blood stage immunity markers associated with the prolonged protection in children vaccinated with RTS,S/AS02A.
- Pilot study of a hospital-based surveillance system for the detection of severe malaria and morbidity in children between two months and four years of age that will facilitate the implementation of a multicentre efficacy phase III clinical trial of the GSK malaria vaccine candidate RTS,S/AS01E (PreMal055).
- Comparison of two methods for quantifying the density of *P. falciparum* in human peripheral blood – an ancillary study to PreMal055.



Malaria Vaccines

PHASE I/IIb RANDOMISED, DOUBLE BLIND AND CONTROLLED, PROOF OF CONCEPT CLINICAL TRIAL TO EVALUATE THE SAFETY, IMMUNOGENICITY AND EFFICACY OF THE GSK VACCINE CANDIDATE RTS,S/AS02D IN INFANTS LIVING IN MALARIA ENDEMIC AREAS

A randomised and double blind phase I/IIb clinical trial was undertaken, involving 214 Mozambican children. The children were randomly assigned to receive three doses of RTS,S/AS02S or the hepatitis B vaccine, Engerix-B, administered at 10, 14 and 18 weeks of age, as well as receiving all routine EPI vaccines at 8, 12 and 16 weeks of age.



The Kaplan-Meier curves showing the accumulative percentage of the study participants with at least one episode of malarial infection. The RTS,S/AS02D vaccine resulted in greater immunity compared with Engerix-B.

The primary objective of this study was to measure the safety of the RTS,S/AS02D vaccine during the first six months of life, through intention to treat analysis. The secondary objectives were to measure the immunogenicity and the efficacy against new infections of *P. falciparum* during a three-month follow-up period after the administration of the third dose of the vaccine.

The time until the first infection between the groups was compared through a protocol analysis using Cox regression

models (this study is registered with the ClinicalTrials.gov, number NCT00197028).

From each group, 17 children (15.9%, 95% CI 9.5–24.2) experienced serious adverse effects. After the follow-up, which ended March 6th, 2007, a total of 31 adverse effects in the RTS,S/AS02D group and 30 in the Engerix-B group were detected, none of which were related with the vaccine. Four deaths occurred during the same follow-up period (two in each group), all after the final period of active detection of the infection (in the sixth month).

The RTS,S/AS02D induced high titres of anti-circumsporozoite antibodies. A total of 68 first infections of *P. falciparum* were detected, 22 in the RTS,S/AS02D group and 46 in the control group; the adjusted vaccine efficacy was 65.9% (95% CI 42.6–78.9%, $p < 0.0001$).

Principal Investigator

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Principal Co-investigator

John Aponte

Co-investigators

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In collaboration with:

– GlaxoSmithKline Biologicals, Rixensart (Belgium).

Funding agencies

The PATH Malaria Vaccine Initiative (MVI), Bethesda (U.S.A.).

Duration of the project

2005 – 2007.

Publication

Aponte JJ, Aide P, Renom M, Mandomando I, Bassat Q, Sacarlal J, Manaca MN, Lafuente S, Barbosa A, Leach A, Lievens M, Vekemans J, Sigauque B, Dubois MC, Demoitie MA, Sillman M, Savarese B, McNeil JG, Macete E, Ballou WR, Cohen J, Alonso PL. Safety of the RTS,S/AS02D candidate malaria vaccine in infants living in a highly endemic area of Mozambique: a double blind randomised controlled phase I/IIb trial. *Lancet*. 2007 Nov 3;370(9598):1543-51.

STUDY OF THE ASEXUAL BLOOD STAGE IMMUNITY MARKERS ASSOCIATED WITH THE PROLONGED PROTECTION IN CHILDREN VACCINATED WITH RTS,S/AS02A

In one cohort of a controlled and randomised clinical trial of the malaria vaccine candidate RTS,S/AS02A in Manhiçan children between one and four years of age, it was observed that the vaccine-induced protection was maintained for up to four years (Alonso et al. Lancet 2005; 366 (9502): 2012-8).



Information session for Mozambican women and their children on the context of the clinical trial.

RTS,S is a pre-erythrocytic vaccine, that is, it acts on *P. falciparum* from the moment of inoculation of the parasite by the mosquito and during liver infection. When the vaccine is effective, there should be the capacity for control of parasitemia at this level, however, as it reduces its effect and – provided there is no intervention – there are new infections, the parasites emerge from the liver in the blood (blood stage of *P. falciparum*).

During the period of partial efficacy of the RTS,S vaccine, the levels of parasites in the blood will be low for a period of weeks or months. It is believed that these low levels of

parasites that pass to the blood cause the optimal stimulation of the immune system that helps to establish lasting protection against the disease; this is termed “natural boosting”.

The main objective of this study is to investigate the hypothesis of the “natural boosting” of the blood stage immunity to describe the mechanisms of the lasting protection induced by RTS,S. Another objective of the study is to confirm if the protection of the vaccine continues until five years after the vaccination.

Thus, five years after vaccination, a cross-sectional survey of both vaccine cohorts was conducted in which parasitemia was measured by microscopy and plasma was collected from the participants to investigate the blood stage immunogenicity against MSP-1, AMA-1 and EBA-175 with multiplex (Luminex®) techniques. Currently, the responses to the antibodies against the live blood stage parasites are also being assessed by growth inhibition techniques and against various surface antigens (VSA) by FACS assay.

The data of this study are currently under analysis, but it is clear that the contribution of the “natural boosting” in the context of partially protective vaccines is especially important in understanding the possible mechanisms of lasting vaccine-induced protection, as well as general understanding of naturally acquired immunity against malaria in African children.

Principal Investigators

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Funding agencies

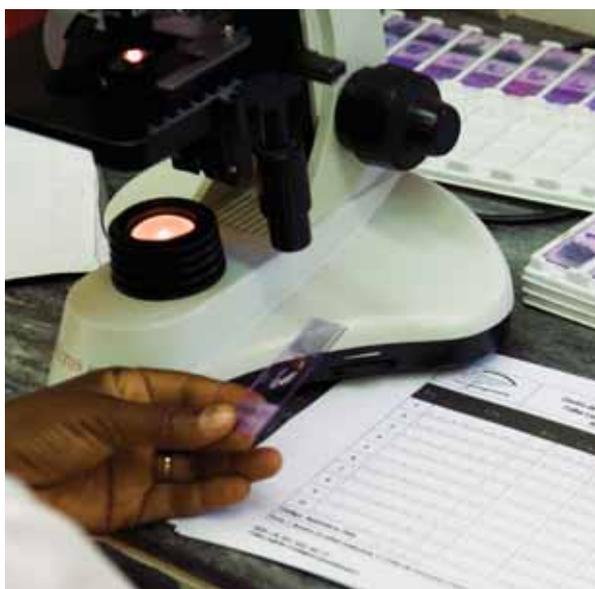
The PATH Malaria Vaccine Initiative (MVI), Bethesda (U.S.A.).

Duration of the project

2008 – 2010.

PILOT STUDY OF A HOSPITAL-BASED SURVEILLANCE SYSTEM FOR THE DETECTION OF SEVERE MALARIA AND MORBIDITY IN CHILDREN BETWEEN TWO MONTHS AND FOUR YEARS OF AGE THAT WILL FACILITATE THE IMPLEMENTATION OF A MULTICENTRE EFFICACY PHASE III CLINICAL TRIAL OF THE GSK MALARIA VACCINE CANDIDATE RTS,S/AS01E (PREMAL 055)

Phase III clinical trials of the malaria vaccine candidate RTS,S/AS01E are expected to start in the second half of 2009. This is a multicentre study to evaluate the effectiveness of this vaccine against malaria in infants and children. Various centres in Africa will participate in this study (11 centres in seven African countries), which will allow the investigation of the efficacy of the vaccine in areas with different levels of malarial transmission.



A CISM researcher visualising microscopic slides of blood films for the diagnosis of *P. falciparum* infection (Manhiça).

To commence the phase III study it is necessary to conduct preliminary work since, in many centres, the setting up of a surveillance system will require the strengthening of the diagnostic processes and the infrastructure of the emergency rooms and inpatient units. This study (PreMal 055) will serve to pilot a hospital surveillance system for the detection of severe malaria and morbidity in children between two months and four years of age.

The preparatory work for the phase III clinical trial is in progress. To build-up capacities in the participating institutions, the training of personnel, as well as the development and implementation of processes relating to diagnostic X-rays and microbiological cultures, is being carried out. This study will also test the algorithm for the evaluation of sick children and the data management processes, as well as allowing the standardisation of the observational methods.

Principal Investigator

Jahit Sacarlal

Co-investigators

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In collaboration with:

– GlaxoSmithKline Biologicals, Rixensart (Belgium).

Funding agencies

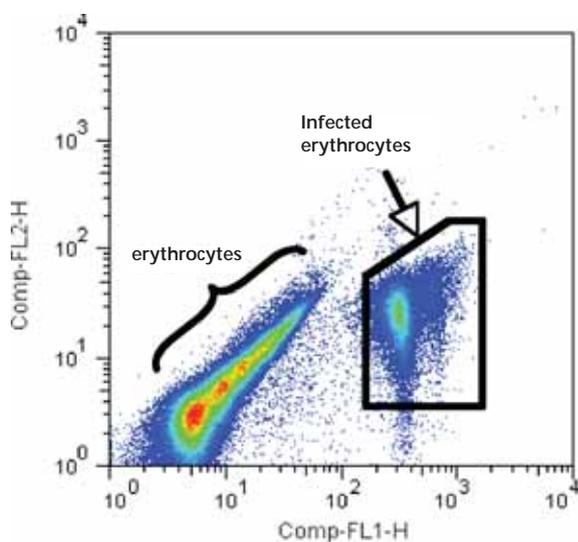
The PATH Malaria Vaccine Initiative (MVI), Bethesda (U.S.A.).

Duration of the project

2008.

COMPARISON OF TWO METHODS FOR QUANTIFYING THE DENSITY OF *P. FALCIPARUM* IN HUMAN PERIPHERAL BLOOD – AN ANCILLARY STUDY TO PREMAL 055

Currently, in studies of *P. falciparum*, the microscopic analysis of blood smears is the most commonly used method for determining parasitemia and parasite density. This method is, however, subjective and labour intensive, complicating its use in evaluating large-scale programs.



Human blood stained with YOYO-1 and visualised bi-dimensionally (FL1 [YOYO-1] vs. FL2 [autofluorescence]). The population of erythrocytes infected with *P. falciparum* are displaced from the population of normal erythrocytes. In this way, the frequency of infected erythrocytes can be calculated and compared with the frequency of non-infected erythrocytes to define the parasitic density by FACS techniques.

Flow cytometry is a useful technique in high-performance analysis, but due to the limited specificity that is achievable with the currently available flow cytometry techniques, it has not been used until now in clinical trials. An application of flow cytometry that uses DNA staining of the parasites is now available. This technique has been shown to be a

possible alternative for the measurement of peripheral blood parasitemia in humans.

The present study aims to assess and explore a new flow cytometry technique developed by the group of Jiménez-Díaz and Angulo-Barturen at the GSK Research Centre in Tres Cantos (Madrid). This method estimates parasitemia in rodent models as a function of infected red blood cell count, through autofluorescence and DNA content, measured after staining with YOYO-1.

Throughout 2008, a strategy has been designed to perform this procedure on blood samples that were initially collected from 100 individuals enrolled at the *Hospital Distrital de Manhica* (Mozambique) who are participating in the PreMal 055 study.

The blood drops were collected by finger prick, fixed in glutaraldehyde and incubated with RNAase before staining with YOYO-1. The measuring of red blood cells infected with *P. falciparum* parasites was conducted by the analysis of two-dimensional graphics data (instead of classical one-dimensional analysis); this process is currently in the final phase.

Principal Investigator

Joseph Campo

Principal Co-investigators

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Co-investigators

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In collaboration with:

- Inigo Angulo-Barturen. GlaxoSmithKline, Infectious Diseases CEDD, Diseases of the Developing World, Tres Cantos, Madrid (Spain).

Funding agencies

The PATH Malaria Vaccine Initiative (MVI), Bethesda (U.S.A.).

Duration of the project

2008 – 2009.



1.12. CLINICAL DEVELOPMENT OF ANTIMALARIAL DRUGS

- Evaluation of four artemisin-based combinations for the treatment of uncomplicated malaria in African children.
- A randomised, investigator-blind, parallel group multi-centre study to compare the efficacy, safety and tolerance of the soluble formulation of Coartem® versus six doses of Coartem® tablets in the treatment of uncomplicated *P. falciparum* malaria in children

Clinical development of antimalarial drugs

EVALUATION OF FOUR ARTEMISIN-BASED COMBINATIONS FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN AFRICAN CHILDREN

The overall goal of this project is to compare the efficacy and safety of four artemisinin-based combination therapies (ACTs: amodiaquine-artesunate, dihydroartemisinin-piperaquine, artemether-lumefantrine and chlorproguanil-dapsone-artesunate) for the treatment of uncomplicated malaria in children.



The mother of a Mozambican child participating in the clinical trial signs the informed consent form before the witness and one of the medical staff of the study.

This is a multicentre, randomised and open, phase IV clinical trial with three arms of treatment that involve five African research centres (Burkina Faso, Nigeria, Zambia, Gabon and Mozambique). The East African Network is responsible for the monitoring of the antimalarial treatment (EANMAT); this is an active network present in Kenya, Tanzania, Uganda, Rwanda and Burundi, and has five European partners (Belgium, Germany, France, United Kingdom and Spain).

Principal Investigator

Clara Menéndez

Co-investigators

Raquel González, Eusébio Macete, Quique Bassat, Sonia Machevo, Montse Renom

The objectives of this study are to establish the safety and efficacy of these new artemisinin-based combined therapies during 28 days after treatment and to determine the rate of re-treatment necessary for each regime during the six months after treatment.

A total of 5,100 children with uncomplicated malaria were randomly assigned to receive one of the four ACTs. The proposed sample size (170 children per arm per centre) will help to determine whether the treatments are equivalent in terms of efficacy (90%), assuming that the differences between them are not higher than 10%. When considered together, the trials will allow the detection of significant differences in the efficacies of the treatments of 4–6%, and important adverse effects occurring at a frequency of at least 1–2%.

This study has finalised the recruitment of participants and is currently in the final stages of clinical follow-up.

In collaboration with:

- Umberto D'Alessandro. Institute of Tropical Medicine, Antwerp (Belgium).

Funding agencies

European & Developing Countries Clinical Trial Partnership, EDCTP (European Union). Fondo de Investigación Sanitaria (FIS), Instituto de Salud Carlos III (ISCIII), Madrid (Spain).

Duration of project

2007 – 2009.

Clinical development of antimalarial drugs

A RANDOMISED, INVESTIGATOR-BLIND, PARALLEL GROUP MULTICENTRE STUDY TO COMPARE THE EFFICACY, SAFETY AND TOLERABILITY OF THE SOLUBLE FORMULATION OF COARTEM[®] VERSUS SIX DOSES OF COARTEM[®] TABLETS IN THE TREATMENT OF UNCOMPLICATED *P. FALCIPARUM* MALARIA IN CHILDREN

The aim of this clinical trial was to show the non-inferiority of the new dispersible formulation of artemether-lumefantrine (Coartem[®]) against the conventional tablet for the treatment of young children with uncomplicated malaria in Benin, Kenya, Mali, Mozambique and Tanzania.



A shop that has been converted into a unit for the clinical trials, located inside the area of the *Hospital Distrital de Manhica*, and medical staff of the study.

The primary objective was to measure the parasitological recovery rate over 28 days, corrected by PCR, with the aim to show the non-inferiority (with a margin of -5%) of the dispersible formulation compared to the tablet. An asymptotic, one-sided confidence interval (CI) of 97.5% was constructed for the difference in cure rates and a computer-generated randomised list was used to ensure that the researchers did not know the medication administered. A modified intention to treat analysis was used (this trial is registered with: ClinicalTrials.gov, number NCT00386763).

In the study, 899 children aged 12 years or younger were randomly assigned to receive the dispersible formulation (n=447) or the tablets (n=452). Over 85% of patients in

each treatment group completed the study; 812 children qualified for the intention to treat analysis (n=403 vs. n=409).

The rate of cure at 28 days, corrected by PCR, was 97.8% (95% CI 96.3–99.2) in the dispersible formulation group and 98.5% (97.4–99.7) in the group that received the tablets. The lowest value of the 97.5% IC in a row was -2.7%. Vomiting was the most frequently reported adverse effect (n=33 [7%] and n=42 [9%], respectively). No relevant signs of ototoxicity or cardiotoxicity were observed.

In collaboration with:

- David Ubben. Medicines for Malaria Venture (MMV), Geneva (Switzerland).

Funding agencies

Novartis Vaccines, Basel (Switzerland).
Medicines for Malaria Venture (MMV), Geneva (Switzerland).

Duration of the project

2006 – 2007.

Publication

Abdulla S, Sagara I, Borrmann S, D'Alessandro U, Gonzalez R, Hamel M, Ogutu B, Martensson A, Lyimo J, Maiga H, Sasi P, Nahum A, Bassat Q, Juma E, Otieno L, Bjorkman A, Beck HP, Andriano K, Cousin M, Lefevre G, Ubben D, Premji Z. Efficacy and safety of artemether-lumefantrine dispersible tablets compared with crushed commercial tablets in African infants and children with uncomplicated malaria: a randomised, single-blind, multicentre trial. *Lancet*. 2008 Nov 22;372(9652):1819-27.

Principal Investigator

Clara Menéndez

Co-investigators

Raquel González, Quique Bassat, Montse Renom, Inácio Mandomando



1.13. INDOOR RESIDUAL SPRAYING (IRS) IN AFRICA

Indoor residual spraying (IRS) is a small-scale anthropological study, carried out mainly in Manhiça (Mozambique), in collaboration with the *Centro de Investigação em Saúde de Manhiça* (CISM). Through this study the socio-cultural factors that influence the response, both positive and negative, to the implementation of IRS with residual insecticides will be explored.



Typical house in a rural area of Mozambique.

The focus of this study is expanded to deal with the same factors, but on a global scale, investigating how political, regional and international influences contribute to the local response to this intervention.

The analysis of the data from this project is still being completed, however, the preliminary results, presented in two conferences, suggest that the acceptability of the IRS in rural areas of Mozambique is based on a sense of citizenship based on a group, resulting from the decentralisation policy after the war, in these areas.

Despite doubts about its benefits, some accept IRS as part of their duty as good citizens, and others do so in order to ensure they can access other services administered by the state, such as health care. The success of this intervention is not limited to the perception of the effectiveness or the physical effects of the spraying process, but is related with broader socio-cultural issues, such as citizenship, identity and rights.

Principal Investigator
Robert Pool

In collaboration with:

- Khatia Munguambe. Centro de Investigação em Saúde de Manhiça, Manhiça (Mozambique).
- Catherine Montgomery. London School of Hygiene and Tropical Medicine, London (U.K.).
- Samuel Mabunda. National Malaria Control Programme, Ministry of Health, Maputo (Mozambique).

Funding agencies

Bill & Melinda Gates Foundation, through the London School of Hygiene and Tropical Medicine, London (U.K.).

Duration of the project

2007 – 2009.

RESEARCH PROGRAMMES

- MALARIA
- **HIV/AIDS**
- ACUTE RESPIRATORY INFECTIONS
- DIARRHOEAL DISEASES
- CHAGAS DISEASE
- OTHER RESEARCH PROJECTS





HIV/AIDS

2. HIV/AIDS

Almost 95% of the estimated 40 million people infected with HIV worldwide live in developing countries, and more than half in of those in sub-Saharan Africa.

Pregnant women are especially vulnerable to HIV infection and the risk of infecting their children during pregnancy is elevated. In areas of sub-Saharan Southeast Africa the prevalence of HIV in pregnant women exceeds 40% and the rate of HIV transmission from mother to child is between 10 and 50%. This alarming situation is compounded by the problem of treatment; in developing countries approximately 12% of AIDS patients who require treatment actually receive anti-retroviral therapy.

A large part of the research on HIV/AIDS that has been developed at the CRESIB, in collaboration with the *Centro de Investigação em Saúde de Manhiça* (CISM), aims to study HIV transmission, antiretroviral treatment and the relationship of HIV/AIDS with other common infections in impoverished countries. The CRESIB has expanded its research programme in this area in parallel with the development and support of an HIV clinical platform, providing diagnostics, counselling and health care for the population of Manhiça. This specialised clinical

platform is supported by the *Agència Catalana de Cooperació al Desenvolupament*.

Research in the area of AIDS is aimed at determining the influence of other infections in the presence of HIV/AIDS and drugs commonly used in this environment (mainly antimalarial) to establish treatment guidelines. Maternal and child health is also a key area in HIV/AIDS research particularly with regard to optimization of breastfeeding and interventions to prevent mother-to-child transmission of HIV.

HIV leads to extensive damage of the immune system. The CRESIB thus has a line of research focussed on the restoration of immunity and risk factors associated with immune reconstitution inflammatory syndrome.

Before addressing intervention studies with drugs or vaccine candidates, it is necessary to conduct epidemiological and anthropological studies, which include elements such as the monitoring and treatment compliance. Clinical trials in this area are aimed at assessing the effectiveness of different combinations of antiretroviral drugs as well as other new drugs that are under development, and preventive or therapeutic candidate vaccines.

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Training Fellow

José Machado

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2.1. TIMNETVIR: EFFECT OF INTERMITTENT PREVENTIVE TREATMENT WITH SULPHADOXINE-PYRIMETHAMINE IN HIV-POSITIVE PREGNANT WOMEN IN MOZAMBIQUE ON THE PREVENTION OF TRANSMISSION FROM MOTHER TO CHILD

The impact of malaria on the transmission of HIV-1 from mother to child (Mother to Child Transmission, MTCT) appears to be more complicated than was initially indicated. Discrepancies in the few studies available reflect the complex relationship that exists between the maternal immune responses against malaria and HIV.

The antimalarial immune response may stimulate HIV-1 replication in the placenta, increasing the plasma viral load of HIV-1. Alternatively, this response could lead to control of HIV-1 replication through production of chemokines.

The aims of the present project are:

1. To characterise the molecular epidemiology of HIV-1 in pregnant women in Manhiça (Mozambique) and analyse the genetic evolution between 1999 and 2004.
2. To evaluate the impact of intermittent preventive treatment (IPTp) for malaria with two doses of SP during the second and third trimesters of pregnancy on the vertical transmission of HIV-1.
3. To evaluate the relationship between maternal *P. falciparum* malaria and the activation of the immune system during childbirth in HIV-positive pregnant women.

This study was integrated in a randomised and double blind clinical trial described in the section on research on malaria: Intermittent Preventive Treatment in Pregnancy in the context of Insecticide Impregnated Mosquito Nets, TIMNET, section 1.10). The monitoring of patients and the collection of samples was completed in 2007 and the analysis and interpretation of the data was carried out in 2008.

Principal Investigator

Denise Nanche

Co-investigators

Clara Menéndez, Azucena Bardaji, Maria Lahuerta

The molecular epidemiological results obtained to date revealed a large genetic variation with the existence of different circulating sublineages of the subtype C. No significant differences were observed in the maternal viral loading or in the risk of HIV-1 MTCT between the SP and placebo groups. It was found that the women with anaemia that participated in this study had a risk of HIV-1 MTCT up to four times higher than women without anaemia; this relationship was independent of the plasma viral load of HIV-1. In the multivariate analysis, placental malaria (both active and past) was associated with a lower risk of HIV-1 MTCT.

In collaboration with:

- Miguel Angel Martinez. Fundació IrsiCaixa, Hospital Germans Trias i Pujol, Badalona (Spain).

Funding agencies

Ministerio de Educación y Ciencia, I+D, Madrid (Spain).

Duration of the project

2004 – 2008.

Publications

- Lahuerta M, Aparicio E, Bardaji A, Marco S, Sacarlal J, Mandomando I, Alonso PL, Martinez MA, Menéndez C, Nanche D. Rapid spread and genetic diversification of HIV type 1 subtype C in a rural area of southern Mozambique. *AIDS Res Hum Retroviruses*. 2008 Feb; 24(2): 327-35.
- Nanche D, Lahuerta M, Bardaji A, Sigauque B, Romagosa C, Berenguer A, Mandomando I, David C, Sanz S, Aponte JJ, Ordi J, Alonso P, Menéndez C. Mother-to-child transmission of HIV-1: association with malaria prevention, anaemia and placental malaria. *HIV Med*. 2008 Oct;9(9):757-64.

2.2. ENIC: ASSESSMENT OF IMMUNOLOGICAL PARAMETERS AND HEALTH INDICATORS DURING THE FIRST YEAR OF LIFE IN HIV-NEGATIVE CHILDREN BORN TO HIV-POSITIVE MOTHERS

It has been suggested that exposure to HIV induces haematological and immunologic abnormalities, and causes HIV-specific cellular immunity in HIV-negative children born to HIV-positive mothers.

It is particularly important to understand the impact of HIV exposure in HIV-negative children born to HIV-positive mothers in Africa. This is relevant in the African context with a predominance of the HIV-1 type C virus, with a high burden of co-infections and a predominantly breast-feeding population.

This project will assess the immunological parameters and the response to routine vaccinations, during the first year of life in HIV-negative children born to HIV-positive mothers. Furthermore, HIV-specific CD8 T cell responses will be assessed in infants who are not infected in the first year of life, to investigate whether a correlation exists between the HIV-specific CD8 cells and a lack of infection during the breastfeeding period.

The recruitment of patients for the study began in August 2008 and will continue until June 2009. The sample processing will continue until June 2010, however, preliminary results are expected in late 2009.



Recruitment of children younger than one year with their mothers for the study (CISM, Mozambique).

Principal Investigator

Denise Naniche

Co-investigators

Clara Menéndez, Montse Renom, Cinta Moraleda, Celia Serna

In collaboration with:

- Montse Plana. Service of Infectious Diseases and HIV Unit, Hospital Clínic de Barcelona, Barcelona (Spain).
- Nilsa de Deus. Centro de Investigação em Saúde de Manhica (CISM), Manhica (Mozambique).

Funding agencies

Fondo de Investigación Sanitaria. Instituto de Salud Carlos III, Madrid (Spain).

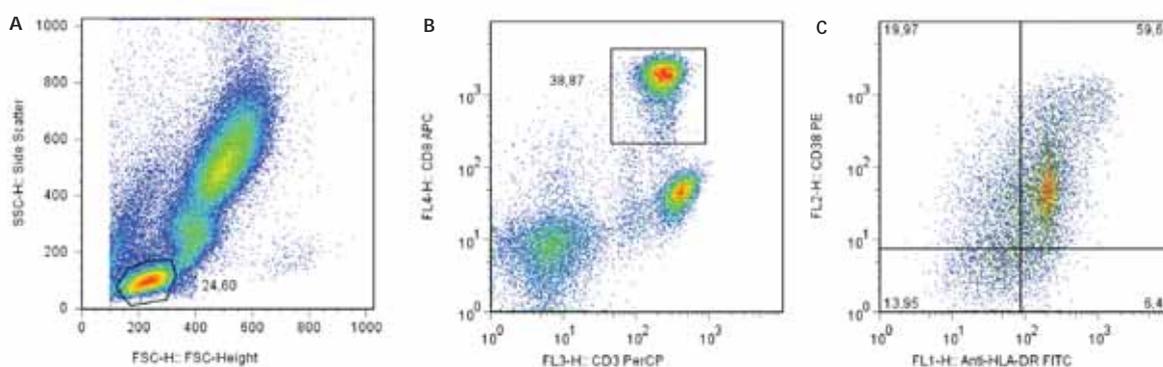
Duration of the project

2007 – 2010.



2.3. RITA: EVALUATION OF IMMUNE RECONSTITUTION AFTER THE COMMENCEMENT OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) IN MANHIÇA, MOZAMBIQUE

In conjunction with the WHO, Mozambique has proposed to treat 132,000 adults with AIDS in 2008. This is an ambitious objective and although there is ample experience in the implementation of highly active antiretroviral therapy (HAART) in western countries, this does not translate directly to African countries. Moreover, the burden of co-infections is much greater in sub-Saharan Africa and could lead to different responses to such treatment.



Activated T CD8+ lymphocytes from adults infected with HIV before the start of the combined antiretroviral treatment (cART). Plot after analysis by flow cytometry, showing: (A) subpopulation of lymphocytes in the blood, (B) subpopulation of T CD8+ cells in the lymphocytes and (C) the expression of the HLA-DR and CD38 activation markers in the subpopulation of T CD8+ lymphocytes.

Thus, to design innovative strategies in the antiretroviral treatment specific for Africa, it is absolutely necessary to have basic information on the kinetics of immune restoration after HAART, the dynamics of opportunistic diseases, the adherence to the treatment and the generation of resistance to medicines for the HIV virus subtypes present in Africa. The impact of the most common co-infections in this continent is also of key importance.

As requested by the protocol of this study, an assessment of immune parameters in the HAART programme, recently implemented in Manhiça (Mozambique), was conducted over a period of 16 months. This assessment included the study of:

1. The dynamics of restoring a functional immune system.
2. The incidence and morbidity associated with immune reconstitution inflammatory syndrome (IRIS).

3. The evaluation of resistance mutations to HAART.

The monitoring of patients and sample collection were carried out between 2006 and 2008.

From the preliminary data obtained, an elevated level of IRIS associated with Kaposi sarcoma (IRIS-KS), as well as a series of risk factors for the development of IRIS-KS, were identified. The in-depth analysis of the clinical and immunological data is ongoing.

Principal Investigator

Denise Naniche

Co-investigators

Emili Letang, José Machado

In collaboration with:

- José Maria Miró. Service of Infectious Diseases and HIV Unit, Hospital Clínic de Barcelona, Barcelona (Spain).
- Thomas Campbell. Division of Infectious Diseases, University of Colorado Health Sciences Center, Colorado (U.S.A.).
- Cláudia Carrilho. Department of Pathology, Hospital Central de Maputo, Maputo (Mozambique).
- Rui Bastos. Department of Dermatology, Hospital Central de Maputo, Maputo (Mozambique).

Funding agencies

Fundació "la Caixa", Barcelona (Spain).

Duration of the project

2005 - 2008.

2.4. AFREVACC: ESTABLISHMENT OF A EUROPEAN–AFRICAN NETWORK FOR THE PREPARATION OF AFRICAN SITES FOR HIV VACCINE CLINICAL TRIALS

Researchers from institutions based in Africa and Europe have proposed the development of a network to investigate vaccines against HIV and the possibility to develop an efficacy trial with a vaccine candidate to prevent HIV-1 infection.

The three-year objectives of the network are:

- To consolidate an integrated and functional network, linking the distinct disciplines required to operate and make decisions.
- To construct capacities for the development of future clinical trials of HIV vaccines.

The above objectives include feasibility studies, prevalence and incidence of HIV studies in the populations where the risk of infection is supposedly higher, the development of strategies for recruitment and retention of volunteers, and the study of the structural changes associated with the vaccine trials. Further objectives include initiating training activities and other activities designed to assure the quality of the procedures with cellular immunology samples, and to standardise and validate the protocols of immunology for the detection of the cellular and humoral immune responses to the vaccine candidates.

The research component of this project is developed in three areas: epidemiology, viability of a clinical trial of an HIV vaccine and characterisation of patients with acute or recent HIV infection.

The epidemiology and viability studies are in the preparation phase and will commence in 2009. The study of acute and recent HIV infections began in 2008. The identification and monitoring of patients with acute HIV identifications is underway; the recruitment of patients with recent HIV infections will begin in 2009.

Principal Investigator

Denise Nanche

Co-investigators

Clara Menéndez, Robert Pool, Raquel González.

In collaboration with:

- Khatia Mungambe. Centro de Investigação em Saúde de Manhica, Manhica (Mozambique).
- Jonathan Weber. Imperial College, London (U.K.).
- Gita Ramjee. HIV Prevention Research Unit, Medical Research Council South Africa, Durban (South Africa).
- Helen Rees. Wits Health Consortium (Pty) Ltd trading as the Reproductive Health & HIV Research Unit, Johannesburg (South Africa).
- Marie-Louise Newell. University of KwaZulu-Natal/Africa Centre for Health and Population Studies, Somkhele (South Africa).
- Leonard Maboko. Mbeya Medical Research Program, Mbeya (Tanzania).
- Michael Hoelscher. Kliniken der Universität München, Munich (Germany).
- Josefo João Ferro. Universidade Católica de Moçambique, Beira (Mozambique).
- Joep Lange. Centre for Poverty-related Communicable Diseases, Amsterdam (Netherlands).
- Wendy Stevens. Contract Laboratory Services, Johannesburg (South Africa).
- Giuseppe Pantaleo. Division of Immunology and Allergy/EuroVacc, Centre Hospitalier Universitaire Vaudois, Lausanne (Switzerland).
- Sheena McCormack. Medical Research Council, Clinical Trial Unit, London (U.K.).
- Jim Tartaglia. Sanofi Pasteur SA, Lyon (France).
- Hans Wolf. Institute for Medical Microbiology and Hygiene, Universität Regensburg, Regensburg (Germany).

Funding agencies

European & Developing Countries Clinical Trial Partnership, EDCTP (European Union).

Duration of the project

2007 – 2010.



2.5. MDP: SOCIAL SCIENCE COMPONENT OF MICROBICIDES DEVELOPMENT PROGRAMME

A multicentre clinical trial of vaginal microbicides for HIV infection is being conducted in six participating sites in four countries (Uganda, South Africa, Zambia and Tanzania). Feasibility and pilot studies have been completed and the phase III clinical trial is currently half completed.



Vaginal microbicide gel studied in the prevention of HIV infection.

The clinical trial includes an important integrated social component, focused on studying the adherence and acceptability of the product, the understanding of informed consent and the greater accuracy of key data. Although the analysis of the results is not yet finalised, the preliminary results have been presented in several communications. Some work has also been presented for publication, based on data already collected.

This project has shown that the use of mixed methods and triangulation can generate data on adherence and compliance which are of higher accuracy compared with quantitative questionnaires.

Principal Investigator
Robert Pool

In collaboration with:

- Gita Ramjee. HIV Prevention Research Unit, Medical Research Council, Durban (South Africa).
- Helen Rees. Reproductive Health and HIV Research Unit, Chris Hani Baragwanath Hospital, Johannesburg (South Africa).
- Claire Moffat. LSHTM/NIMR/AMREF Collaborative Research Projects, Muanza (Tanzania).
- Anatoli Kamali. MRC Programme on AIDS, Uganda Virus Research Institute, Entebbe (Uganda).
- Maureen Chisembele. University Teaching Hospital, Lusaka (Zambia).
- Mitzzy Gafos. Africa Centre for Health and Population Studies, KwaZulu-Natal (South Africa).
- Jonathan Weber. Imperial College, London (U.K.).
- Richard Hayes. London School of Hygiene and Tropical Medicine, London (U.K.).
- Khatia Munguambe. Centro de Investigação em Saúde de Manhica, Manhica (Mozambique).
- Sibone Mocumbi. Maputo Central Hospital, Maputo (Mozambique).
- Sheena MacCormack. Medical Research Council, London (U.K.).
- Andrew Nunn. St. George's Hospital Medical School, London (U.K.).
- St George's Hospital Medical School, London (U.K.).
- Richard Mutemwa. University of Southampton, Southampton (U.K.).
- Charles Lacey. University of York, York (U.K.).

Funding agencies

U.K. Department for International Development (DFID), London and Glasgow (U.K.).

Duration of the project

2006 – 2009.

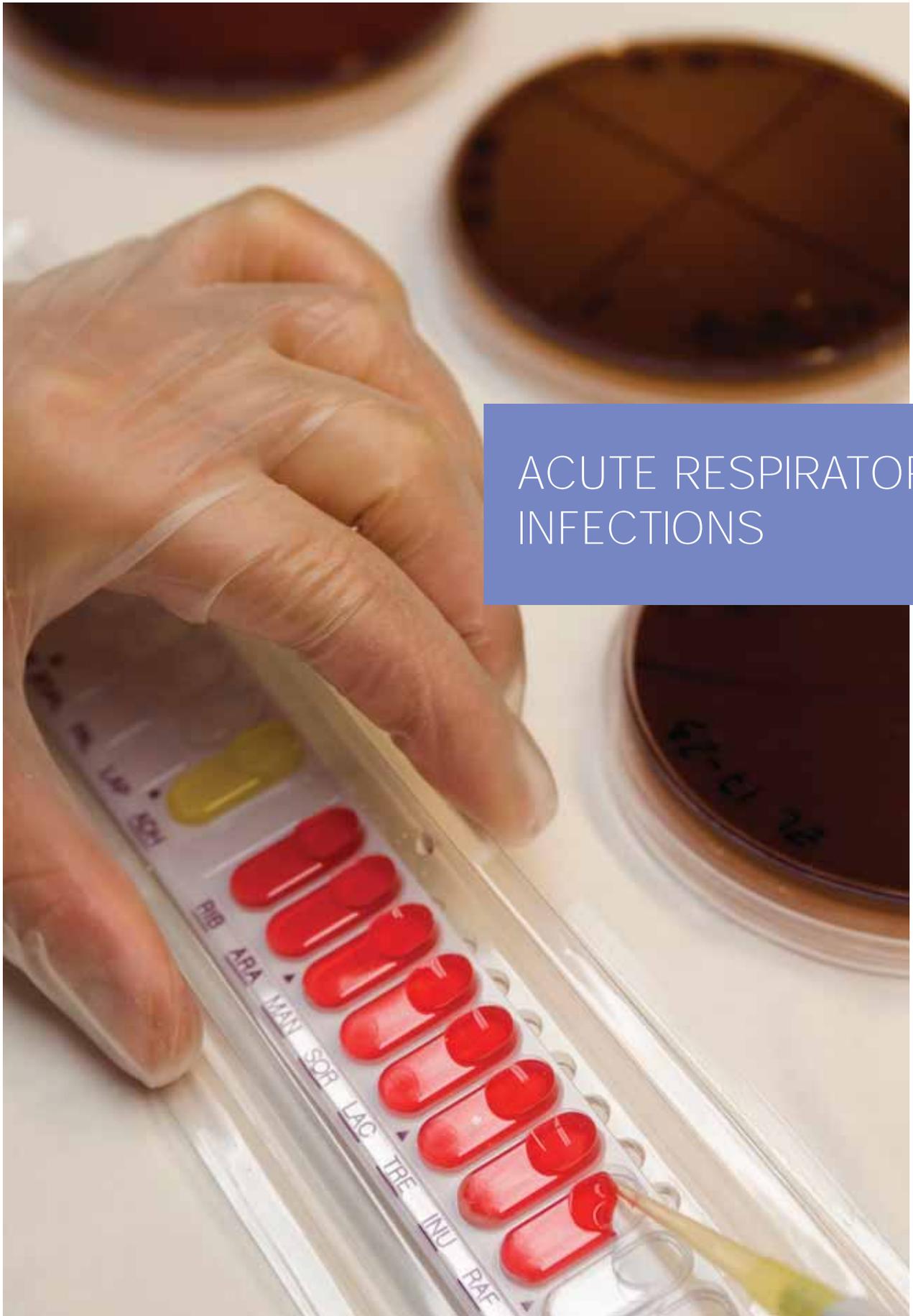
Publications

- Ndinda C, Chimbwete C, McGrath N, Pool R. Perceptions of anal sex in rural South Africa. *Cult Health Sex.* 2008 Feb;10(2):205-12.
- Montgomery CM, Lees S, Stadler J, Morar NS, Ssali A, Mwanza B, Mntambo M, Phillip J, Watts C, Pool R. The role of partnership dynamics in determining the acceptability of condoms and microbicides. *AIDS Care.* 2008 Jul;20(6):733-40.
- Some issues arising from the use of qualitative methods in clinical trials. Pool R. In Ader, HJ & Mellenbergh GJ. Eds. *Proceedings of the 2007 KNAW Colloquium: Advising on Research Methods.* Amsterdam: KNAW.

RESEARCH PROGRAMMES

- MALARIA
- HIV/AIDS
- **ACUTE RESPIRATORY INFECTIONS**
- DIARRHOEAL DISEASES
- CHAGAS DISEASE
- OTHER RESEARCH PROJECTS





ACUTE RESPIRATORY INFECTIONS

3. ACUTE RESPIRATORY INFECTIONS

According to the latest information of UNICEF/WHO (Pneumonia, the forgotten killer of children), pneumonia is the leading cause of death in children around the world, including sub-Saharan Africa. This disease is responsible for up to 19% of all deaths, at a global level, in children younger than five years.

A high proportion of pneumonia cases and deaths are due to bacterial infections, mainly caused by pneumococcus and Haemophilus influenzae type B (Hib), although viruses (such as respiratory syncytial virus (RSV) and different varieties of the influenza virus) also play an important role.

Early treatment of pneumonia with appropriate antibiotics saves numerous lives. The clinical diagnosis of this disease is, however, difficult, mainly due to the low specificity of signs and symptoms of these infections, and limited laboratory infrastructure in many areas. Moreover, the abuse of antibiotics has contributed to accelerating the development of resistance, an especially important issue in areas where there is a shortage of antibiotics other than those most commonly used. In addition, although there are efficient interventions to reduce the number of deaths

in children (including the use of combined vaccines against pneumonia and Hib, which are highly effective in the high risk age groups, i.e., children below two years), they do not reach the majority of the populations most at risk living in countries where the burden of the disease is the highest.

Given this scenario, the aim of the CRESIB is to help overcome the important challenges in public health in the areas where pneumonia is present by generating data of epidemiological surveillance (burden of the disease, aetiology, antibiotic susceptibility) that permit the prioritising of health interventions in populations with limited resources. The CRESIB also aims to evaluate the impact of interventions, such as the introduction of new vaccines in the Expanded Programme on Immunization (EPI), and to improve the diagnosis in populations with limited clinical and laboratory infrastructure.

The CRESIB is currently developing descriptive and analytical studies in the field of pneumonia in collaboration with the *Centro de Investigação em Saúde de Manhica* (CISM, Mozambique).

Research Professors

Pedro L. Alonso

Assistant Research Professors

Anna Roca

Post-Doctoral Fellows

Anna Belen Ibarz, Ruth Aguilar

Medical Research Fellow

Quique Bassat

Training fellows

Betuel Sigauque, Inácio Mandomando, Luis Morais,

Sonia Machevo

PhD Students

Cristina O'Callaghan, Núria Díez

Biostatisticians

Llorenç Quintó, Sergi Sanz



Purulent cerebrospinal fluid in a case of bacterial meningitis.

3.1. STUDY TO DETERMINE THE BURDEN OF DISEASE ASSOCIATED WITH PNEUMONIA AND THE PNEUMOCOCCUS IN CHILDREN UNDER TWO YEARS ADMITTED TO THE *HOSPITAL DISTRITAL DE MANHIÇA*

Pneumococcus is the leading bacterial cause associated with pneumonia in children. Clinical data for the diagnosis of pneumococcal pneumonia are non-specific and, in addition, pneumococcus is isolated in only a small percentage of cases.



Child aged three years with bacterial pneumonia showing symptoms of respiratory difficulties and intercostal retractions.

The lack of highly sensitive definitions for the diagnosis of pneumococcal pneumonia has hampered the epidemiological study of the burden of the disease and the assessment of the efficacy in intervention studies (such as the evaluation of new vaccines). For these reasons the WHO has standardised a generic protocol that defines radiologically confirmed pneumonia as an indirect measure to assess the burden associated with pneumococcal. This definition has been used in several efficacy trials of a pneumococcal conjugate vaccine in children under two years of age.

Principal Investigator

Pedro L. Alonso

Co-investigators

Anna Roca, Betuel Sigauque, Llorenç Quintó, Luis Morais

The aim of this present study is to evaluate the above definitions for measuring the burden of pneumococcal pneumonia in epidemiological studies and to assess the burden potentially prevented by vaccination. For this, an epidemiological surveillance study was conducted on pneumonia in children less than two years admitted to the *Hospital Distrital de Manhica*.

The initial results obtained in the study indicate that approximately one in three children below two years of age admitted to this hospital presented the clinical criteria of severe pneumonia, of which 43% of these cases were radiologically confirmed. According to the data obtained, pneumococcus is the bacterial agent most prevalent in both groups: clinical pneumonia and radiologically confirmed pneumonia; serotypes 1 and 5 being those found with higher frequency. The prevalence of HIV infection in these children was greater than 20%.

In collaboration with:

- Brendan Flannery, Montse Soriano-Gabarró, Anne Schuchat, Gloria M. Carvalho and Bernard Bell, Centers for Disease Control and Prevention, Atlanta (U.S.A.).

Funding agencies

World Health Organisation (WHO).

Duration of the project

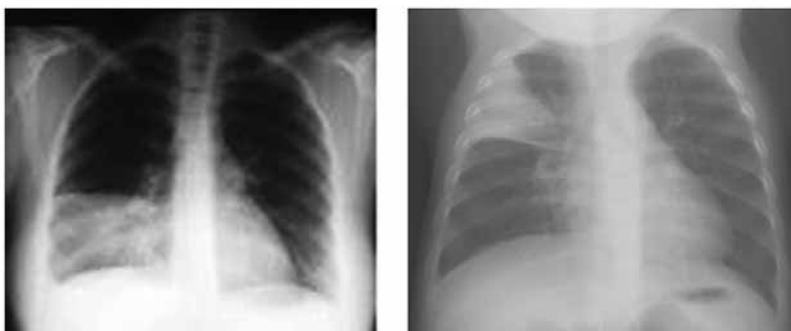
2003 – 2007.

Publications

- Morais L, Carvalho MG, Roca A, Flannery B, Mandomando I, Soriano-Gabarró M, Sigauque B, Alonso PL, Beall B. Sequential multiplex PCR for identifying pneumococcal capsular serotypes from south-Saharan African clinical isolates. *J Med Microbiol.* 2007 Sept; 56(Pt 9):1181-4.

3.2. OVERLAP: SURVEILLANCE STUDY TO DETERMINE THE ACTUAL DIAGNOSIS AND AETIOLOGY OF SUSPECTED PNEUMONIA CASES IN CHILDREN ADMITTED TO THE *HOSPITAL DISTRITAL DE MANHIÇA* (MOZAMBIQUE)

Although previous studies have shown the extent of overlap between the clinical presentation of pneumonia and malaria, there is not yet sufficient evidence to suggest modifications to the current WHO clinical guidelines and political recommendations in malaria-endemic areas.



Chest X-rays showing consolidation (left) and pleural effusion (right) in two children of the study.

Few places in rural Africa are equipped with the infrastructure necessary to accurately diagnose malaria and pneumonia. Both the *Hospital Distrital de Manhiça* and the CISM in Mozambique are equipped with laboratories and X-ray equipment that allow the diagnosis of both diseases.

The aim of this study is to assess the extent of overlap between pneumonia and malaria in children admitted to this hospital and whose clinical presentation is compatible with both diseases. For this, the final

diagnosis will be determined in these children after all available diagnostic tests have been performed.

The preliminary results of the study have confirmed the high prevalence of co-infections (malaria and bacterial and/or viral pneumonia) in children with clinical presentations compatible with both. Moreover, the data obtained also shows that the prevalence of HIV infection was higher in the cases where a bacterial agent was isolated as the cause of the infection.

Principal Investigator

Anna Roca

Co-investigators

Quique Bassat, Cristina O'Callaghan, Sonia Machevo, Luis Morais, Betuel Sigáúque, Llorenç Quintó, Sergi Sanz

Funding agencies

World Health Organisation (WHO).

Duration of the project

2006 – 2008.

3.3. EXPLORING THE USE OF BIOMARKERS FOR THE DIAGNOSIS OF COMMON INFECTIONS IN AFRICA

Health workers in facilities in rural Africa are confronted each day with the necessity to diagnose and treat children with infectious diseases.

The shortage of appropriate diagnostic tools presents major difficulties for the correct diagnosis in cases that present fever and other unspecific symptoms in this environment. As a consequence, infectious diseases are often incorrectly diagnosed, resulting in deficient health provisions.

Principal Investigator

Anna Roca

Principal Co-investigator

Pedro L. Alonso

Co-investigators

Sonia Machevo, Quique Bassat, Núria Díez, Cristina O'Callaghan, Betuel Sigauque, Luis Morais, Ruth Aguilar, Llorenç Quintó

Through this project, the levels of different cytokines and other proteins will be measured in the blood to explore the possibility of markers to diagnose malaria and other bacterial and viral infections in developing countries. The study will involve children who are admitted to the *Hospital Distrital de Manhica* with unspecific signs and symptoms of illness and fever.

The preliminary studies indicate that there is a difficulty in identifying markers to differentiate the infectious diseases indicated above.

Funding agencies

Bill & Melinda Gates Foundation, Seattle (U.S.A.).

Duration of the project

2007 – 2009.

3.4. AETIOLOGY, EPIDEMIOLOGY AND CLINICAL PRESENTATION OF ACUTE RESPIRATORY INFECTIONS IN MOZAMBICAN CHILDREN UNDER FIVE YEARS OLD

Respiratory distress or suffering is highly prevalent in children younger than five years who attend Hospital Distrital de Manhica. This study aims to evaluate the respiratory viruses associated with this common clinical presentation.

Between September 2006 and 2007, nasopharyngeal aspirates of children under five years, admitted to the *Hospital Distrital de Manhica* with respiratory distress and fever, were collected to carry out the diagnosis of respiratory viruses with molecular biology techniques.

The viral detection included the following pathogens: respiratory syncytial virus (RSV), influenza and para-influenza viruses, metapneumovirus, rhinovirus, adenovirus and enterovirus. The study patients were also tested for invasive bacterial infections (through blood culture), evidence of HIV

infection, evidence of malarial parasites and were submitted to chest radiography.

As expected, viral aetiologies were associated with almost half of the children participating in the study, the most prevalent being rhinovirus and adenovirus. The prevalence of HIV was 23% and the mortality of the subjects with viral infection was 9%. According to the data of this study, the prevalence of bacterial infections varied greatly depending on the type of virus detected.

Principal Investigator

Anna Roca

Co-investigators

Cristina O'Callaghan, Núria Díez, Quique Bassat, Sonia Machevo, Llorenç Quintó, Luis Morais

Funding agencies

Fondo de Investigación Sanitaria (FIS), Instituto de Salud Carlos III (ISCIII), Madrid (Spain).

Duration of the project

2005 – 2008.

3.5. STRENGTHENING THE SURVEILLANCE OF BACTERIAL MENINGITIS IN CHILDREN UNDER FIFTEEN YEARS ADMITTED TO THE *HOSPITAL DISTRITAL DE MANHIÇA* (MOZAMBIQUE)

Acute bacterial meningitis (ABM) is one of the most severe illnesses in sub-Saharan Africa, with more than one million estimated cases in the continent each year. The mortality rate and the long-term consequences are high; the infantile population is the most at danger.

In 1998, an ABM monitoring system was launched in the *Hospital Distrital de Manhiça*, where it was found that the pneumococcus *Haemophilus influenzae* and the meningococcal pathogens were those most commonly isolated after the neonatal period. The actual burden of the disease associated with the ABM is, however, higher than the initial data showed.

This study aims to support the collection of samples, by lumbar puncture, from children with suspected ABM, and thus improve the microbiological diagnosis through complementary tests. In this way, the diagnosis for both clinical and epidemiological purposes (epidemiological surveillance) will be improved. The strengthening of the microbiological diagnosis

has allowed the more accurate evaluation of rates of illnesses associated with ABM; this method is more than four times superior to previously described methods.

During the last years an increase of meningococcal meningitis has also been detected.

Principal Investigator

Anna Roca

Co-investigators

Quique Bassat, Luis Morais, Betuel Sigaúque, Inácio Mandomando, Cristina O'callaghan

Funding agencies

Pneumococcal Vaccines Accelerated Development and Introduction Plan (PneumoADIP), John Hopkins University, Baltimore (U.S.A.).

Duration of the project

2006 – 2008.

Publication

Sigauque B, Roca A, Sanz S, Oliveiras I, Martinez M, Mandomando I, Valles X, Espasa M, Abacassamo F, Sacarlal J, Macete E, Nhacolo A, Aponte JJ, Levine MM, Alonso PL. Acute bacterial meningitis among children, in Manhiça, a rural area in Southern Mozambique. *Acta Trop.* 2008 Jan;105(1):21-7.

3.6. TEN-YEAR SURVEILLANCE OF INVASIVE INFECTIONS CAUSED BY NEISSERIA MENINGITIDIS IN THE RURAL POPULATION OF MANHIÇA (MOZAMBIQUE)

Since 1998, the surveillance of invasive bacterial infections in children younger than 15 years admitted to the *Hospital Distrital de Manhiça* has been conducted through the CISM.

The data obtained since 1998 shows that meningococcus is the third cause of acute bacterial meningitis and a major cause of bacteremia. From 2006, a significant increase in annual cases of meningococcal infection has been observed. The current rate of annual incidence is elevated in areas that are outside the meningitis belt.

The aim of the present study is to perform the molecular

characterisation of the meningococcal strains isolated in the CISM in recent years to evaluate the potential coverage of vaccines against meningococcus.

The characterisation of the serotypes was performed by PCR techniques and the preliminary results obtained to date indicate that more than 95% of isolated meningococcus is of the genotype W-135.

Principal Investigator

Anna Roca

Co-investigators

Ana Belén Ibarz, Luis Morais, Betuel Sigaúque, Inácio Mandomando

Funding agencies

GlaxoSmithKline Biologicals, Rixensart (Belgium).

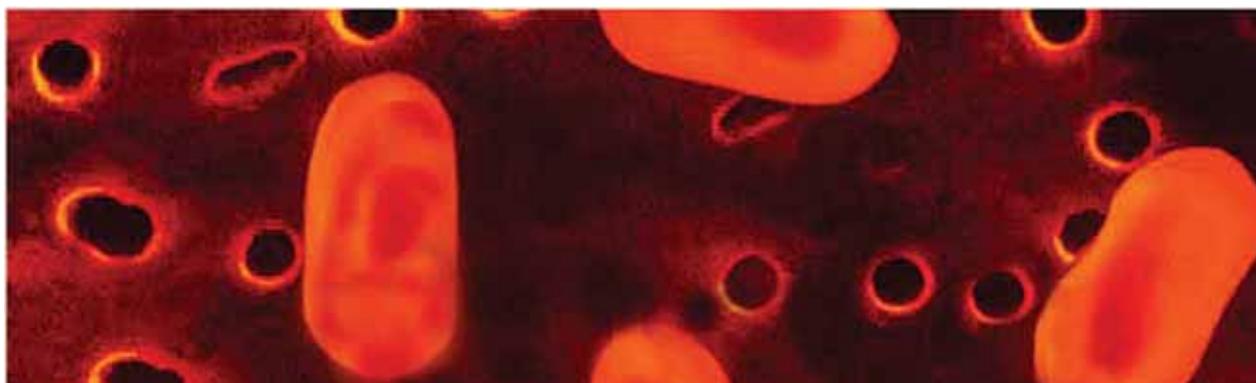
Duration of the project

2008 – 2009.



3.7. STUDY TO MEASURE THE EFFECTIVENESS OF AN HIB VACCINE CONJUGATE IN THE ROUTINE VACCINATION SCHEME IN MOZAMBIQUE

The Hib vaccine has not yet been introduced into some countries of sub-Saharan Africa, including Mozambique.



Haemophilus influenzae isolated from cerebrospinal fluid (CSR) viewed by microscopy.

Although this vaccine has been shown to be very effective in developed countries, the main obstacles to its introduction in economically weak countries (especially in Africa) are its high cost and the lack of surveillance data in certain regions with a high burden of the disease.

With the support of the preliminary data on the burden of invasive Hib illnesses generated by the CISM, Mozambique will introduce the Hib vaccine in mid-2009, within the EPI, with financial support from the Global Alliance for Vaccines and Immunisations (GAVI).

The present study aims to evaluate the impact, in terms of the burden of the disease and death, of the introduction of the Hib vaccine under the EPI in Mozambique through different strategies:

- To decrease the burden of invasive Hib disease in the Manhica district, comparing values before and after the introduction of the vaccine.
- To study the risk factors of Hib infection (invasive Hib and radiologically confirmed pneumonia).
- To look at trends in mortality in children between two and 24 months in the Manhica study zone before and after the Hib vaccine.

Principal Investigator

Anna Roca

Co-investigators

Sonia Machevo, Ana Belén Ibarz, Luis Morais, Betuel Sigauque, Llorenç Quintó

Funding agencies

The Hib Initiative, John Hopkins University, Baltimore (U.S.A.).

Duration of the project

2008 – 2011.

Publication

Roca A, Quinto L, Abacassamo F, Morais L, Valles X, Espasa M, Sigauque B, Sacarlal J, Macete E, Nhacolo A, Mandomando I, Levine MM, Alonso PL. Invasive *Haemophilus influenzae* disease in children less than 5 years of age in Manhica, a rural area of southern Mozambique. *Trop Med Intern Health*. 2008 Jun;13(6):818-26.

RESEARCH PROGRAMMES

- MALARIA
- HIV/AIDS
- ACUTE RESPIRATORY INFECTIONS
- **DIARRHOEAL DISEASES**
- CHAGAS DISEASE
- OTHER RESEARCH PROJECTS





DIARRHOEAL DISEASES

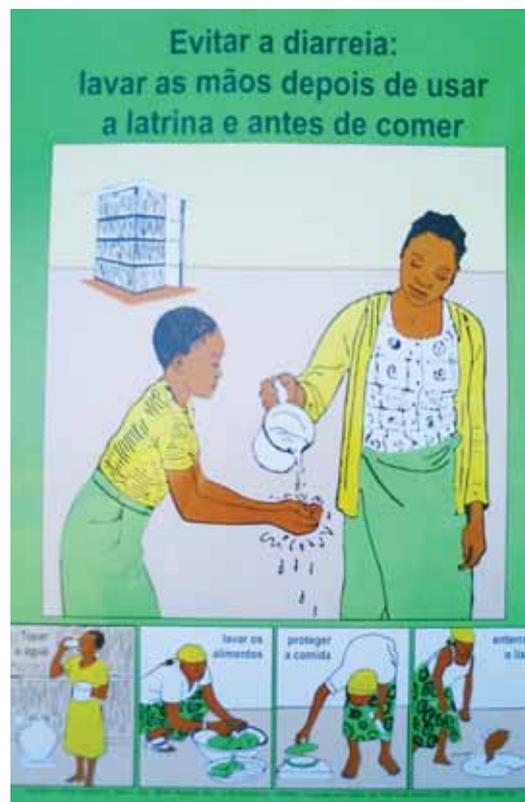
4. DIARRHOEAL DISEASES

Diarrhoeal diseases impose a heavy burden on the developing countries, each year there are 1.5 billion cases registered in children younger than five years, almost all in the impoverished areas where sanitation is poor, the hygiene is insufficient and the water consumed is not potable.

In many developing countries, epidemics of diarrhoeal diseases, such as cholera and dysentery, affect both adults and children. These diseases are very widespread in these countries, so much so that the parents don't recognise the danger signs and their children die because their bodies are often debilitated by the rapid loss of fluids and malnutrition.

The research conducted by the CRESIB in this field is focused on the study of the diarrhoeal diseases and persistent diarrhoea in these places, with particular emphasis on the aetiology of these infections and the clinical picture of the disease. Also under study are the consequences of these infections in terms of malnutrition among children and the resistances that they develop against antibiotics (mechanisms and patterns).

From the epidemiological point of view, the burden posed by diarrhoeal diseases and their prevalence in low-income countries will be investigated. These studies are of special importance in areas where there is the desire to conduct vaccine clinical trials or any other prevention, treatment and control strategies.



Poster with advice for the prevention of diarrhoea in Mozambique.

Research Professors

Pedro L. Alonso, Joaquim Gascon

Assistant Research Professors

Quim Ruiz

Medical Research Fellow

Montse Renom

Training Fellows

Inácio Mandomando, Tacilta Nhamposa, Dinis Jaintilal

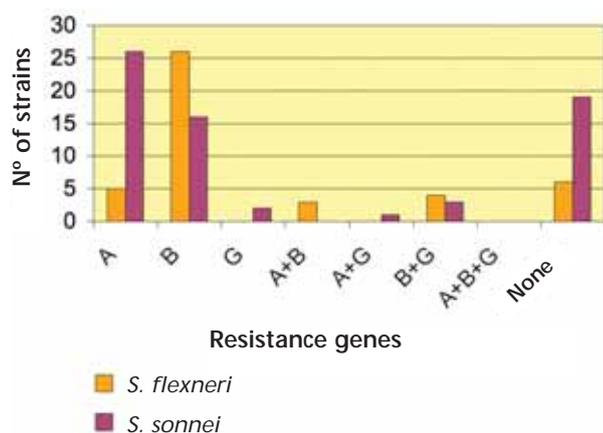
PhD Students

M^a Jesús Pons



4.1. EPIDEMIOLOGICAL–MOLECULAR CHARACTERISATION OF DIARRHOEAGENIC PATHOGENS

The objectives in this area include the characterisation of the composition and evolution of the normal intestinal microbiota, the aetiology of diarrhoea in children from low-income countries and in international travellers, and the development of studies focused on the search for alternative treatments to those currently used, both antibiotic (rifaximin) and non-antibiotic (studies on fimbriae, potential antigenic agents) treatments.



Tetracycline resistant genes detected in strains of *Shigella* spp. causing diarrhoea in travellers. A: *tetA*. B: *tetB*. G: *tetG*.

In this study, an identification using conventional techniques in patients with diarrhoea who have travelled to the tropics will be conducted to determine the resistance to antibiotics, the underlying mechanisms, the genes implicated in virulence, the clonal relationship and the rates of change in the normal composition of the microbiota. For this, PCR, colourimetric tests, sequencing, RFLP, *in vitro* mutation obtention, isoelectric focusing and field-pulse gel electrophoresis techniques will be used.

Both the diarrhoeal strains isolated in Manhiça, as well as those isolated from travellers with diarrhoea, have shown elevated levels of resistance to conventional antibiotics. In Manhiça, a large diversity of genes implicated in the development of this resistance phenotype has been detected. Likewise, in samples from both Manhiça and travellers with diarrhoea, a different prevalence of genes were detected depending on the species studied suggesting the existence of different mechanisms of dispersion (e.g., the differential presence of tet genes in *Shigella* species).

Principal Investigator

Quim Ruiz

Co-investigators

Pedro L. Alonso, Joaquim Gascon, M^a Jesús Pons, Inácio Mandomando

The results obtained also showed that different diarrhoeal pathogens coming from India have elevated levels of quinolone resistance; a motive for studying alternatives to antibiotics. Thus, the good activity of rifaximin *in vitro* has been confirmed, despite the fact that this therapeutic agent easily develops stable resistance (frequency of mutation of the order of 10^{-8}). In resistant strains obtained *in vitro*, the presence of mutations in the *rpoB* gene (especially at the codon for the amino acid 516), changes in the profile of the Outer Membrane Proteins (OMPs) and the presence of hyperactive efflux pumps were detected.

Funding agencies

Fondo de Investigación Sanitaria (FIS), Instituto de Salud Carlos III (ISCIII), Madrid (Spain).

Duration of the project

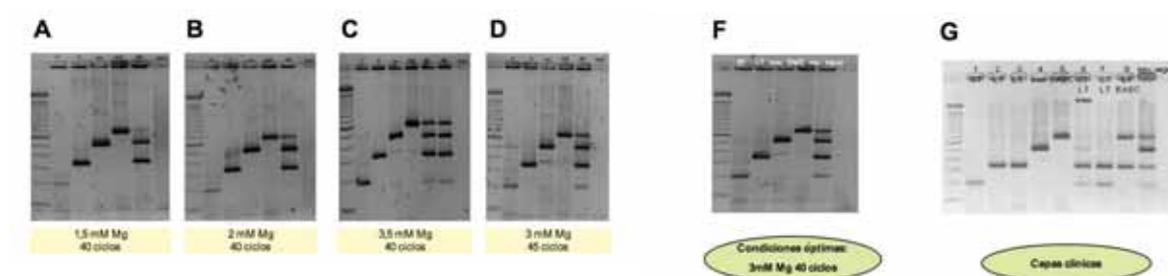
2006 – 2008.

Publications

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- Ruiz J, Mensa L, Pons MJ, Vila J, Gascon J. Development of *Escherichia coli* rifaximin-resistant mutants: frequency of selection and stability. *J Antimicrob Chemother*. 2008 May;61(5):1016-9.
- Mensa L, Marco F, Vila J, Gascon J, Ruiz J. Quinolone resistance among *Shigella* spp isolated from travellers returning from India. *Clin Microbiol Infect*. 2008 Mar;14(3):279-81.
- Ruiz J, Herrera-Leon S, Mandomando I, Macete E, Puyol L, Echeita MA, Alonso PL. Detection of *Salmonella enterica* serotype Typhimurium DT104 in southern Mozambique. *Am J Trop Med Hyg*. 2008 Dec;79(6):918-20.

4.2. EPIDEMIOLOGICAL–MOLECULAR RELEVANCE AND CHARACTERISATION OF ENTEROAGGREGATIVE *ESCHERICHIA COLI* AS A CAUSE OF DIARRHOEA IN CHILDREN YOUNGER THAN FIVE YEARS

The main aim of this research is to deepen the knowledge of the pathogenesis of the enteroaggregative strains of *Escherichia coli* (EAEC), to establish its relevance, to characterise both the virulence mechanisms that it presents, as well as the mechanisms of resistance to antibiotics, and, moreover, to establish the relationship that may exist between resistance and virulence.



PCR–Multiplex detection of *Escherichia coli* diarrhoeal strains. (A–F) The search for ideal conditions. In each photo: lane 1: molecular weight size marker; lane 2: detection of the ST strain; lane 3: detection of the LT strain; lane 4: detection of the eae strain; lane 5: detection of the EAEC strain; lane 6: simultaneous detection in PCR–Multiplex. (G) Application of the ideal conditions to diarrhoeal strains of clinical origin.

The results obtained to date show a large heterogeneity in the presence of virulence factors. High levels of resistance to antibiotics were found in the Manhiça area, as well as an elevated ease of the EAEC strain to form biofilms, which is associated with the presence of the aggR gene and can result in the development of persistent diarrhoea and in problems of poor intestinal absorption.

Furthermore, the presence of these strains has been detected as a major cause of bacteraemia in this geographical area (30% of the total bacterial strains of *E. coli* have been detected), making this the first description of such strains as the causative agent of diarrhoea in children under five.

Principal Investigator

Quim Ruiz

Co-investigators

M^a Jesús Pons, Inácio Mandomando,
Dinis Jaintilal

Funding agencies

Fondo de Investigación Sanitaria (FIS), Instituto de Salud Carlos III (ISCIII) Madrid (Spain).

Duration of the project

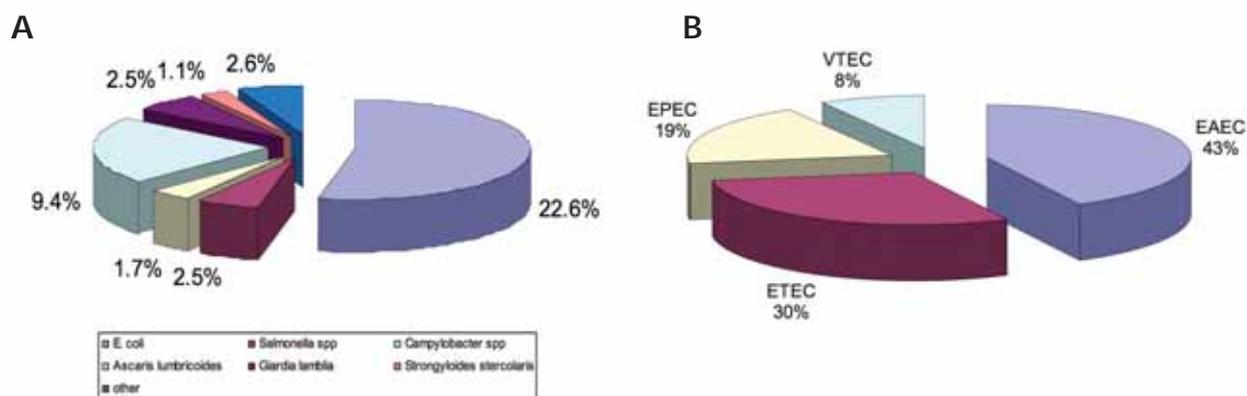
2006 – 2009.

Publications

- Ruiz J. Iniciativas para facilitar el acceso a publicaciones científicas a países en vías de desarrollo. Papeles Médicos. 2007;16:4–5.
- Mendez-Arancibia E, Vargas M, Soto S, Ruiz J, Kahigwa E, Schellenberg D, Urassa H, Gascon J, Vila J. Prevalence of different virulence factors and biofilm production in enteroaggregative *Escherichia coli* isolates causing diarrhoea in children in Ifakara (Tanzania). Am J Trop Med Hyg. 2008 Jun;78(6):985-9.

4.3. GEMS: DIARRHOEAL DISEASES IN CHILDREN FROM LOW-INCOME COUNTRIES

Diarrhoea is one of the most relevant causes of infant mortality in low income countries, in fact, it is considered the second leading cause of infant mortality in these countries, preceded by perinatal deaths and followed by respiratory diseases.



(A) Aetiology of childhood diarrhoea in Manhiça (data from 2001).

(B) The breakdown in the different pathotypes of the diarrhoeagenic strains of *E. coli* (data from 2001).

The objective of this multicentre, case-controlled study is to establish the different aetiological causes of this disease (bacterial, viral and parasitic), in children younger than five years, stratifying the monitored population into three age subgroups.

The results obtained to date in the population of Manhiça have shown an elevated number of isolated *Giardia* and rotavirus cases, as well as diarrhoeal strains of *E. coli*.

Principal Investigator

Pedro L. Alonso

Principal Co-investigator

Quim Ruiz

Co-investigators

Tacilta Nahmposa, Inácio Mandomando, Dinis Jaintilal, Montse Renom

In collaboration with:

- Karen L. Kotloff. Center for Vaccine Development, Baltimore (U.S.A.).
- Richard Adegbola. Medical Research Council (U.K.) The Gambia, Fajara (Gambia).
- Robert F. Breiman. Kenya Medical Research Institute, Kisumu (Kenya).
- Samba Sow. Center for Vaccine Development-Mali, Bamako (Mali).
- Sujit Bhattacharya. National Institute of Cholera and Enteric Disease, Kolkata (India).
- Abu S.G. Faruque, International Centre for Diarrhoeal Disease Research, Dhaka (Bangladesh).
- Anita K.M. Zaidi. Aga Khan University, Karachi (Pakistan).

Funding agencies

Bill & Melinda Gates Foundation, Seattle (U.S.A.).

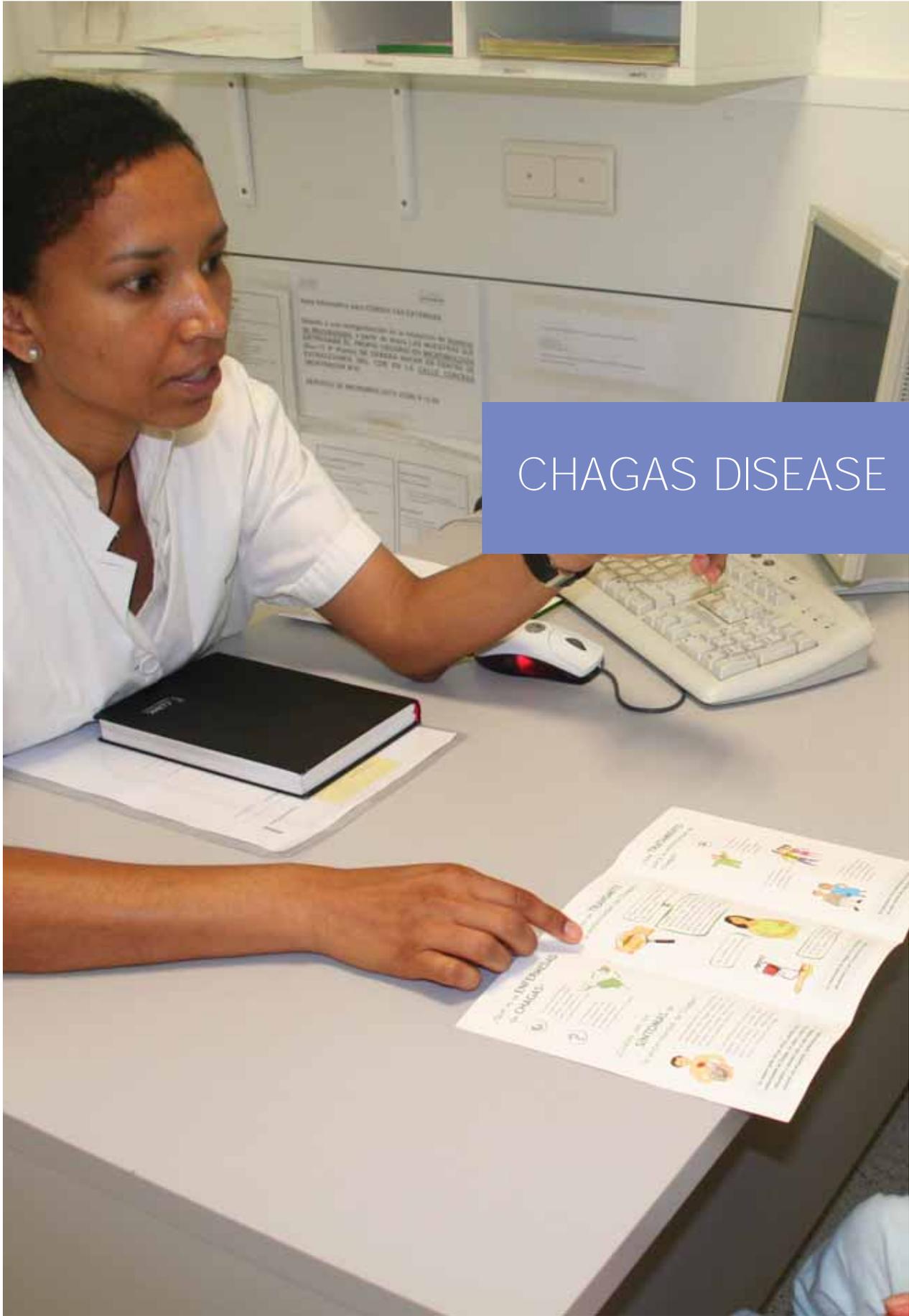
Duration of the project

2007 – 2011.

RESEARCH PROGRAMMES

- MALARIA
- HIV/AIDS
- ACUTE RESPIRATORY INFECTIONS
- DIARRHOEAL DISEASES
- **CHAGAS DISEASE**
- OTHER RESEARCH PROJECTS





CHAGAS DISEASE

5. CHAGAS DISEASE

Chagas disease, also called American trypanosomiasis, is a parasitic disease caused by the flagellate protozoan, *Trypanosoma cruzi*. This disease, traditionally linked to rural environments in impoverished regions of Central and South America, has changed its epidemiological profile due to migratory movements and now can be diagnosed in urban areas of non-endemic countries.

Because of its potential for transmission and its chronic health complications, Chagas disease has clear implications on the health of Latin American immigrants living in Spain, with a resulting impact on the Spanish health system. To cope with this situation it is necessary to better the knowledge about this disease and the physiopathological processes that determine the chronic forms. This is currently a priority, although the accessibility to more and better treatments against this parasite is also important (safer drugs with proven efficacies in the chronic phase of the infection), and the identification of biological markers of disease progression.

In non-endemic countries, one of the main proposed objectives is to determine the impact of the burden of Chagas disease in the immigrant population, which is necessary to better the control methods and avoid transmission of the disease by blood transfusion, organ transplants and vertical transmission from mother to child.

To assess the burden of Chagas disease in the immigrant population in metropolitan Barcelona, a network on imported diseases has been established in this city, involving seven institutions/departments: the Department of Cardiology at *Hospital Clínic de Barcelona*, the Perinatal Infections Unit of the Department of Maternal-Foetal Medicine at *Hospital Clínic de Barcelona*, the Tropical Medicine and International Health Unit of *Drassanes*, the *Hospital Sant Joan de Deu*, the Department of Parasitology within the Faculty of Pharmacy at *Universitat de Barcelona*, the Catalan Blood and Tissue bank at *Hospital del Vall d'Hebrón* and the Tropical Medicine and International Health Services at *Hospital Clínic de Barcelona*.

Through this network, a series of studies have been designed to determine the prevalence of the *Trypanosoma cruzi* infection in Latin American immigrants, to characterise the clinical forms of the disease, to study biological markers of disease progression, to develop new diagnostic control tools for the detection of the parasite, and to define and characterise clinical and social aspects of the immigrant population in relation to Chagas disease.

Research Professors

Joaquim Gascon, Robert Pool, Montserrat Portús,
Montserrat Gállego

Associate Research Professor

Marjolein Gysels

Medical Research Fellows

M^a Jesús Pinazo, José Muñoz

Research Assistants

Elizabeth Posada, Christopher Pell



5.1. STUDY OF *TRYPANOSOMA CRUZI* INFECTION IN THE IMMIGRANT POPULATION OF CENTRAL AND SOUTH AMERICA

The main objectives of this research were to determine the prevalence of Chagas disease in the population of potential blood donors and pregnant women from Central and South America and, as a consequence, to know the risk of transmission of the disease, both horizontally and vertically, in Spain. Another priority objective was to determine the prevalence of the infection in people of Latin American origin that consulted clinics specialising in imported pathology in Barcelona.

The participants of the study were pregnant women from areas in which Chagas disease is endemic, treated at two maternity wards in the Barcelona area, people from countries where Chagas disease is endemic and who went to donate blood at the Blood Bank, and/or people attending the specialised clinical services in imported pathology in the Barcelona area.

The diagnostic tests involved the serological screening of all patients for *T. cruzi* BioELISA Chagas®, serological tests (ELISA in-house®, Ortho® ELISA from Ortho Clinical Diagnosis), parasitological testing (microhematocrit in neonates whose mothers were seropositive) and PCR techniques.

Furthermore, the clinical monitoring and treatment, when appropriate, was also realised for both seropositive adults and children. In this way, in addition to knowing the prevalence of this disease in our environment, criteria were established for the creation of protocols for the diagnosis and treatment of this disease, as well as for the screening of maternity wards and blood banks.

The results obtained to date from the studies conducted in the blood bank indicate that 0.66% (10/1524) of blood donors from Latin America and 0.42% (1/209) of Spanish donors that have lived in risk areas tested positive for *T. cruzi*.

According to the study of maternity wards, 3.4% (46/1350) of pregnant women from Latin America were positive for Chagas disease, with Bolivian women having the highest prevalence (27.5%). The rate of vertical transmission (the percentage of infants infected whose mothers were seropositive) was 7.3% (3/46).

The results of the study conducted at centres specialising in imported pathology indicated that 41% (202/489) of the patients who visited were positive for *T. cruzi*, with 19%

(30/202) having the chronic cardiac form and 9% (15/202) the chronic digestive form.

In collaboration with:

- Silvia Sauleda. Banc de Sang i de Teixits, Barcelona (Spain).
- Oriol Coll. Gynaecology, Obstetrics and Neonatal Services, Hospital Clínic de Barcelona, Barcelona (Spain).
- Victoria Fumadó. Hospital Sant Joan de Deu, Barcelona (Spain).
- Jordi Gómez Prat. UMTSID, Institut Català de la Salut, Barcelona (Spain).

Funding agencies

Agència d'Avaluació de Tecnologia i Recerca Mèdiques de Catalunya (AATRM) Generalitat de Catalunya, Barcelona (Spain).
Fundació Bayer, Barcelona (Spain).

Duration of the project

2005 – 2008.

Publications

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- Piron M, Fisa R, Casamitjana N, López-Chejade P, Puig L, Vergés M, Gascon J, Gómez J, Portús M, Sauleda S. Development of a real-time PCR assay for *Trypanosoma cruzi* detection in blood samples. *Acta Trop.* 2007 Sep;103(3):195-200.
- Gascon J, Albajar P, Canas E, Flores M, Gomez i Prat J, Herrera RN, Lafuente CA, Luciardi HL, Moncayo A, Molina L, Muñoz J, Puente S, Sanz G, Trevino B, Sergio-Salles X. Diagnosis, management and treatment of chronic Chagas' heart disease in areas where *Trypanosoma cruzi* infection is not endemic. *Rev Esp Cardiol.* 2007;60(3):285-93.
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- Piron M, Vergés M, Muñoz J, Casamitjana N, Sanz S, Maymo RM, Hernandez JM, Puig L, Portús M, Gascon J, Sauleda S. Seroprevalence of *Trypanosoma cruzi* infection in at-risk blood donors in Catalonia (Spain). *Transfusion.* 2008 Sept;48(9):1862-8.

Principal Investigator

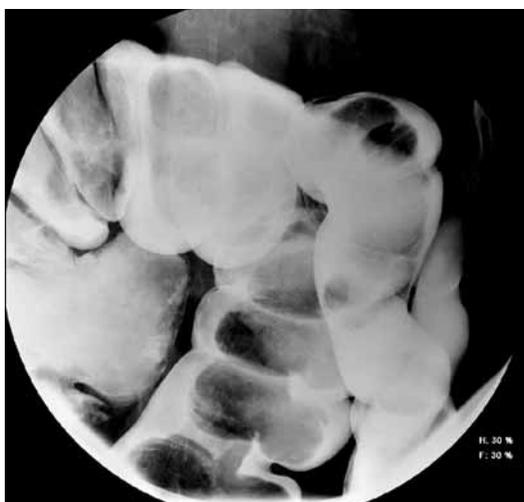
Joaquim Gascon

Co-investigators

Montserrat Gállego, Montserrat Portús, José Muñoz

5.2. SOCIAL APPROXIMATION OF THE NUTRITIONAL PROFILE IN BOLIVIAN IMMIGRANTS WITH CHAGAS DISEASE THAT ATTEND THE TROPICAL MEDICINE DEPARTMENT OF HOSPITAL CLÍNIC DE BARCELONA

After several years of research in Chagas disease in the Latin American immigrant population at the Tropical Medicine Department of *Hospital Clínic de Barcelona*, some issues about this condition have surfaced that need to be addressed from a social perspective.



Digestive affectation (megacolon) in a patient with Chagas disease.

Throughout 2008, 350 patients infected with *T. cruzi* were examined, 88% of which were of Bolivian origin, 56 of these were selected to participate in this present study. The main aim of this study was to identify and describe the changes in the dietary habits of patients with Chagas disease who reported constipation in their consultation, and to identify the causal relationship of the digestive affectation with the pathology itself. For this, three groups were organised: patients who were serologically positive for *T. cruzi* and clinically compatible with gastrointestinal involvement (constipation, alteration of the depositional rate, dysphagia, n=28), patients who were serologically positive for *T. cruzi* that show no symptoms compatible with digestive affectation (n=28)

Principal Investigator

Joaquim Gascon

Co-investigators

Elizabeth Posada, Marjolein Gysels, M^a Jesús Pinazo, Christopher Pell, Robert Pool

and a control group of patients who were serologically negative for *T. cruzi* (n=18).

Qualitative and quantitative methods were used, including semi-structured interviews and a 24-hour dietary recording for all patients and, depending on the clinic, a radiological study and a simplified colonic transit were also conducted.

The results of this study showed that the dietary culture of the group is heterogeneous due to the coexistence of different styles of nutrition in the country of origin and previous migratory experiences (21%). This cultural and nutritional diversity has been influenced by social and work factors; the type of work and the working times influenced the nutritional profile of the participants in the study. Moreover, the changes in nutritional habits detected between the patients can be a cause of the constipation. It is noteworthy that the definition of constipation perceived is different to the clinical definition, which can be a factor of confusion when dealing with gastrointestinal involvement of Chagas disease.

In collaboration with:

- Faust Gimeno. Radiology Services, Hospital Clínic de Barcelona, Barcelona (Spain).
- Ignasi Elizalde. Gastroenterology Services, Hospital Clínic de Barcelona, Barcelona (Spain).

Funding agencies

Agència de Gestió d'Ajuts Universitaris i Recerca (AGAUR), Ayudas para incentivar la investigación aplicada y la formación universitaria en materia de inmigración en Catalunya (ARAFI), Generalitat de Catalunya, Barcelona (Spain).

Duration of the project

2008 – 2009.

5.3. CHARACTERISATION AND EVALUATION OF THE PROTHROMBOTIC STATE IN CHAGAS DISEASE AS A PREDICTIVE MARKER OF RECOVERY AFTER THE TREATMENT WITH BENZNIDAZOLE

The association of thromboembolic disease with Chagas disease was described in the first publications on the latter disease. Historically, the cardiopathology of Chagas disease has been associated with the presence of dilation of the heart chambers, ventricular aneurysms and intracavitary thrombosis, which would favour thrombus formation. In recent years, however, the existence of other factors, such as endothelial dysfunction or the presence of associated prothrombotic factors that could influence the formation of thromboembolism, have been postulated.



Patient with her son during a visit to the clinic for Chagas disease at the Hospital Clinic de Barcelona.

One of the main problems in the management of Chagas disease is the absence of progression markers of the infection. The decrease in, or a negative result for conventional serological titres, which indicate the cure of the disease, take years to occur and are not useful in the short term. Until now, progression markers of the disease after drug therapy do not exist, neither do other factors that helps predict which patients will develop Chagas disease and which will remain without repercussions as a

consequence. Thus, it highlights the need to study new markers of disease evolution and assess, in our environment, the therapeutic response to conventional therapy.

In view of the above, a research project has been launched on the pathophysiology of thromboembolic events associated with infection by *Trypanosoma cruzi*, which consists of two phases. The first phase is a

comparative and cross-sectional case-controlled study, which was designed to measure the association between *T. cruzi* infection and/or Chagas disease with prothrombotic markers. The control group consists of *T. cruzi*-negative individuals from the same geographical area as the *T. cruzi*-positive patients from the case group; this will maximally reduce the genetic variability of the values of the prothrombotic markers.

The second phases consist of a cohort study, which will assess the evolution of the studied marker levels after the administration of benznidazole treatment in patients infected with *T. cruzi*. The sample size of this study,

assuming a 20% loss in the case group, was estimated to be 40 cases and 33 controls.

The enrolment of all patients and controls necessary for the study has been completed and, currently, the monitoring of the study participants is being conducted.

Principal Investigator

Joaquim Gascon

Co-investigators

María Jesús Pinazo, Elizabeth Posada,
José Muñoz, Montserrat Portús,
Montserrat Gállego

In collaboration with:

- Joan Carles Reverter and Dolors Tàssies. Hospital Clinic de Barcelona, Barcelona (Spain).
- Roser Fisa. Parasitology Laboratory, Faculty of Pharmacy, Universitat de Barcelona, Barcelona (Spain).

Funding agencies

Fundación Mundo Sano, Madrid (Spain).

Duration of the project

2007 – 2011.



5.4. PLATFORM FOR THE COMPREHENSIVE CARE OF PATIENTS WITH CHAGAS DISEASE IN COCHABAMBA (BOLIVIA) AND BARCELONA

The public health problem posed by Chagas disease in Latin America has spread to other areas and continents, mainly due to migration from the area.



Rehabilitation and works on the building that will house the Chagas Medical Centre in Cochabamba (Bolivia).

As in almost all diseases linked to poverty and migration, there is still a great ignorance of basic aspects of the disease and a scarcity of tools to improve the monitoring and medical surveillance of patients suffering from these ailments.

This project proposes to help reinforce and consolidate programmes in Chagas disease in both Bolivia and Catalonia. The primary mechanism of both health systems is to improve comprehensive care for the patients and increase the knowledge on epidemiological, clinical, immunological, diagnostic and therapeutic aspects of Chagas disease. For this, it was planned to develop a global intervention strategy, centred on the Chagas programmes, that combines measures of direct medical assistance to the patients,

specialist training of health professionals from both systems and the development of research protocols.

An agreement of cooperation has already been signed between the *Hospital Viedma* and the Biomedical Research Department in the Faculty of Medicine at the *Universidad Mayor de San Simón* (both in Cochabamba, Bolivia). Currently, work is being done on the suitability of infrastructures and the development of protocols for the integrated management of patients with Chagas disease in Bolivia.

Principal Investigator

Joaquim Gascon

Co-investigators

M^a Jesus Pinazo, Elizabeth Posada, Montserrat Portús, Montserrat Gállego, Robert Pool, Christopher Pell

In collaboration with:

- Faustino Torrico. Institute of Biomedical Research, Faculty of Medicine, Universidad Mayor de San Simón, Cochabamba (Bolivia).
- Hospital Viedma, Cochabamba (Bolivia).
- National Chagas Programme, Ministerio de Salud y Deportes, Cochabamba (Bolivia).

Funding agencies

Agència Catalana de Cooperació al Desenvolupament (ACCD), Generalitat de Catalunya (Spain).

Duration of the project

2008 – 2009.

RESEARCH PROGRAMMES

- MALARIA
- HIV/AIDS
- ACUTE RESPIRATORY INFECTIONS
- DIARRHOEAL DISEASES
- CHAGAS DISEASE
- **OTHER RESEARCH PROJECTS**



6.1. PRISMA: REFLECTING THE POSITIVE DIVERSITIES OF EUROPEAN PRIORITIES FOR RESEARCH AND MEASUREMENT IN THE END OF LIFE CARE

PRISMA is a programme that aims to inform best practice for care cancer patient and to harmonise research in palliative care (diseases in advanced and terminal phases) in Europe through the comparison and interchange of approaches, experiences and research priorities.

This is a coordination project that is carried out through meetings, workshops, networking and expert networks, activities that have been developed during 2008.

In the first half of the year (May 2008), an initial meeting was held in Amsterdam to create a European network of experts on the subject and to define the direction of work to be carried out in this project. A questionnaire was designed and distributed to the experts in the field of terminal care that participated in the meeting.

Currently, evidence is being collected (through the revision and analysis of the relevant literature) on culture and terminal care in the countries participating in the PRISMA

project (Spain, United Kingdom, Italy, Portugal, Germany, Netherlands, Belgium and Norway). As part of the project, a study will also be conducted in five hospitals in Kenya (a partner country of this programme). The study, already designed, explores the concerns and cultural significances of terminal care, diseases, death and suffering in patients with incurable terminal diseases and in health professionals. Data collection will take place in 2009; the protocol has already been submitted to the ethics committees of the lead institutions.

In addition, PRISMA plans to launch a website for the publication of results and to disseminate, in a final conference, the programme's conclusions obtained in this.

In collaboration with:

- Richard Harding, Irene J. Higginson, Sue Hall and Fliss Murtagh. King's College London (KCL), London (U.K.).
- Stein Kaasa, Dagny Faksvaag Haugen and Anne Kvikstad. Norges Teknisk-Naturvitenskapelige Universitet, Trondheim (Norway).
- Claudia Bausewein. Deutsche Gesellschaft für Palliativmedizin (DGP), Berlin (Germany).
- Peo Lopes Ferreira. Centro de Estudos e Investigação em Saúde da Universidade de Coimbra, Coimbra (Portugal).
- Luc Deliens, Bregje Onwuteaka-Philipsen, Michael Echteld, Miel Ribbe and Jenny van der Steen. Vrije Universiteit Medisch Centrum (VUMC), Amsterdam (Netherlands).
- Lucas Ceulemans, Noël Derycke, Bart van den Eynden and Tine De Vlioger. Universiteit Antwerpen (UA), Antwerpen (Belgium).
- Ana Barros Pinto. Hospital Santa Maria, Lisbon, (Portugal).
- Julia Downing. African Palliative Care Association, Kampala (Uganda).
- Franco Toscan. Istituto di Ricerca in Medicina Paliativa, Cremona (Italy).
- Paul Vanden Berghe, Johan Menten and Trudie van Iersel. Federatie Palliatieve Zorg Vlaanderen, Wemmel (Belgium).

Funding agencies

FP7 Programme, European Union.

Duration of the project

2008 – 2011.

Principal Investigator

Marjolein Gysels

Co-investigators

Robert Pool, Arantza Meñaca, Natalie Evans

RESEARCH SUPPORT SERVICES



BIOSTATISTICS UNIT

The Biostatistics Unit has as its mission, the processing of the data, from an integral perspective, obtained from the studies on health research in the design, statistical analysis and interpretation phases, assuring its quality and excellence. In addition, they collaborate in the training activities of the CRESIB and give support to the researchers in the field of biostatistics.

The functions of the unit are:

- Development of analytical programs
- Implementation of studies
- Data cleaning programming
- Sample calculations
- Statistical analysis
- Process automation
- Resolution of queries
- Training (Statistical courses, R)

Biostatistics Unit Team:

Head of Unit

John Aponte

Biostatisticians

Sergi Sanz, Edgar Ayala, Llorenç Quintó, Santiago Pérez-Hoyos

LABORATORY MANAGEMENT

Some 30 researchers work in the CRESIB research laboratories, utilising different techniques, such as ELISA, Real time PCR, sequencing, transcriptional analysis, in vivo imaging and NMR, and Bioplex.

The centre has a laboratory management structure, which provides the necessary support to researchers in the development of their activities. The person responsible for the laboratory management is Laura Puyol.

The principal functions of the laboratory management are:

- Ordering materials and laboratory equipment
- Stock maintenance
- Managing shipments
- Laboratory support for the CISM
- Equipment and infrastructure maintenance and management
- Laboratory coordination
- Support for new staff
- Coordination with the platforms and scientific/technical services of the Campus

OFFICE OF INTERNATIONAL COOPERATION

The Office of International Cooperation (OIC) is a service of the Fundació Clínic per a la Recerca Biomèdica (FCRB), whose role is primarily administrative. The OIC offers support to the activities of the CRESIB and ensures the transparency, responsiveness, compliance and efficiency in the management of resources and of both internal and external funding.

OIC Staff: (see Annex III).

TRAINING & INTERNATIONAL COOPERATION



TRAINING

On the basis of research experience, focused on the generation of knowledge, the CRESIB has the mission to be a reference institution and facilitator in the field of international health training. The centre develops training programmes, also in collaboration with different institutions, with four fundamental objectives:

1. To promote and increase awareness about global health problems.
2. To train highly qualified researchers in specific areas related to international health (mainly through master, doctoral and continuing education programmes), with special emphasis on personnel from less developed countries.
3. To improve the training of health professionals, both in developed countries for the management of imported diseases, and in low and middle incomes countries, to address the health problems endemic in these places.
4. To train technicians, doctors and scientists from the poorest countries.

The CRESIB, together with the Faculty of Medicine at the *Universitat de Barcelona*, is a member of the TropED network for the education and training in the field of international health (www.tropED.org).

The centre is currently developing the following training programmes in relation to International Health:

POSTGRADUATE TRAINING

1. MASTER'S PROGRAMMES

The CRESIB collaborates in the master programmes with the following universities:

- ***Universitat de Barcelona***: Master of Tropical Medicine and International Health, Master in Internationalisation, Master in Advanced Microbiology
- ***Universitat Autònoma de Barcelona***: Master in International Health and Tropical Medicine
- ***Universitat Pompeu Fabra***: Official Master of Public Health

2. DOCTORAL PROGRAMMES

The CRESIB participates as a research centre in the doctoral programmes of the Faculty of Medicine at the *Universitat de Barcelona*.

The centre also collaborates with the *Fundació Clínica per a la Recerca Biomèdica* in a "Training Fellows" programme, which aims to provide scientific training to young graduates in Morocco and Mozambique (the latter is developed in collaboration with the CISM), to initiate them to medical research. This programme provides support for postgraduate, master and/or doctorate studies.

DOCTORAL THESES

Since its creation in 2006, and until 2008, the researchers of the CRESIB, through their training programmes, have presented the following doctoral theses:

Dr. Samuel José Alves Mabunda

Thesis: The Epidemiology and the burden of malaria in Mozambique
Director: Pedro L. Alonso
Faculty of Medicine, Universitat de Barcelona
Date: October 2006

Dr. Francesc Xavier Vallès i Casanova

Thesis: Epidemiology of pneumococcal invasive disease in children under five years of age in a rural area of Southeast Africa. The association with genetic polymorphisms in the lectin pathway of complement activation (MBL2 and MASP2).
(Thesis written in Catalan)
Director: Pedro L. Alonso
Faculty of Medicine, Universitat de Barcelona
Date: October 2006

Dr. María Cleofé Romagosa Pérez-Portabella

Thesis: Maternal mortality in sub-Saharan Africa: an autopsic study.
Director: Clara Menéndez
Faculty of Medicine, Universitat de Barcelona
Date: May 2007

Dr. Eusebio Víctor Macete

Thesis: Evaluation of new control measures against Malaria in a rural area of Mozambique.
(Thesis written in Spanish)
Director: Pedro L. Alonso
Faculty of Medicine, Universitat de Barcelona
Date: January 2008

OTHER TRAINING PROGRAMMES

The CRESIB organises short courses and continuous training programmes, seminars, workshops, conferences and similar activities on their own and in collaboration with other institutions.

These training programmes include weekly seminars and the workshops on Chagas disease that were organised by the centre and constitute an authentic platform for the updating and sharing of research expertise.

The seminars are taught by expert researchers from around the world, and are open to the public (the details of the seminars are given in Annex II).

The workshops on Chagas disease, organised annually by the CRESIB (the third and fourth workshop on imported Chagas disease were given in 2007 and 2008, respectively) bring together international researchers and experts of this disease. These workshops result in the generation and editing of consensus documents and

clinical guidelines endorsed by the *Sociedad Española de Medicina Tropical y Salud Internacional* (SEM-TSI).

PROJECTS OF COOPERATION IN THE FIELD OF INTERNATIONAL HEALTH RESEARCH

PROGRAMME TO SUPPORT THE CREATION OF A SPECIALISATION IN EPIDEMIOLOGY AND BIostatISTICS IN THE INSTITUTO NACIONAL DE ADMINISTRACIÓN SANITARIA (INAS) IN MOROCCO

Participating entities: Fundació Clínic per a la Recerca Biomèdica, CRESIB, Universitat de Barcelona, Universitat Pompeu Fabra, Institut Nacional d'Administración Sanitaire de Marruecos and Agencia de Salud Pública de Barcelona

Funder: Fundació La Caixa

Quantity: €200,000

Period: 2007 – 2008

TRAINING PROGRAMME IN HEALTH SCIENCES IN MOZAMBIQUE: SKILL DEVELOPMENT AND STRENGTHENING OF ACADEMIC CAPACITIES IN THE FACULTY OF MEDICINE AT THE UNIVERSIDADE EDUARDO MONDLANE

Participating entities: Fundació Clínic per a la Recerca Biomèdica, CRESIB, Universitat de Barcelona, Centro de Investigação em Saúde de Manhiça and Universidade Eduardo Mondlane

Funder: Fundació La Caixa

Quantity: €272,000

Period: 2008 – 2009

UNIVERSITY SCHOLARSHIP PROGRAMME FOR MOZAMBIKAN WOMEN

Participating entities: CRESIB, Fundació Clínic per a la Recerca Biomèdica and Fundação para o Desenvolvimento da Comunidade (FDC)

Funder: Fundació La Caixa

Quantity: €300,000

Period: 2008–2013

INTERNATIONAL COOPERATION

Mozambique

An important part of the research developed at the CRESIB is carried out in collaboration with the *Centro de Investigação em Saúde de Manhiça* (CISM) in Mozambique and with other institutions in Africa, India, Papua New Guinea and Latin America, with which the CRESIB has established agreements of collaboration. These programmes are essential not only to establish specific research programmes (particularly in the long

term), but to ensure that the results of the research have a high international impact and can be translated into clinical practice and health policies for the most disadvantaged countries, thus improving the health of these populations.

The cooperative activities of the CRESIB are developed through the *Fundació Clínic per a la Recerca Biomèdica* (FCRB), which has had a fundamental role in the creation and development of the CISM in Mozambique (www.manhica.org) and the *Fundação Manhiça*, recently formed to manage the centre. Also of importance is the role of the FCRB in the "Programme of strengthening the national strategies of maternal and child health, research and specialist training" in Morocco (www.fundacioclinic.ma).



The *Centro de Investigação em Saúde de Manhiça* (CISM, Mozambique).

The CISM, created in 1996, is the first Mozambican biomedical research centre and is the result of the cooperation between the Spanish and Mozambican governments. The centre is currently managed through the *Fundação Manhiça*, which has a board of trustees constituted by the governments of Mozambique and Spain, and the FCRB. The presence of *Fundação Manhiça* is a response to the strategic association of the CISM with the *Hospital Clínic de Barcelona*, the *Universitat de Barcelona* and the CRESIB. The CISM operates with funding from the *Agencia Española de Cooperación Internacional para el Desarrollo* (AECID), the Bill & Melinda Gates Foundation, and other relevant public and private organizations.

Currently this centre employs more than 270 workers, organised into different departments (Administration, Clinical, Laboratory, Demographics, Data Centre and Social Sciences). This is one of the few research centres in rural Africa that is in direct contact with the health problems affecting its population and also providing health care. The CISM also provides training to researchers, and technical and health personnel from Mozambique.

The CISM is now one of the most advanced research platforms in the African continent, with the capacity to generate relevant results and translate these into direct benefits for the population. Their research activities are

mainly oriented to the study, from a multidisciplinary perspective, of malaria, AIDS, tuberculosis, pneumonias and diarrhoeal diseases, the illnesses that constitute the priority health problems in this country.

Morocco

The cooperation with Morocco is carried out through the FCRB and was possible through the bilateral agreements between Spain and this country, articulated by the AECID. The Memorandum of Understanding, signed by both countries, considers the improvement of maternal and child health, and the Safe Motherhood Programme, with the collaboration of the Ministry of Health. Moreover, they provide for the implementation of the maternity unit of the *Hospital Español de Tetuán* to be used by the provincial public health network of this country.



Women in a maternity consultation at the Hospital Español de Tetuán (Morocco).

Currently this model of cooperation is in the consolidation phase and responds to priority lines of action for the Moroccan Ministry of Health, such as the specialised training of professionals of the National Health System and the research of some of the diseases prevalent in Morocco. In this respect, an operational research platform is being created between specialists from both countries on transmissible diseases and maternal and perinatal conditions; this will constitute an essential tool for the development of research studies in this area. The main priorities are the study of the aetiological causes of diarrhoeal diseases in children less than five years, as well as those of acute respiratory infections (ARI). This study is in collaboration with the Hôpital d'Enfants of the *Centro Hospitalario Universitario Ibn Sina de Rabat*.

In the area of training, the creation and operation of a Diploma in Epidemiology of Public Health at the national level, in collaboration with the *Institut National d'Administration Sanitaire* (INAS), will strengthen the epidemiological surveillance and the epidemic response capacities of the Moroccan Ministry of Health through the Department of Epidemiology and Disease Control. The postgraduate training of health professionals of the Moroccan National Health System within the *Universitat de Barcelona* and *Universitat Pompeu Fabra* is also supported.

With respect to maternal and child care, the CRESIB is planning to collaborate with the Centro Nacional De Salud Reproductiva (CNSR)/Maternité des Oranger (MO) of Rabat, to improve the quality of the services for the attention and the promotion of maternal and neonatal health in the city of Rabat-Salé. The implementation of a perinatal programme in the CNSR/MO aims to improve care at delivery and postpartum for both the mother and the child. The good practices and procedures developed in the CNSR and the improvement in the coordination and continual assistance between the health centres and the maternity units of reference will be extended to other zones of the country, especially in the province of Tetuán.

Bolivia

Bolivia is the country with the greatest number of people with Chagas disease; it is estimated that 40% of the population are infected and that the area endemic with this disease covers 60% of the country. Although, it is estimated that Chagas disease is responsible for 13% of all deaths among people aged 15 to 75 years, the measures and programmes for its diagnosis and treatment are insufficient in both Bolivia and Barcelona.



A doctor of the CRESIB attends to a patient in a pilot consultation on Chagas disease in Cochabamba (Bolivia).

The programme of cooperation with Bolivia is carried out in coordination with the *Universidad Mayor de San Simón* in Cochabamba and the National Chagas Programme of the Ministry of Health. This programme aims to contribute to the effective and applicable knowledge of Chagas disease, in close collaboration with the health system and its specialists. In the long term, the programme seeks to promote the creation and consolidation of a joint platform between teams from both countries to contribute to improving care, training and research on priority diseases and key health issues between Cochabamba and Barcelona.

The initial phase of action in Bolivia has been funded by the *Agencia Catalana de Cooperación al Desarrollo* (ACCD) and the *Agencia Española de Cooperación Internacional para el Desarrollo* (AECID).

ANNEX I CRESIB PUBLICATIONS



ORIGINAL ARTICLES 2007

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LETTERS TO THE EDITOR

OTHER PUBLICATIONS 2007-2008

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3. La práctica de la medicina en países con escasos recursos. Gascon J. In: JANO (2007). ISSN02X, N° 1669, pag. 16-19.
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ANNEX II CRESIB SEMINARS



10/1/2007

Pneumococcal disease in Papua New Guinea

Dr. Suparat Phuanukoonnon, Papua New Guinea Institute of Medical Research, Goroka (Papua New Guinea)

17/1/2007

Pooled analysis of efficacy of intermittent preventive treatment in infants in Africa

Dr. John J. Aponte, CRESIB (Spain)

24/1/2007

Malaria in a district hospital in rural Mozambique

Dr. Caterina Guinovart, CRESIB (Spain)

31/1/2007

Impact of intermittent preventive treatment for malaria on mother-to-child-transmission of HIV in Mozambique

Dr. Denise Nanche, CRESIB (Spain)

7/2/2007

Desarrollo de un candidato a vacuna contra Plasmodium vivax

Dr. Hernando del Portillo, CRESIB/Department of Parasitology, Universidade de São Paulo (Spain/Brazil)

14/2/2007

Expresión diferencial de receptores de macrófagos de bazo en infecciones experimentales de ratones Balb/c con P. chabaudi y P. yoelii

Ms. Anna Rosanas Urgell, Department of Parasitology, Universidade de São Paulo (Brazil)

21/02/2007

Seguridad del candidato de vacuna de malaria RTS,S/AS02 en niños en Mozambique

Dr. Jahit Sacarlal, CISM (Mozambique)

28/2/2007

Descripció clínic-epidemiològica de la tuberculosi en un Districte rural de Moçambic

Dr. Mateu Espasa, CRESIB/HC/CISM (Spain/Mozambique)

7/3/2007

Escherichia Coli enteroagregativa (EAEC), un patògeno emergente

Mrs. Eva Méndez, Department of Microbiology, Hospital Clínic de Barcelona (Spain)

14/3/2007

Estudios epidemiológicos e inmunológicos de enfermedad de Chagas y de malaria

Ms. Diana Barrios, CRESIB (Spain)

21/3/2007

Characterization of a DBL-alpha domain from PfEMP-1 expressed by a rosetting parasite

Dr. Alfredo Mayor, CRESIB (Spain)

28/3/2007

Social responses to indoor residual spraying (IRS) for malaria prevention in Manhica, Mozambique

Dr. Robert Pool, CRESIB (Spain)

11/4/2007

La intervenció en salut materno infantil a la provincia de Tetuan; fets i mites de les contribucions a la reducció de la mortalitat materna

Mr. Enric Grau, Office of International Cooperation, CRESIB/Fundació Clínic per a la Recerca Biomèdica, Barcelona (Spain)

18/4/2007

Epigenetic silencing of Plasmodium falciparum genes linked to erythrocyte invasion

Dr. Alfred Cortes, Institut de Recerca Biomèdica, Barcelona (Spain)

25/4/2007

Underlying Molecular Mechanisms of Presymptomatic, Asymptomatic and Symptomatic Phases of Viral Illnesses

Dr. Jesús Fco. Bermejo, Mucosal Immunology Lab, Institute of Biology and Molecular Genetics, Faculty of Medicine, Universidad de Valladolid (Spain)

2/5/2007

Combination of interventions for malaria prevention in pregnancy

Dr. Clara Menéndez, CRESIB (Spain)

16/5/2007

Invasive Haemophilus influenzae disease among young children in rural Mozambique: from surveillance to public health interventions

Dr. Anna Roca, CRESIB (Spain)

23/5/2007

Maternal Mortality in Maputo Hospital, Mozambique: Clinico-pathological correlation

Dr. Jaume Ordi, Pathology Service, Hospital Clínic de Barcelona/Universitat de Barcelona (Spain)

6/6/2007

Mutilation of female genitals: human rights, tradition and identity. A methodological proposal for a change. Initiation without mutilation

Dr. Adriana Kaplan, Department of Social and Cultural Anthropology, Universitat Autònoma de Barcelona (Spain)

12/6/2007

Host genetic factors in relation to malaria during pregnancy

Dr. Jaume Bertranpetit, Evolutionary Biology Research Unit, Universitat Pompeu Fabra, Barcelona (Spain)

13/6/2007

Use of Multilocus Sequence Typing for Epidemiological and Evolutionary Studies in *Neisseria meningitidis*

Dr. Ana Belen Ibarz Pavon, The Peter Medawar Building for Pathogen Research, University of Oxford (United Kingdom)

20/6/2007

Tracking Human Viruses That Contaminate Environments

Dr. Rosina Girones, Department of Microbiology, Faculty of Biology, Universitat de Barcelona (Spain)

27/6/2007

Malaria vaccines against *Plasmodium knowlesi* tested in rhesus monkeys

Dr. Walter Weiss, Malaria Program, Naval Medical Research Center, Maryland (U.S.A.)

12/9/2007

Estudio y caracterización de cepas bacteriémicas de *Salmonella enterica* en Manhica, Mozambique

Dr. Joaquim Ruiz, CRESIB, (Spain)

19/9/2007

Autoagglutination of *Plasmodium falciparum* and role of a new platelet receptor

Dr. Abdul Ahfiz, International Centre for Genetic Engineering and Biotechnology, New Delhi (India)

26/9/2007

Clinical and Epidemiological features of Chagas Disease in Barcelona

Dr. Jose Muñoz, CRESIB (Spain)

3/10/2007

Detección por PCR de *P. falciparum* en mujeres embarazadas de Manhica: infecciones submicroscópicas y recurrencia en episodios consecutivos

Dr. Alfredo Mayor, CRESIB (Spain)

10/10/2007

Impact of Intermittent Preventive Treatment with sulfadoxine-pyrimethamine on immune responses to malaria in Mozambican children

Dr. Carlota Dobaño, CRESIB (Spain)

17/10/2007

Elevated basal hepcidin levels in the liver may inhibit the development of malaria infection

Dr. Albert Oliveras Vergés, Universitat Politècnica de Catalunya, Catalonia (Spain)

24/10/2007

Vigilancia epidemiológica de virus respiratorios y vacunas anuales de gripe

Dr. Pilar Pérez-Breña, Instituto de Salud Carlos III, Majadahonda, Madrid (Spain)

31/10/2007

Immune Responses to Tuberculosis Vaccines (old and new)

Dr. Helen Fletcher, Nuffield Department of Medicine, Oxford (United Kingdom)

14/11/2007

Giardiasis: epidemiología y genotipado

Dr. Pilar Goñi, Universidad de Zaragoza (Spain)

21/11/2007

Assessment of cellular immune responses in infants participating in a RTS,S/AS02D phase I/II B Trial in Mozambique

Dr. Arnaldo Barbosa, CISM (Mozambique)

28/11/2007

Gene expression analysis of *Anopheles gambiae* towards an assessment of transcriptomic divergence between lab and field mosquitoes

Dr. Ruth Aguilar, CRESIB (Spain)

5/12/2007

Innovations in improving access to malaria treatment in rural Tanzania: the ACCESS Programme

Dr. Manuel Hetzel, Swiss Tropical Institute/Ifakara Health Research and Development Centre (Tanzania)

12/12/2007

Using sero-conversion rates to measure malaria transmission levels

Dr. Chris Drakeley, London School of Hygiene and Tropical Medicine – Kilimanjaro, (Tanzania)

18/12/2007

Transport and Metabolism of Host Essential Nutrients by Malaria Parasites

Dr. Choukri Ben Mamoun, Department of Genetics and Developmental Biology, University of Connecticut Health Center (U.S.A.)

09/01/2008

Proteomic approaches for the discovery of new drug and vaccine targets against American trypanosomiasis

Dr. Igor Almeida, Department of Biological Sciences, University of Texas, El Paso (U.S.A.)



16/01/2008

CRESIB LECTURA DE TESIS

Dr. Eusebio Macete, CISM (Mozambique)

23/01/2008

Caracterización molecular de la resistencia a antimicrobianos en bacterias de origen alimentario, animal y humano

Dr. Yolanda Saenz, Universidad de La Rioja (Spain)

30/01/2008

Transmisión vertical de la enfermedad de Chagas

Dr. Faustino Torrico, Universidad Mayor de San Simón, Cochabamba (Bolivia)

06/02/2008

The role of ethnicity and immune responses to Plasmodium falciparum antigens (MSP1-19, AMA-1 and CSP) in determining malaria risk in a seasonal transmission area in The Gambia

Dr. Azucena Bardaji, CRESIB (Spain)

13/02/2008

Secular trend of HPV types in invasive cervical cancer, 1920-2005

Dr. Laia Alemany, Epidemiology Service and Cancer Register, Institut Català d'Oncologia, Barcelona (Spain)

20/02/2008

Lípidos y dianas terapéuticas

Dr. Amadeu Llebaria, Research Unit on Bioactive Molecules, Department of Biological Organic Chemistry, IQAB-CSIC (Spain)

25/02/2008

Active infection, latency, genome evolution and diagnosis: some approaches to understand the tubercle bacilli

Dr. Leiría Salazar, Molecular Biology Laboratory, Department of Structural Biology, Instituto Venezolano de Investigaciones Científicas, Caracas (Venezuela)

27/02/2008

Development of Russian doll nanovectors for the targeted drug delivery of antimalarials

Dr. Xavier Fernández-Busquets, Institute for Bioengineering of Catalonia, Barcelona Science Park, Universitat de Barcelona (Spain)

05/03/2008

Squamous Intraepithelial lesion prevalence and HPV type distribution among Sex Workers and General Population in the Department of Escuintla, Guatemala

Dr. Xavier Valles, Epidemiology Service and Cancer Register, Institut Català d'Oncologia, Barcelona (Spain)

11/03/2008

Computational and System Biology Approaches for Transcriptome Analysis

Dr. Ricardo Vencio, Institute for Systems Biology, Seattle (U.S.A.)

12/03/2008

Reduced mortality among vaccinated children in Kenya

Dr. Carol Rao, Centers for Disease Control and Prevention, Atlanta (U.S.A.)

26/03/2008

CRESIB/CISM Fundación Manhiça

Dr. Pedro L. Alonso/Joan Vives, CRESIB/FCRB (Spain)

01/04/2008

P. falciparum malaria: How the infected erythrocyte adheres in the placenta

Dr. Mats Wahlgren Karolinska Institutet and Smittskyddsinstitutet, Stockholm (Sweden)

09/04/2008

Genética de la adaptación del mosquito Anopheles al medio ambiente y sus implicaciones en la transmisión

Mr. Diego Ayala, Institut de Recherche pour le Développement, Montpellier (France)

16/04/2008

Scientific misconduct

Dr. Toni Trilla, Hospital Clínic de Barcelona/Institut de Recerca Biomèdica (CEIC), Barcelona (Spain)

21/04/2008

The role of toll-like receptors in malaria: Implications from murine models

Dr. Jakob P. Cramer, University Medical Center Hamburg-Eppendorf/Bernhard-Nocht Institute for Tropical Medicine, Hamburg (Germany)

23/04/2008

Malaria in the Americas: control versus eradication

Dr. Leopoldo Villegas, International Public Health Consultant, Asociación Civil Impacto Social, Tumeremo (Venezuela)

30/04/2008

Immune Reconstitution Inflammatory Syndrome Associated with Kaposi Sarcoma during antiretroviral therapy in a rural area of Mozambique

Dr. Emili Letang, CRESIB/CISM (Spain/Mozambique)

07/05/2008

Salmonella enterica serotipo Typhimurium fagotipo DT104: pasado, presente y futuro

Dr. Silvia Herrera-Leon, Instituto de Salud Carlos III de Madrid (Spain)

14/05/2008

A household costs estimate of hospital care associated with low birth weight infants in Manhica

Dr. Elisa Sicuri, CRESIB (Spain)

20/05/2008

Immunological mechanisms controlling erythropoietic suppression and severe anaemia in rodent malaria

Dr. Louis Schofield, The Walter & Eliza Hall Institute, Melbourne (Australia)

21/05/2008

Antimalarial drug efficacy trials in children in Manhica, Mozambique

Dr. Raquel Gonzalez, CRESIB (Spain)

28/05/2008

Exploring the use of Biomarkers for the diagnosis of common infections in Africa

Dr. Anna Roca, CISM/CRESIB (Mozambique/Spain)

04/06/2008

Long-term safety and efficacy of the RTS,S/AS02A malaria vaccine in Mozambican children

Dr. John Aponte/Dr. Jahit Sacarlal, CISM/CRESIB (Mozambique/Spain)

05/06/2008

Revitalizing HIV/AIDS prevention in Thailand

Dr. Laoha Siritwong Wongsa, Faculty of Public Health, Khon Kaen University (Thailand)

11/06/2008

Glycosyltransferases in Trypanosoma brucei

Dr. Luis Izquierdo, Division of Biological Chemistry and Drug Discovery, University of Dundee (United Kingdom)

18/06/2008

Antimicrobial susceptibility evolution in rural hospital, southern Mozambique: A 5 years surveillance

Dr. Inacio Mandomando, CISM (Mozambique)

19/06/2008

Plasmodium immunomics and vaccine development

Dr. Denise Doolan, The Queensland Institute of Medical Research, Brisbane (Australia)

25/06/2008

Community-Acquired Bacteremia Among Children Admitted to a Rural Hospital in Mozambique

Dr. Betuel Sigauque, CISM/CRESIB (Mozambique/Spain)

02/07/2008

CRESIB Increased expression levels of the multidrug resistance genes pvcrt-o and pvmdr1 in a patient with severe Plasmodium vivax malaria

Dr. M^a Jesús Pinazo/Dr. Carmen Fernández, CRESIB/Hospital Clínic de Barcelona, Barcelona (Spain)

03/07/2008

Molecular systematics of Anopheles species with emphasis on the Anopheles barbirostris Subgroup. Implications for the identification of disease vectors

Dr. Claudia Caterina Paredes-Esquivel, Vector Group, Liverpool School of Tropical Medicine (United Kingdom)

09/07/2008

Cellular IL-6, TNF- α and IFN- γ : correlates of immunity and risk of re-infection and symptomatic malaria in Papua New Guinean children

Ms. Leanne Robinson, The Walter & Eliza Hall Institute, Melbourne (Australia)

16/07/2008

IPTi Cost Effectiveness: Progress, Preliminary Results, Challenges

Dr. Lesong Conteh, Swiss Tropical Institute, Basel (Switzerland)

10/09/2008

Resistencia a glicopéptidos en Enterococcus. Mecanismos, epidemiología e implicaciones ecológicas

Dr. Carmen Torres, Universidad de La Rioja/CIBIR (Spain)

15/09/2008

Post-translational regulation of the adenosine transporter CNT2

Dr. Isabel Huber, Institut d'Investigacions Biomèdiques de Barcelona (IBUB)/Department of Biochemistry and Molecular Biology, Universitat de Barcelona (Spain)

30/09/2008

Quantitative proteomics of the intra-erythrocytic life stages of the malaria parasite Plasmodium falciparum

Dr. Bernardo Javier, Foth School of Biological Sciences, Nanyang Technological University (Singapore)

08/10/2008

Quantum simulation of the molecular mechanisms underlying biological processes

Dr. Carme Rovira, Computer Simulation & Modeling Laboratory, Parc Científic de Barcelona (Spain)

20/10/2008

Global Health Supply Chains: Structural Issues and Incentives for Multiple First Line Treatments.

Dr. Prashant Yadav, Professor of Supply Chain Management MIT, Zaragoza International Logistics Program, Zaragoza Logistics Center (Spain)

29/10/2008

Chagas congénito: aspectos diagnósticos, clínicos y terapéuticos: la experiencia en Bolivia

Dr. Marisol Córdova, Hospital Materno infantil Germán Urquidí, Cochabamba (Bolivia)



05/11/2008

The bioinformatics approach in biomedical research

Dr. Xavier de la Cruz, Institut de Recerca Biomèdica, Barcelona (Spain)

12/11/2008

Humoral immunity patterns in children with severe and uncomplicated malaria

Dr. Eduard Rovira, Malaria Laboratory, CRESIB (Spain)

18/11/2008

Malaria Vaccine Development/B Cell Biology and Malaria

Dr. Louis H. Miller / Dr. Sue Pierce, Head of Malaria Cell Biology Section, NIAID, NIH/Head of Immunogenetics Laboratory, NIAID, NIH (U.S.A.)

19/11/2008

Diving into the mRNA decay: The role of K-homology Splicing Recognition Protein

Dr. Irene Díaz-Moreno, Instituto de Bioquímica Vegetal y Fotosíntesis (IBVF) Universidad de Sevilla-CSIC (Spain)

26/11/2008

Demand and household costs for malaria treatment in Papua New Guinea

Dr. Elisa Sicuri, Postdoctoral Fellow in Health Economics, CRESIB (Spain)

03/12/2008

Leishmaniasis: Immune responses and vaccination strategies in the canine model

Dr. Alhelí Rodríguez, Unit of Veterinary Pharmacology, Department of Pharmacology, Therapeutics and Toxicology, Universitat Autònoma de Barcelona (Spain)

10/12/2008

Targeting HIV-1 CCR5 and CXCR4 Coreceptors: Antagonists Characterization and Mechanisms of Viral Escape

Ms. Gemma Moncunill, Fundació irsiCaixa Retrovirology Laboratory, Hospital Universitari Germans Trias i Pujol, Badalona (Spain)

15/12/2008

Briefing on research activities of CRESIB in Papua New Guinea

Dr. Anna Rosanas, Postdoctoral Fellow, CRESIB (Spain)

17/12/2008

Biomarkers of Obstructive Lung Diseases: a Longitudinal Population-Based Study

Dr. Stefano Guerra, Centre de Recerca en Epidemiologia Ambiental (CREAL), Barcelona (Spain)

ANNEX III
CRESIB PERSONNEL

EXECUTIVE MANAGEMENT

Pedro Alonso Director
Núria Casamitjana Deputy Director
Joan Vives Economic and Financial Director

OFFICE OF SCIENTIFIC COORDINATION AND TRANSLATIONAL RESEARCH

Enric Jané Scientific Coordinator
Eva Casamitjana Assistant Manager

SCIENTIFIC DIVISION

Research Professors

Pedro Alonso
Hernando A. del Portillo (ICREA Research Professor)
Joaquim Gascon
Ned Hayes
Clara Menéndez
Robert Pool

Associate Research Professors

John Aponte
Marjolein Gysels

Assistant Research Professors

Carlota Dobaño
Carmen Fernández-Becerra
Alfredo Mayor
Denise Naniche
Santiago Pérez-Hoyos
Anna Roca
Quim Ruiz

Researchers from other institutions collaborating with CRESIB

Montserrat Gállego (Universitat de Barcelona)
Jaume Ordi (Hospital Clínic, Universitat de Barcelona)
Montserrat Portús (Universitat de Barcelona)

Post-Doctoral Fellows

Ruth Aguilar
Tamara Berthoud
Marina Bruçet
Ana Belén Ibarz
Lorena Martín
Arantza Meñaca
Anna Rosanas
Edmilson Rui
Elisa Sicuri

Medical Research Fellows

Arnoldo Barbosa
Azucena Bardaji
Quique Bassat
Raquel González
Caterina Guinovart
Nayra Gutiérrez
Sarah Lafuente (UASP)
Emili Letang

Cinta Moraleda
José Muñoz
María Jesús Pinazo
Montserrat Renom
Cleofé Romagosa

Research Assistants

Joseph Campo
Natalie Evans
Mauricio Humberto Rodríguez
María Maixenchs
Christopher Pell
Elizabeth Posada
Lianne Straus
Helen Street

Training Fellows

Pedro Aide (CISM, Mozambique)
Dinis Jaintilal (CISM, Mozambique)
Eusebio Macete (CISM, Mozambique)
José Machado (CISM, Mozambique)
Sonia Machevo (CISM, Mozambique)
Maria Nélia Manaca (CISM, Mozambique)
Inácio Mandomando (CISM, Mozambique)
Luis Morais (CISM, Mozambique)
Augusto Nhabomba (CISM, Mozambique)
Tacilta Nhamposa (CISM, Mozambique)
Diana Quelhas (CISM, Mozambique)
Jahit Sacarlal (CISM, Mozambique)
Esperanza Sevene (Universidade Eduardo Mondlane, Mozambique)
Betuel Sigauque (CISM, Mozambique)
Rachid Ben Messaoud (FCRB, Morocco)
Imane Jroundi (Institut National d'Administration Sanitaire-Ministère de la Santé de Rabat, Morocco)

PhD Students

María Bernabeu
Núria Díez
Mireia Ferrer
María Lahuerta
Cristina O'Callaghan
María Jesús Pons
Eduard Rovira
Celia Serna
Elisa Serra

Biostatistics Unit

John Aponte	Head of Unit
Edgar Ayala	Biostatistics Technician
Santiago Pérez-Hoyos	Biostatistics Technician
Llorenç Quintó	Biostatistics Technician
Sergi Sanz	Biostatistics Technician

I.T. Support

Jordi Deu	I.T. Support Technician
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Project Management

Carolyn Daher	Project Manager
Andrea Egan	Project Manager
Maria Oziemkowska	Project Manager
Janifer Quick	Project Manager

Sònia Tomàs Project Manager
 Ana Aguilera Project Manager-Mozambique (CISM)
 Patricia García Project Assistant
 Montse Pi Project Assistant

Laboratory

Laura Puyol Laboratory Manager
 Pep Astola Laboratory Technician
 Diana Barrios Laboratory Technician
 Pau Cisteró Laboratory Technician
 Alfons Jimenez Laboratory Technician
 Roberto Álvarez Biosafety-CISM
 María José López Laboratory Manager-Quality Control - CISM

Communication and Training Department

Cristina De Carlos Scientific and Communication-Support Technician
 Salut Renom Scientific, Training and Communication-Support Technician, CISM

Administrative Staff

Yolanda Antín Personal Assistant
 Anna Lozano Personal Assistant
 Sònia Tomàs Personal Assistant
 Maribel Espinosa Administrative Assistant
 Marcela Yñesta Administrative Assistant

Administration

Marga Sala Head of Administration

Accounting

Patricia Agusti Management Technician-Accountancy
 Jaume Tarragó Project Management Technician
 Fernando Pizzabocche Controller-CISM

Controlling

Alicia Llamas Management Technician-Controller

Human Resources

María José Merino Management Technician-Human Resources

Secretariat and Purchasing

Esperanza Marin Personal Assistant
 Carla Garrido Administrative Assistant
 Laia Pon Administrative Assistant
 M^a Luz Smaldoni Administrative Assistant
 Noemi Bellanco Documentalist
 Meritxell Graupera Documentalist

ECONOMIC AND FINANCIAL DIVISION - OFFICE OF INTERNATIONAL COOPERATION

Director

Joan Vives Economic and Financial Director

Projects

Enric Grau Project Manager

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 Pep Figueras International Fellow
 Anna Lucas Contract Management Technician
 Pau Balcells Management Technician-Desk Officer
 Marta Cuezva Project Management Technician
 Elena Esteban Project Management Technician
 Mireia Hernández Project Management Technician
 Sam Mardell Management Technician-Desk Officer
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 Anne Lise Sirvain Logistics and Scientific Support Technician-Morocco

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