CRESIB
Annual Report
2009

CRESIB
BARCELONA

Hospital Clínic - Universitat de Barcelona

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The year 2009 was one of growth and consolidation for the Barcelona Centre for International Health Research (CRESIB)

The centre was created and began to take shape in 2006, and has further developed over the last three and a half years. Thanks to its sound and innovative approach, it now stands on its own merit as one of the most important actors in the field of global health at a local, national and global level.

Despite the international economic crisis in 2009, our centre grew in all senses. We expanded our research areas and programmes and consolidated existing lines, such as the *Plasmodium vivax* studies and the development of Phase III of the malaria vaccine, which, once completed, will be sent for registration to the European Medicines Agency. We devoted more resources to research and development in the area of maternal and child health. We also completed the MalERA project —an initiative that brought together over 200 top-level researchers

in 36 different countries specializing in malaria and other diseases, with the aim of developing a research agenda for eradicating malaria in the world.

In 2009 we recruited new staff, strengthened our already close ties with the platforms of Mozambique, Morocco and Bolivia, and established partnerships with new research centers in Spain and abroad. The process of creating and implementing the Manhiça Foundation —a landmark in the management of research centres based in the African continent—received special support from CRESIB throughout the year.

Our scientific production also grew. We increased the number of our publications and invested in more training programmes. The knowledge generated by CRESIB has allowed us to position ourselves as one of the key advisors and consultants on global health issues within Spain and on the international scene.

This has all been possible because we have more partners and funders with the capacity to raise funds and have therefore increased our budget.

The groundwork and conceptual design of the Institute for Global Health of Barcelona (ISGlobal), of which CRESIB will form part in the near future, were also carried out in 2009.

Finally, I would like to highlight one of the most important milestones of 2009: CRESIB's 2010-2013 Strategic Plan, designed to drive us forward and to bring us closer to the goals stemming from our mission: to improve global health through research and training, in order to contribute to the development of the most disadvantaged populations.

Pedro L. Alonso
Director

One of the most outstanding achievements in 2009 was the development of CRESIB's 2010-2013 strategic plan

In addition to research, training and cooperation, of which details are offered in this report, other outstanding achievements in 2009 were the development of CRESIB's 2010-2013 strategic plan, the negotiation of the programme contract and the groundwork for the new Institute for Global Health of Barcelona (ISGlobal).

In its initial stage, CRESIB carried out scientific activities within a very linear research programme based on the ongoing activities at the time of its creation. However, within its strategic plan the centre has now reached sufficient maturity to develop a new research plan that better reflects its mission and its multidisciplinary and translational spirit. Unlike other research centres, CRESIB considers that health problems must be approached from a multidisciplinary perspective. Its ultimate goal is that its research results should have a measurable impact on health policies and on the health of the most disadvantaged populations, which are the hardest hit. For this reason, our scientific work is conducted in teams of researchers with different views and disciplines, and our field work is always based on the priorities of the populations and countries in which we work.

The strategic plan was developed through a participatory and consensual system, in which we all learned and gained knowledge of our centre and its activities. I would like to express my thanks for the ideas, work and time dedicated to this major project by all those involved. Now we finally have a proposal to submit to our governing bodies (the Executive Board and the Board of Trustees) and to the Scientific and Technical Advisory Committee (STAC), which has provided us with advice throughout the process.

In the 2010-2013 strategic plan we started by defining our mission: to improve global health through research and training, in order to better respond to what we are and wish to be. The research plan laid down in the strategic plan has a matrix structure with areas and programmes that are intertwined and that reflect the reality of our research. Our annual reports will be based on this structure as of next year. The areas consist of disciplines focusing on health systems or health problems relevant to population groups, whereas the programmes consist of well defined diseases or disease groups. Based on this initial approach, all the research activities —and all the resources necessary for carrying them out— are developed. The new strategic plan is intended to be a dynamic, open-ended document that responds and adapts continuously to the prevailing needs and conditions. From now on, this plan will be detailed in annual action plans.

In 2009 we also worked with the Ministry of Health and the Ministry of Innovation, Universities and Enterprise of the Catalan

government (Generalitat de Catalunya) to establish the programme contract for the next four years (2010-2013). This contract should ensure economic support for the lines of action laid down in the strategic plan and thus allow CRESIB to achieve its mission. The conclusion of negotiations with the ministries and the final approval of this contract are scheduled for the first half of 2010.

In addition to the scientific activity carried out by CRESIB, great efforts have been devoted to training researchers and specialists in international health. The training consisted of master's and doctoral programmes, as well as seminars, workshops and lectures on the research topics that are developed and followed by the centre. These training programmes are attended by students, researchers and specialists of all the centres and countries with which CRESIB collaborates, and especially by trainee researchers in Mozambique and Morocco.

CRESIB's experience in the field of research has also been associated with health cooperation and the training of health professionals in the countries in which the centre conducts research and training —mainly Mozambique, Morocco and Bolivia—. The new Health Policy Unit set up in 2009 mostly carried out global health advocacy and provided support and advice for the development of new projects.

Finally, together with the Fundació "la Caixa", CRESIB promoted and participated in the process of consultation and conceptual design for the Institute for Global Health of Barcelona (ISGlobal), culminating in its constitution in early 2010. The aim of this new institute is to take advantage of the presence of global health on international political agendas, and of the experience and potential existing in Barcelona in this field, in order to develop the value chain of generation, management, transmission and application of knowledge on global health. ISGlobal will be divided into four areas: research, which will continue within the framework of CRESIB; training; a think tank, analysis and prospecting; and consulting/advisory services. The aim is to create synergies among these areas.

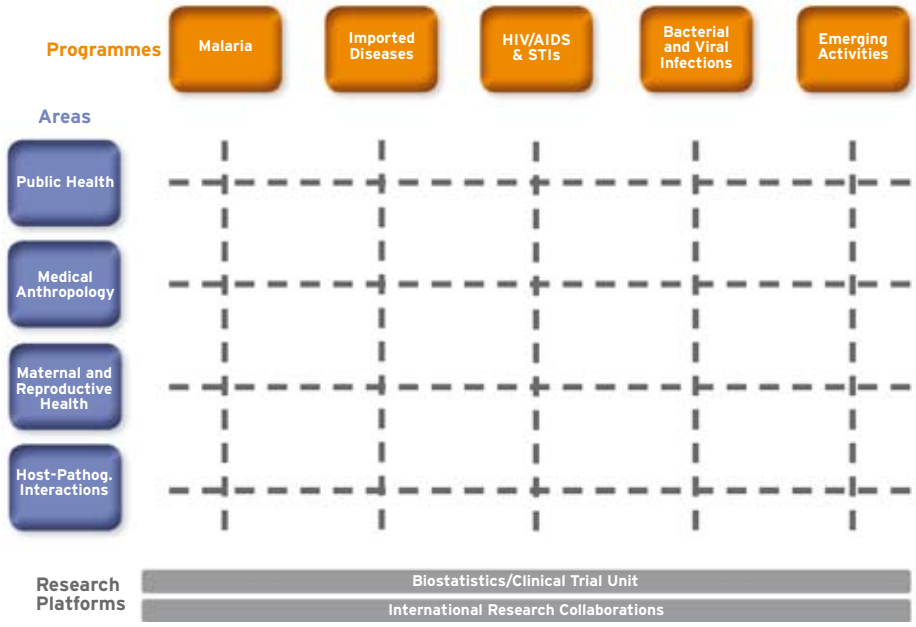
Before closing, I would like to point out that in 2009, despite the general economic context, income from international health activity showed a 28% increase over the previous year, allowing us to improve the support structure for research and to gain efficiency and competitiveness.

All the above results and achievements would not have been possible without the dedication and enthusiasm of all those who work at CRESIB.

Núria Casamitjana
Technical Director

2010-2013 Strategic Plan Preview

Four years after its creation, the Barcelona Centre for International Health Research (CRESIB, Hospital Clínic – Universitat de Barcelona) has adopted a new organizational model which is a more faithful reflection of the multidisciplinary and cross-cutting nature of the Centre's research and contributes better to achieving its mission: ***To improve global health through research and training.*** CRESIB has been re-organized into a matrix organization to maximize interaction between researchers and to better coordinate and promote multidisciplinary, translational and cross-cutting research, as shown in the following chart:



CRESIB's new scientific matrix organization

This matrix contains:

Areas as disciplines focused on health systems or health problems of relevant population groups:

- Public Health (Effectiveness and Safety of Preventive Interventions)
- Medical Anthropology
- Maternal and Reproductive Health
- Host-Pathogen Interactions

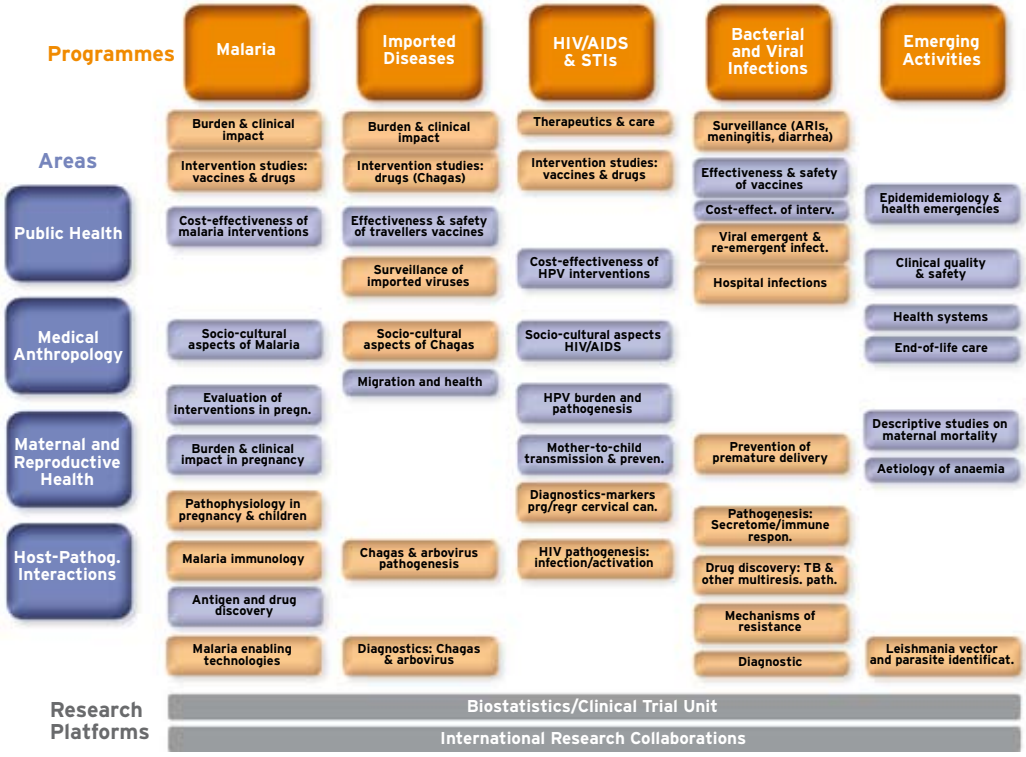
Programmes focusing on particular diseases or groups of diseases:

- Malaria
- Imported Diseases
- HIV/AIDS and Sexually Transmitted Infections
- Bacterial and Viral Infections
- Emerging Activities

Platforms providing specific research support services that can have their own (methodological) research:

- Biostatistics and Clinical Trial Unit
- International Research Collaborations

Specifically, each research programme or area has its own lines of research which can include researchers from different disciplines, as shown in the following chart, where the colour indicates where the principal investigator is. **"Emerging Activities"** have been added to include those projects/lines which are not cross-cutting and whose track record does not yet allow them to be considered as programmes or areas.



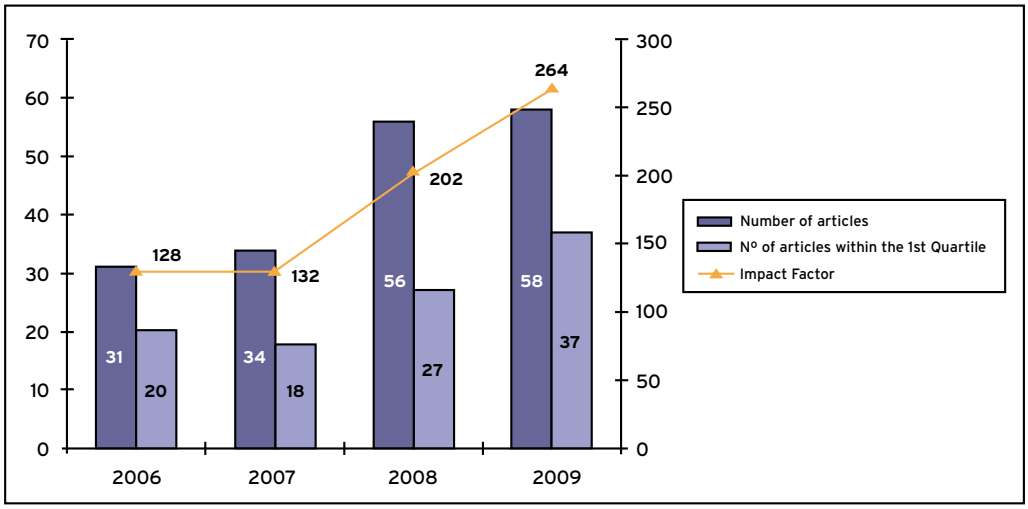
CRESIB's lines of research by programmes and areas.

By implementing this Strategic Plan, CRESIB aims to play a fundamental role in achieving the following key public health goals:

- In collaboration with CRESIB's partners, registration of the RTS,S malaria vaccine.
- Establishment of a Chagas control programme in Catalonia.
- Approval of new prevention and treatment strategies to prevent malaria among pregnant women.
- Better control strategies of emergent viral diseases in Catalonia.
- Setting up of a new educational offer to confront the main global health problems.
- Establishment of a new global health institute in Barcelona.

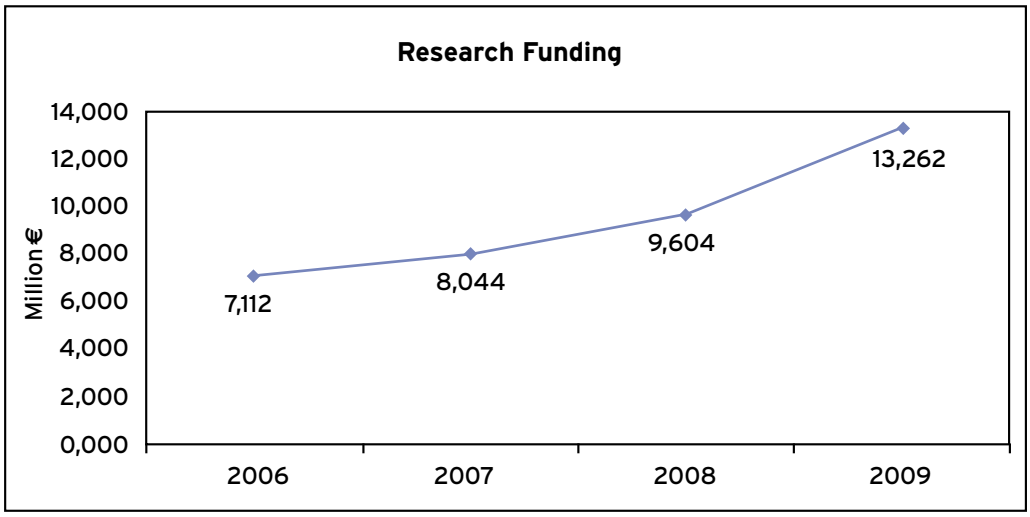
Facts & Figures

CRESIB Publications



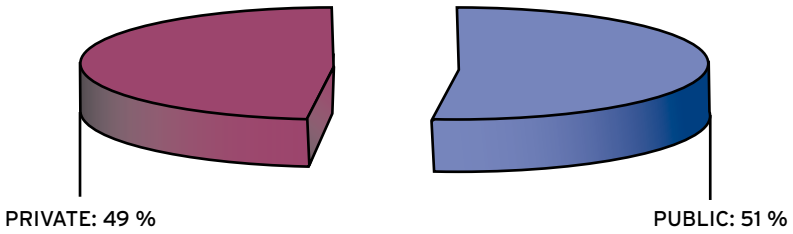
Number of articles, impact factor and articles in the first quartile of the speciality published by CRESIB's researchers since its foundation.

Research Funding



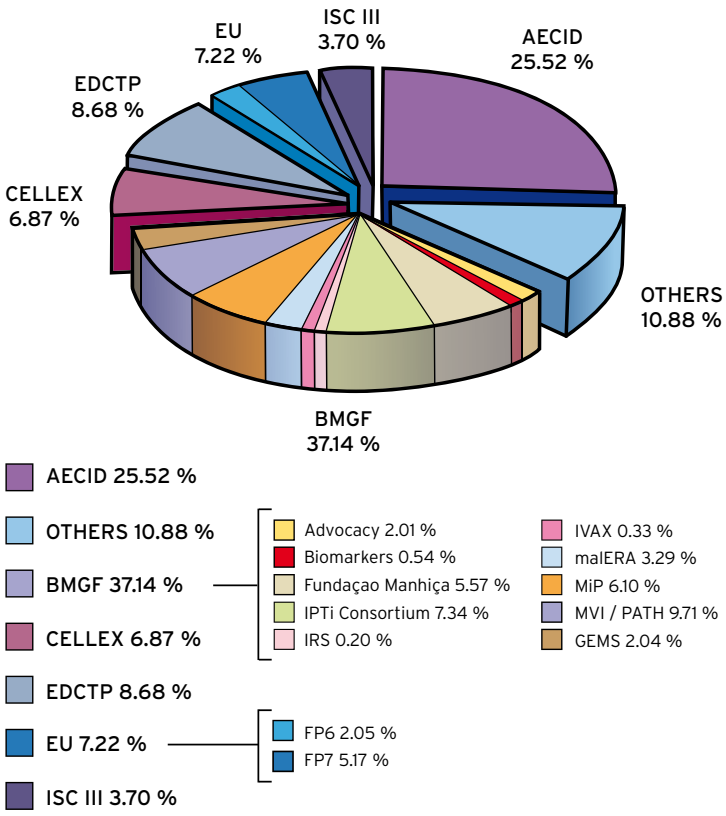
Evolution of CRESIB's annual executed budget since its foundation. The Centre is financed through health research funds (competitive and structural funds) awarded to CRESIB, to its founding institutions (Hospital Clínic de Barcelona, IDIBAPS, Universitat de Barcelona) and to the Fundació Clínic per a la Recerca Biomèdica (FCRB), acting as the organization responsible for managing these funds.

Sources of Funding



Sources of funding (public or private) for project funds and grants active in 2009.

Main Funders



Major funders of CRESIB, taking into account project funds and grants active in 2009. These include health research funds awarded to CRESIB, to its founding institutions (Hospital Clínic, IDIBAPS, Universitat de Barcelona) and to the Fundació Clínic per a la Recerca Biomèdica (FCRB). (AECID, Agencia Española de Cooperación Internacional para el Desarrollo; BMGF, Bill and Melinda Gates Foundation; EDCTP, European and Developing Countries Clinical Trials Partnership; EU, European Union; ISCIII, Instituto de Salud Carlos III; IPTi consortium, Intermittent preventive treatment in infants consortium; IRS, Indoor Residual Spraying; *IVAX*, Interdisciplinary *P. vivax* Research Consortium Planning Grant; malERA, malaria Eradication Research Agenda; MiP, Malaria in Pregnancy Consortium; MVI/PATH, Malaria Vaccine Initiative/Programme for Appropriate Technology in Health; GEMS, Global Enteric Multi-Centre Study; FP6/7, Framework Programme 6/7.)

CRESIB in the News 2009

CRESIB organizes the fifth workshop on Chagas disease: "Vectorial Transmission and Neurologic Complications "

Dr. Gascon (CRESIB) during the workshop on Chagas disease.

As in previous editions, on February 2009 CRESIB organized the fifth workshop on Chagas disease in Barcelona under the title "Vectorial Transmission and Neurological Complications", with the support of the Fundación Mundo Sano. This event was coordinated by the researcher and specialist in Chagas disease, Dr. Joaquim Gascon (CRESIB, Hospital Clínic-Universitat de Barcelona), within the framework of the centre's training programme. The workshop was attended by international research experts, including Dr. Faustino Torrico from the University Mayor de San Simón de Cochabamba in Bolivia and Dr. Jean Jannin from the World Health Organization (WHO) in Geneva.

At the end of this meeting a consensus document initiated by the Working Group on Chagas disease of the Spanish Society of Tropical Medicine and International Health (SEMTSI) and CRESIB (HC-UB) was drafted for publication in Enfermedades Infecciosas y Microbiología Clínica, the official journal of the Spanish society of the same name. This document addresses the diagnosis, management and treatment of neurological manifestations and complications of patients infected by *Tripanosoma cruzi* in Spain.

CRESIB participates in the Malaria Exhibition in Madrid and Barcelona

CRESIB (HC-UB) participated in the design, organization and launching of the Malaria Exhibition, led by the Biblioteca Nacional de España (Spanish National Library) with the support of the Cruz Roja Española (Spanish Red Cross) and Caja Madrid. The aim of this exhibition was supporting the fight against malaria by raising public awareness of the problem in the world today, the burden that it represents and the economic repercussions suffered by countries where the disease is widespread. This exhibition was held from March to June 2009 at the Biblioteca Nacional de España in Madrid, and from October 2009 to June 2010 at CajaMadrid in Barcelona.

(L to R) Carmen Contreras, Obra Social Caja Madrid President; Milagros del Corral, Biblioteca Nacional de España Director; Bernat Soria, Spanish Health Minister; Juan Manuel Suárez del Toro, Cruz Roja President and Pedro L. Alonso, CRESIB Director.

The Malaria Exhibition was a journey in time illustrating the intense battle waged against this illness throughout history, in which Spain has played a prominent role. It was organized in chronological order from the first Hippocratic theories to recent times, including some fundamental historical landmarks: the first concepts of "intermittent fevers" and their treatment; the use of Peruvian bark and the isolation of quinine; the discovery of the parasite/vector agents that cause the illness and its vital cycles; the strategies of the battle since the beginning of the 20th century; the anti-malaria campaigns undertaken in Spain; and the evolution of the illness in recent times.

CRESIB collaborates in a health science training programme with the Universidade Eduardo Mondlane in Mozambique

A group of Mozambican doctors, all of them lecturers at the Universidade Eduardo Mondlane (Mozambique) during a meeting with Dr. Xavier Carné (Hospital Clínic de Barcelona).

This innovative training programme named "Skills Development and Academic Capacity Building in the Faculty of Medicine of the Universidade Eduardo Mondlane" was initiated in January 2008 under the coordination of the Fundació Clínic per a la Recerca Biomèdica, with the support of the Fundació Obra Social "la Caixa", Barcelona, and the participation of the Centro de Investigaçao em Saúde de Manhiça (Manhiça Health Research Centre, Mozambique), CRESIB (HC-UB) and the Universitat de Barcelona.

The main objective of this programme, involving a collaboration in health sciences between the Universidade Eduardo Mondlane and the Universitat de Barcelona, is to offer capacity-building and qualification to university teachers in order to improve their capabilities in teaching, research, and learning processes with a view to creating a group of highly qualified health professionals in the future. To this end, and as a part of the different activities developed in the context of this project, in May 2009 a group of Mozambican doctors participating in this programme—all of them lecturers at the Universidade Eduardo Mondlane, Mozambique—visited the Hospital Clínic de Barcelona, its Department of Pharmacology and CRESIB in order to receive special training in teaching methodology and the use of technologies applicable to biomedicine and research. After this training of trainers programme, the lecturers will transmit their expertise to their students at the university.

The rate of newborn infants infected with Chagas disease rises within the immigrant population

Scientific experts led by Dr. Joaquim Gascon (CRESIB, HC-UB) worked together with Dr. Oriol Coll, head of the Maternal and Foetal Health Section (Hospital Clínic de Barcelona), the Parasitology Group of the Faculty of Pharmacy (Universitat de Barcelona) led by Dr. Montserrat Portús, and researchers from the Hospital Sant Joan de Déu, Barcelona, lead by Dr. Victoria Fumadó to study mother to child transmission of Chagas disease.

(L to R) Victoria Fumadó, Joaquim Gascon, Oriol Coll and Monserrat Gállego (attended as Monserrat Portús' representative).

The results of the study carried out by this group of researchers were published in the journal Clinical Infectious Diseases (Clin Infect Dis 2009 Jun 15; 48(12):1736-40), and showed that 3.4% of Latin American women who gave birth in Barcelona were infected with Chagas disease and that the transmission rate to newborn infants had risen to 7.3% in Catalonia. Screening programmes of pregnant women have fostered the early detection of infected children and allowed a more

efficacious administration of treatments. This is especially important because drug treatments are very effective (close to 100%) in the newborn and prevent infected children from developing complications related to Chagas disease in adulthood.

The Chagas platform: a collaborative project between Barcelona and Bolivia

Faustino Torrico, first from the right, and Joaquim Gascon, fourth from the right, during the inauguration ceremony of the Centre for specialized care of adults with Chagas disease in Cochabamba (Bolivia).

Fundació Clínic per a la Recerca Biomèdica and CRESIB (HC-UB) promoted a new Centre for specialized care of adults with Chagas disease in Cochabamba, Bolivia, with the support of the Catalan Agency for Development and Cooperation and the Spanish Agency for International Development and Cooperation. Dr. Joaquim Gascon, head of the Tropical Medicine Service at the Hospital Clínic de Barcelona and Research Professor at CRESIB, and Dr. Faustino Torrico, full professor at the University Mayor de San Simón, Cochamba, Bolivia—both experts on Chagas disease—have led the creation of this centre, inaugurated on July 2009 and which is part of the National Guidelines for Chagas Control issued by the Bolivian Ministry of Health.

The aim of this project is to share a management model similar to that of the Chagas Disease Clinic at the Hospital Clínic de Barcelona, and to promote research projects sharing working groups in order to advance in the biomedical research field and optimize the diagnosis and treatment of this condition. In 2009 the Bolivian centre had three clinics, a pharmacy, a laboratory and a room for basic equipment for clinical evaluation and control of the Chagas infection, such as electrocardiographs and echocardiographs.

MalERA (Malaria Eradication Agenda) launched its website to promote the participation of the community involved in the malaria control process

In May 2009 the Malaria Eradication Research Agenda (malERA), a consultative initiative aimed at identifying current knowledge gaps and new tools needed for malaria eradication, launched its Stakeholder Commons webpage (http://malera.tropika.net), aimed at gathering input and innovative ideas from scientists and implementers in order to define a malaria R&D agenda for the long-term goal of eradication.

MalERA Young Investigator's meeting, Boston October 8-9th, 2009.

MalERA is a natural continuation of the Global Malaria Action Plan launched in September 2008 by the Roll Back Malaria Initiative. It consisted of a consultative process that will culminate in the publication of a white paper proposing how current malaria R&D should change

with the goal of global malaria eradication. The agenda will be developed from the results of seven consultative groups who worked from mid-2008 until 2009, and it will focus on different disciplinary sectors of malaria control. It will also draw on input received through the website, which has been developed in collaboration with TropIKA.

The site includes meeting summaries and background documents for review. The malaria community was encouraged to visit the malERA web page, to participate in the process and to respond to key questions from the consultative

CRESIB and CISM researchers published the results of a clinical trial which demonstrates the long-term safety and efficacy of the malaria vaccine candidate RTS,S/ASO2A in Mozambican children

Jahit Sacarlal -in the middle- talking to two workers from the Centro de Investigação em Saúde de Manhiça (CISM).

In August 2009, almost coinciding with the launching of the Phase III trial with the candidate malaria vaccine RTS,S/ASO2A of GlaxoSmithKline Biologicals, the results of a study published in the Journal of Infectious Diseases provided new data on the duration of efficacy of this vaccine candidate in children.

Dr. Pedro Alonso, director of CRESIB, who leads the clinical development of this vaccine, and the first author of the study, Dr. Jahit Sacarlal, a researcher at the Centro de Investigação em Saúde de Manhiça (CISM), Mozambique, released the results of a clinical trial conducted in more than 2.000 Mozambican children between 1-4 years old. According to this study the vaccine remains safe and effective for a period of at least 45 months, reducing clinical malaria episodes overall by 30% and severe cases by 38%. These results reinforce the idea that, if the clinical development is successfully completed, this vaccine could be useful in the prevention of malaria, especially in African children.

Phase III malaria vaccine trial began, final testing of RTS,S

Eusebio Macete, CISM Director (Mozambique), talking to a group of Mozambican mother participants in the Phase III malaria vaccine clinical trial.

The Phase III trial of the most clinically advanced malaria vaccine candidate began on 26 May 2009 with inoculations administered at the Bagamoyo Research and Training Centre of the Ifakara Health Institute in Tanzania, one of the eleven trial sites participating in this multicentre study. In the following months, the trial was initiated in seven other countries across sub-Saharan Africa, including Mozambique, and aims at enrolling a total of 16,000 children and infants.

GlaxoSmithKline Biologicals' RTS,S is the first malaria vaccine to demonstrate promising safety and significant efficacy to warrant Phase III testing and in 2009 it is the leading candidate in the effort to

develop a malaria vaccine.

The full Phase III trial is designed to demonstrate how the vaccine performs in a large group of children and infants in different transmission settings across a wide geographic region. If the required regulatory clearances are granted and international, and African national public health authorities recommend its use, RTS,S could be introduced in 2012 as an antimalarial vaccine for children aged 5 to 17 months.

Intermittent Preventive Treatment in Infants (IPTi) reduced the incidence of clinical malaria in the first year of life by 30%

(L to R) Clara Menéndez, John Aponte and Andrea Egan, (CRESIB) main participants in the study, presented the results obtained during a press conference to celebrate this occasion.

The international IPTi Consortium, an initiative coordinated by CRESIB (HC-UB), is composed of more than 20 institutions in Africa, Europe and the United States, the WHO and UNICEF. IPTi (Intermittent Preventive Treatment in infants) is a new strategy of malaria control applied to infants regardless of whether they are infected or suffering from malaria. The researchers of the consortium, led by Dr. Clara Menendez, evaluated the strategy through a meta-analysis of 6 clinical trials conducted in four African countries between 1999 and 2008. The trials involved almost 8000 children who received the treatment at the time of routine vaccinations under the WHO's Expanded Programme on Immunization.

The results of this research, published by The Lancet on 17 September 2009, showed that IPTi, using the drug combination sulfadoxine-pyrimethamine, reduced clinical malaria by 30%, hospital admissions by 23% and anaemia by 21%. It has thus proven to be a safe, affordable and simple method recommended by the WHO that could prevent 6 million new cases of malaria each year in children under 1 year of age in Africa.

The Hospital Clínic de Barcelona and the Ibn Sina Hospital University Centre (CHU) of Rabat sign a collaboration agreement in Barcelona

In July 2009, the General Director of the Hospital Clínic de Barcelona, Dr. Raimon Belenes, and the General Director of the Ibn Sina CHU of Rabat, Professor Charif Chefchaouni Al Mountacer, signed this collaboration agreement at the Hospital Clínic de Barcelona. Its aim is to exchange management models and promote research projects in transmissible diseases in children under 5 years of age. This project promotes initiatives involving capacity building of healthcare professionals and interaction of working groups.

(L to R) Raimon Belenes (Hospital Clínic de Barcelona), Ghoulam Maichane (Moroccan Consulate), Charif Chefchaouni Al Mountacer (Ibn Sina CHU of Rabat), Angeles Matesanz (AECID), Francesc Cardellach (Universitat de Barcelona), Pedro L. Alonso (CRESIB).

This international agreement is a major step forward resulting from the efforts made in Morocco since 1999 by the Fundació Clínic per a la Recerca Biomèdica,

the Agencia Española de Cooperación Internacional para el Desarrollo (Spanish Agency for International Cooperation and Development, AECID) and the Ministry of Health of the Kingdom of Morocco, as well as by CRESIB (HC-UB).

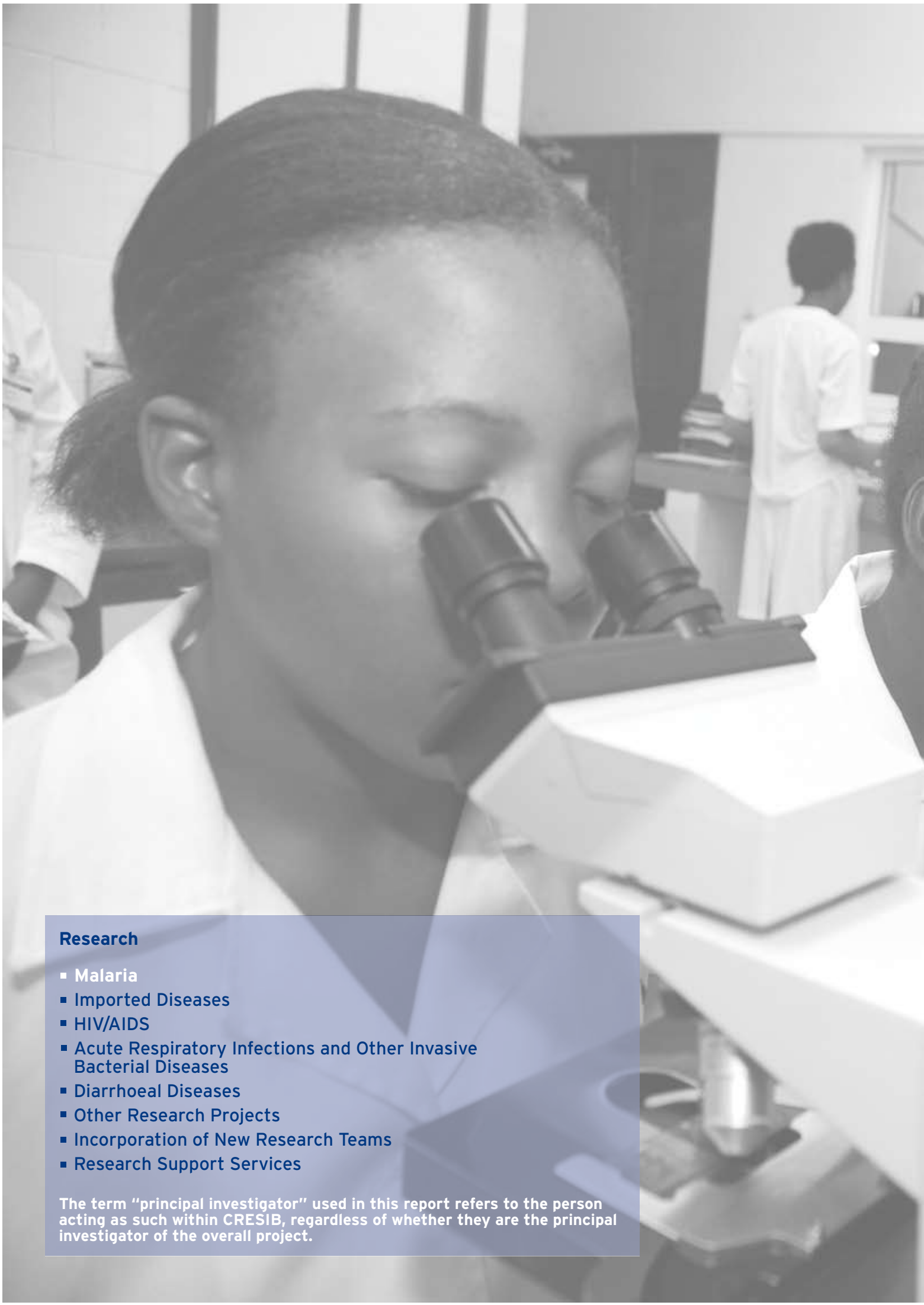
CRESIB will participate in the design and creation of a research laboratory in the Ibn Sina CHU and initially in the implementation of research protocols on epidemiology and aetiology of respiratory and diarrhoeal diseases in children under 5 years of age.

HIV 'prevention' gel PRO 2000 proven ineffective

Robert Pool (CRESIB), coordinator of the social science component of the study.

The largest international clinical trial to date on a preventative HIV gel was carried out between September 2005 and September 2009, with Dr. Robert Pool (anthropologist and Research Professor at CRESIB, HC-UB) as the coordinator of the social science component. The trial found no evidence that the vaginal microbicide PRO 2000 reduces the risk of HIV infection in women. This placebo-controlled trial involved 9,385 women at six research centres in four African countries, and found that the risk of HIV infection in women who were supplied with PRO 2000 gel was not significantly different from that in women supplied with placebo gel. However, although ineffective in providing protection, PRO 2000 gel itself was safe to use.

The trial participants were informed of the outcome, and the full results will be submitted for presentation at international conferences in 2010, as well as for publication in a peer-reviewed scientific journal.



Research

- **Malaria**
- Imported Diseases
- HIV/AIDS
- Acute Respiratory Infections and Other Invasive Bacterial Diseases
- Diarrhoeal Diseases
- Other Research Projects
- Incorporation of New Research Teams
- Research Support Services

The term "principal investigator" used in this report refers to the person acting as such within CRESIB, regardless of whether they are the principal investigator of the overall project.

Research

Malaria

Malaria

Malaria is a mosquito-borne infectious disease caused by parasites of the genus *Plasmodium*. Symptoms of malaria include fever, headache and vomiting, and usually appear between 10 and 15 days after the mosquito bite. Left untreated, malaria can quickly become life-threatening by disrupting the blood supply to vital organs.

According to the 2009 World Malaria Report produced by the World Health Organization (WHO), half of the world's population is at risk of malaria, and an estimated 243 million cases led to approximately 863,000 deaths in 2008. The vast majority of cases (85%) and deaths (89%) were in the African Region, affecting especially children under 5 years of age and pregnant women.

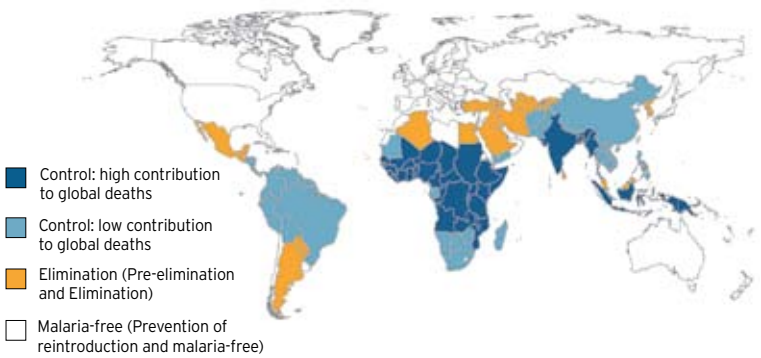
Progress has been made in the last few years thanks to increased implementation of key malaria control interventions, but research is needed along the continuum from innovation (basic research) over validation (clinical trials) to application for impact (effectiveness studies and policy) in order to improve the current tools and develop more effective ones.

Malaria has been one of the major research programs at CRESIB since its foundation. CRESIB's malaria research focuses on developing new enabling technologies to improve malaria research, antigen and drug discovery, pathophysiology of severe malaria and malaria in pregnancy, understanding of natural and acquired immunity to malaria, assessment of the safety, efficacy and effectiveness of malaria vaccines and drugs and description of the burden, clinical presentation and epidemiological features of malaria in different epidemiological settings. Research also includes socio-cultural aspects of malaria and the cost-effectiveness of malaria interventions.

In policy research, CRESIB is leading a consultation process with the malaria research and academic community to develop a multidisciplinary global R&D agenda that can be implemented by research and public health agencies and sponsors (the Malaria Eradication Research Agenda [MalEra]). In addition, CRESIB is leading a planning grant to establish an interdisciplinary *Plasmodium vivax* research consortium with the overall goal of defining a priority research agenda for *P. vivax* and developing a proposal for an interdisciplinary *P. vivax* research consortium (iVAX) that will foster the development of new tools targeting *P. vivax* by addressing the most important gaps in the knowledge of this parasite.

On the following pages all of 2009 CRESIB's ongoing malaria projects are described in order from the more basic research projects to the more clinical and policy-addressed ones. The major consortium programmes are described at the end of the chapter because in most cases they include a comprehensive approach within themselves.

Country categorization by malaria control status and burden



Source: World Malaria Report 2008, Geneva, World Health Organization, 2008; 2006 data.

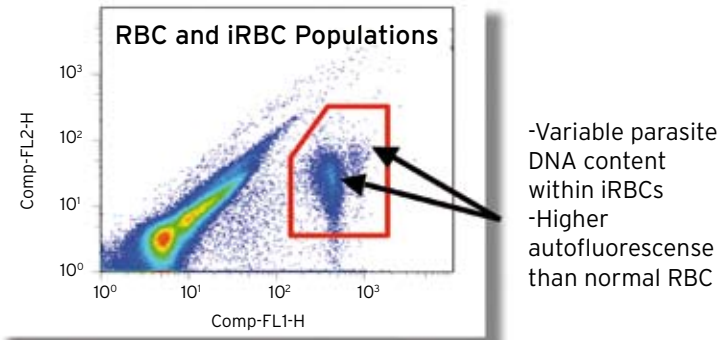
Comparison of two methods for quantifying the density of *Plasmodium falciparum* in human peripheral blood

The microscopic analysis of blood smears is currently the most commonly used method for determining parasitaemia and parasite density in studies of *Plasmodium falciparum*. This method is, however, subjective and labour-intensive, making it complicated to use in evaluating large-scale programmes.

Flow cytometry is a useful technique in high-performance analysis, but due to the limited specificity that is achievable with current 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 measuring peripheral blood parasitaemia in humans.

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

During 2009, the statistical analysis of the data was completed and the manuscript is in preparation. The study was presented at the XIII Jornadas de Saúde (Ministerio da Saúde) in Mozambique and at the 5th MIM Pan-African Malaria Conference in Nairobi, Kenya in November 2009.



Establishing the pattern of *P. falciparum*-infected erythrocytes by flow cytometry

Principal investigator:
Joseph J. Campo

Co-principal investigators:
Carlota Dobaño, John Aponte

Co-investigators:
Augusto Nhabomba, Jahit Sacarlal, Pedro L. Alonso

In collaboration with:
Iñigo Angulo Barturen. GlaxoSmithKline, Infectious Diseases CEDD, Diseases of the Developing World, Tres Cantos, Madrid (Spain).

Funder:
The PATH Malaria Vaccine Initiative (MVI), Bethesda (USA).

Duration of the project:
2008-2009

1.1 Malaria Enabling Technologies

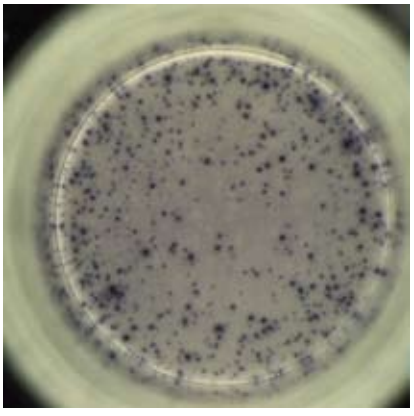
OptiMalVac: Initiative on optimizing malaria vaccine laboratory assay evaluation

A broad range of candidate malaria vaccines derived from several novel technologies have resulted from the multiple approaches being taken by different groups in developing malaria vaccines. The majority of the candidates are recombinant proteins based on complex native antigens found on the surface of the parasite. The vaccine potential of these parasite surface antigens is often supported by epidemiological data, and by the ability to induce measurable antigen-specific antibodies or potential protective responses in animals and later, in humans. *In vivo* assays, such as protection models in mice or non-human primates as well as human sporozoite challenge, provide additional data for some relevant antigens.

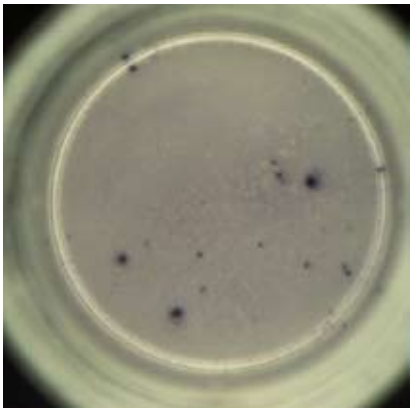
Individual groups have developed assays within the context of the vaccine discovery efforts, with identification of measurable processes for parasite growth and virulence to test specific antigens. In-house assays are strain-, stage- and even process-specific and the ability to compare results between different candidates is further limited by diverse methodologies and assay components such as parasites, cells and reagents. The absence of any level of standardization and harmonization of practices also makes it complex to interpret the meaning and relevance of vaccine research outcomes.

To enable a comparison of different candidate vaccines and approaches in a credible and informed manner, efforts must be made to create an enabling environment by supporting the development of a baseline level of standardization around key assays that can be utilized in the development and evaluation of malaria vaccines. Consistent, reproducible and comparable intra- and inter-lab performance and increased accuracy and precision of assay data will strengthen the quality of the data on vaccine performance and generate greater confidence in the vaccine potential of the candidate.

Representative examples of cytokine responses in human leukocytes after antigen stimulation, by the ELIspot technique that is standardized in the OptiMalVac Consortium.



C10: high immune responder to the *in vitro* stimulation



A3: low immune responder

B8: no responder

The overall goal of this project is to develop harmonized assays to facilitate comparison of results and improve decision making in the following areas:

- Discovery, concept development and rationale
- Product characterization
- Down-selection between candidates and/or formulation
- Clinical development plans

Working groups have been established to address the optimization and harmonization of humoral, functional and T-cell assays in close coordination with existing efforts worldwide. The first consortium meeting was held at the World Health Organization headquarters in Geneva in April 2009.

Principal investigator:
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Co-investigators:
Tamara Berthoud, Gemma Moncunill, Pedro L. Alonso

Laboratory technician:
Pau Cisteró

In collaboration with:
- Odile Leroy. European Vaccine Initiative (EVI)/Statens Serum Institut, Copenhagen (Denmark).
- Patrice Dubois. ImmunoVac Consulting, Brussels (Belgium).
- David Cavanagh. University of Edinburgh, Edinburgh (Scotland)
- Adrian Hill. University of Oxford, Oxford (UK)
- Adrian Luty. Radboud University Nijmegen, Nijmegen (the Netherlands).
- Vasee Moorthy. World Health Organization, Geneva (Switzerland).
- Alan Thomas. Biomedical Primate Research Centre, Rijswijk (the Netherlands).
- Klavs Berzins and Marita Troye-Blomberg. Stockholm University, Stockholm (Sweden).
- Pierre Druilhe. Institut Pasteur UPBM, Paris (France).
- Barry Walker. National Institute for Biological Standards and Control, South Mimms (UK).
- Ya Ping Shi. Centre for Diseases Control (CDC), Atlanta (USA).
- Emily Locke. PATH Malaria Vaccine Initiative (MVI), Bethesda (USA).

Funder:
FP7 Programme, European Union (Coordination Action).

Duration of the project:
2009–2012

1.2 Malaria Pathophysiology

SEVMAL: Severe malaria in children from Manhiça. Role of *Plasmodium falciparum* adhesion and immune responses to the parasite

Severe malaria has been attributed partly to the sequestration of *Plasmodium falciparum*–infected erythrocytes (IEs) in the microvasculature of vital host organs. Defining the cytoadherence phenotypes of IEs that are predictive of severe malaria may lead to the development of novel strategies against life-threatening malaria.

Ninety-two *P. falciparum* isolates from Mozambican children under 5 years of age with severe malaria (cases) and sex/age-matched uncomplicated

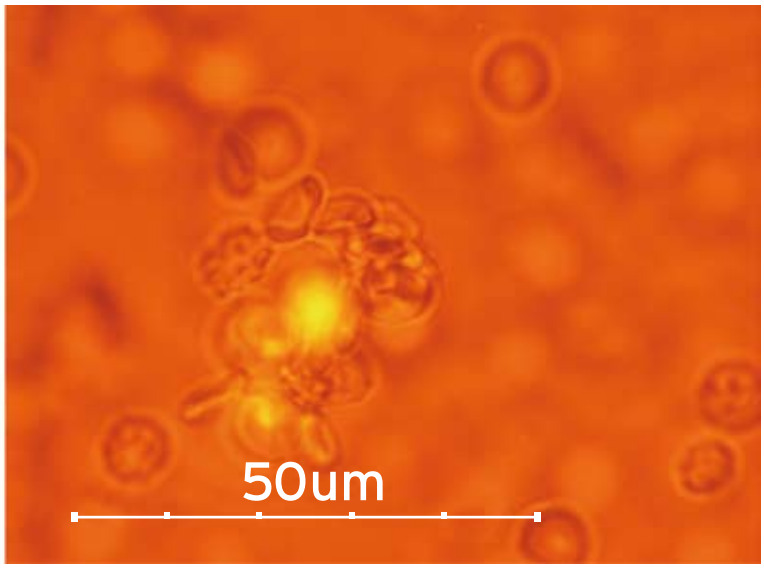
1.2 Malaria Pathophysiology

malaria(controls) were studied. Adhesion to purified receptors (CD36, ICAM1 and gC1qR), rosetting and platelet-mediated clumping were compared between matched pairs by non-parametric tests.

The most common syndrome associated with severe malaria was prostration. Compared with matched controls, prevalence of platelet-mediated clumping was higher in cases (P = .019), in children presenting prostration (P = .049) and in children presenting severe anaemia (P = .025). Prevalence of adhesion to gC1qR was also higher in isolates from cases with multiple seizures than in their matched controls (P = .025).

Our data indicate a role for platelet-mediated clumping and adhesion to gC1qR in the pathogenesis of severe malaria. Inhibition of platelet-mediated clumping and adhesion to gC1qR could improve severe malaria outcomes.

Currently, we are completing the analysis of immune responses in children with severe malaria, as compared to those in children with uncomplicated malaria. These results will provide information on differential immune mechanisms involved in the severity of infection. Part of these data was presented at the 5th MIM Pan-African Malaria Conference in Nairobi, Kenya in November 2009.



Agglutinate of infected erythrocytes in a clinical sample from Manhiça.

Principal investigator:
Alfredo Mayor

Co-principal investigator:
Pedro L. Alonso

Co-investigators:
Eduard Rovira, Quique Bassat, Inácio Mandomando, Betúel Sigaúque, Pedro Aide, Carlota Dobaño, Ruth Aguilar, Clara Menéndez

Laboratory technician:
Pau Cisteró

In collaboration with:
- Chetan Chitnis. International Centre for Genetic Engineering and Biotechnology, New Delhi (India).

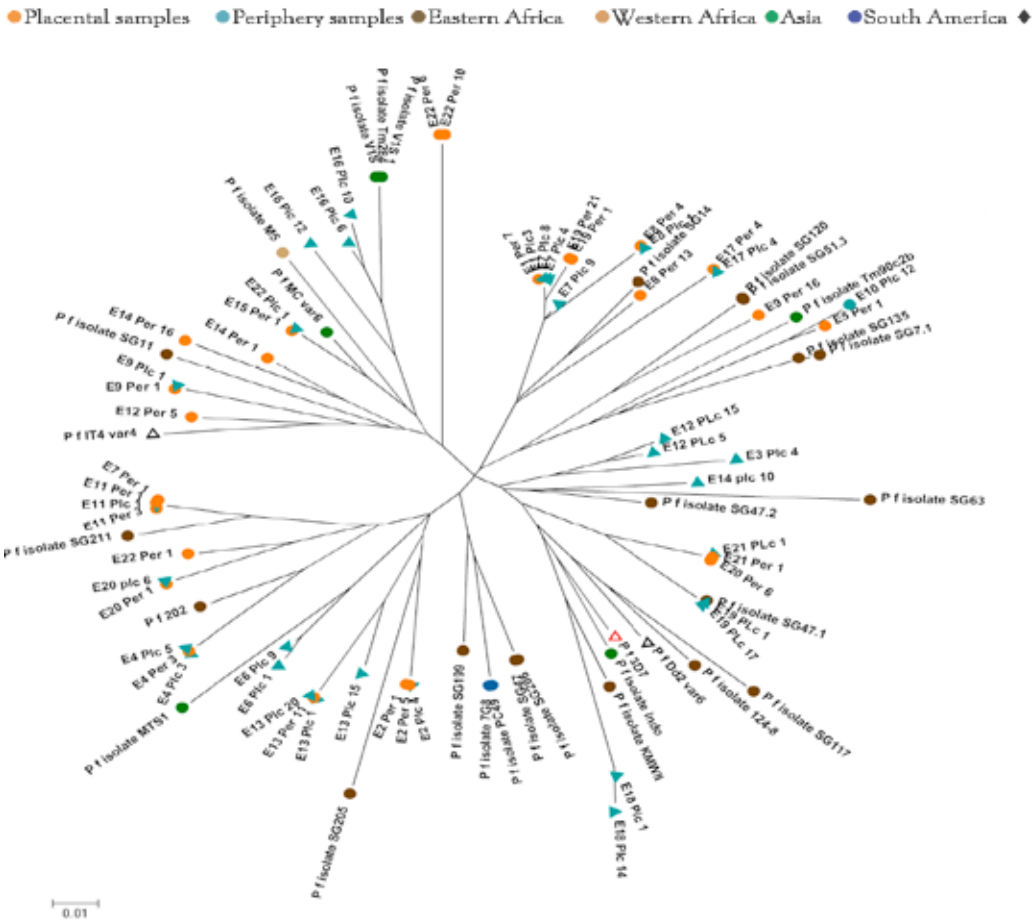
Funder:
Fondo de Investigación Sanitaria (FIS), Instituto de Salud Carlos III (ISCIII), Madrid (Spain).

Duration of the project:
2007-2010

Publications:
- Mayor A, Rovira-Vallbona E, Srivastava A, Sharma SK, Pati SS, Puyol L, Quinto L, Bassat Q, Machevo S, Mandomando I, Chauhan VS, Alonso PL, Chitnis CE. Functional and immunological characterization of a Duffy binding-like alpha domain from *Plasmodium falciparum* erythrocyte membrane protein 1 that mediates rosetting. **Infect Immun.** 2009 Sep;77(9):3857-63.

PREGMAL: Characterization of the *Plasmodium falciparum* ligand involved in adhesion to the placenta and its role in the development of immunity to malaria during pregnancy

Women are at higher risk of *Plasmodium falciparum* infection and disease when pregnant. There is growing evidence that malaria susceptibility in primigravidae could be largely explained by the lack of antibodies that can block adhesion of infected erythrocytes to placental chondroitin sulphate A (CSA). The CSA adhesion phenotype is specific to placental parasites and has been linked to a unique *var* gene (*var2csa*). Immunity to CSA-binding parasites is gender-specific (i.e., men exposed to malaria lack these antibodies) and parity-dependent (i.e., antibodies increase during successive pregnancies), and has been associated with lower risk of placental parasitaemia, maternal anaemia and low birth weight.



Phylogenetic analysis of DBL3X sequences from var2CSA in Manhiça parasites, compared to parasites for other geographic areas.

1.2 Malaria Pathophysiology

In the light of these experimental findings, it has been suggested that *var2csa* may constitute an attractive target for vaccination against malaria in pregnancy. However, a similar parity-dependent profile has been found for antibodies against *P. falciparum* antigens not specifically associated with pregnancy.

The aim of this project is to establish an area dedicated to research on the molecular mechanisms involved in *P. falciparum* adhesion to placenta and on the development of protective immunity against its adverse effects. We examined the effect of parity on maternal antibody responses against *P. falciparum* isolates collected from 15 Mozambican pregnant women and 26 non-pregnant hosts. IgGs against the surface of *P. falciparum*-infected erythrocytes and merozoite recombinant antigens were quantified in plasmas from women at delivery, men and children. Maternal isolates, but not isolates from non-pregnant hosts, were found to transcribe *var2csa*. Placental infection increased recognition by pregnant women of both maternal and non-maternal isolates. Primigravidae without placental infection recognized fewer maternal and non-maternal isolates and had lower levels of IgG against merozoite antigens than multigravidae without infection. They also recognized fewer maternal and non-maternal isolates than men.

These results show that placental infection and parity can modulate maternal immunity against *P. falciparum*. We hypothesize that poor antibody responses in primigravidae might contribute to their increased susceptibility to malaria. Finally, we are also determining the level of genetic and antigenic conservation of *var2csa* and characterizing the humoral response naturally developed by pregnant women in a malaria-endemic area against different domains of *var2csa* and other merozoite antigens. These data were presented at the 5th MIM Pan-African Malaria Conference in Nairobi, Kenya in November 2009.

Principal investigator:
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Co-principal investigator:
Clara Menéndez

Co-investigators:
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Laboratory technicians:
Pau Cisteró, Alfons Jiménez

In collaboration with:
- Chetan Chitnis. International Centre for Genetic Engineering and Biotechnology, New Delhi (India).

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

Duration of the project:
2007-2010

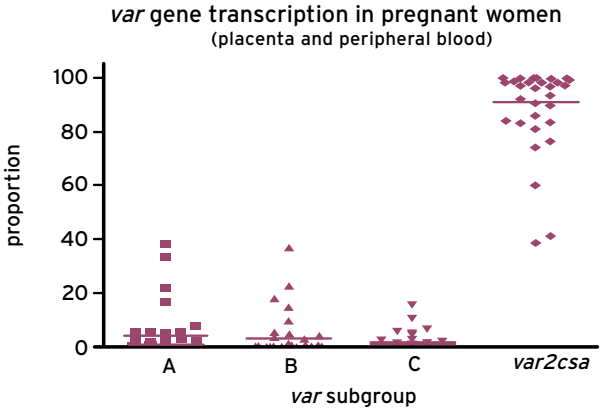
EPIC: Physiopathological mechanisms involved in placental malaria infections and their impact on foetal development

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.

In 2009 the work mainly focused on two sub-studies:

- ***Var* gene transcription in *Plasmodium falciparum* parasites infecting pregnant women**

Malaria in pregnancy (MiP) is characterized by the accumulation of *Plasmodium falciparum* parasites in the placenta, which is thought to contribute to adverse clinical outcomes in the mother and newborn. VAR2CSA, the parasite ligand mediating placental sequestration through binding to chondroitin sulphate A (CSA), may constitute an attractive target for vaccination against MiP. However, further studies are needed to understand the physio-pathological mechanisms of MiP.



Var gene transcript proportions in isolates from Mozambican pregnant women, by real time PCR. The sum of A,B, C and *var2csa* transcripts was assumed to represent the total *var* gene amount in each sample.

To assess the specificity and uniqueness of *var2csa* transcription in pregnancy, *var* gene transcription patterns were measured by real-time polymerase chain reaction in *P. falciparum* parasites infecting 25 pregnant women and 40 non-pregnant controls from Mozambique. Isolates from pregnant women transcribed *var2csa* at higher levels than those in non-pregnant individuals (p=0,0001), although *var2csa* was detected at low levels in 39/40 isolates (98%) from non-pregnant donors. Presence of other *var* gene subgroups in pregnant women was detected in 15/19 placental isolates (78%) and in all peripheral samples. The proportion of A transcripts was significantly higher in children (71% of total *var* genes) than in adults (38%; *P*=0.0001), while B genes were more common in adults (42%) than in children (19%; *P*=0.0002). Whether the presence of parasites transcribing non-*var2csa var* transcripts plays an important role in placental infection and development of immunity during pregnancy needs to be further explored.

To quantify the degree of conservation of expressed *var2csa* variants, the CSA-binding DBL2 and DBL3 domains of *var2csa* were sequenced from 22 placental and 21 peripheral isolates of pregnant women. 388 different sequences for DBL2 and 456 for DBL3 were obtained. Nucleotide diversity was 7.3% for DBL2 and 6.5% for DBL3. The previously reported DBL2-CSA-binding region was more polymorphic than other regions in DBL2. Phylogenetic analysis suggested overlap in *var2csa* sequences between Mozambican and worldwide isolates.

1.2 Malaria Pathophysiology

• Cellular-mediated immune mechanisms involved in placental malaria infection and its impact on foetal outcome

The aim of this research is to evaluate the relationship between immune cell populations and immuno-endocrine mediators and adverse outcomes of MiP. A phenotypic analysis of immune cells from peripheral, placental and cord blood from Mozambican women (50 with active malaria infection in the placenta, 72 with past infection, 50 with no infection and 17 controls) was completed. Cell surface markers included CD3, CD4, CD8, CD20, CD16, CD56, CD57, CD94, CD14 and CD45. Levels of hormones (chorionic gonadotropin, prolactin, 17β-estradiol, progesterone, cortisol) and cytokines (IFNγ, TNFα, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12) were measured in placental plasma.

Principal investigators:
Jaume Ordi, Clara Menéndez

Co-principal investigators:
Alfredo Mayor, Carlota Dobaño

Co-investigators:
Tamara Berthoud, Gemma Moncunill, Cleofé Romagosa, Azucena Bardají, Eusébio Macete, Elisa Serra, Eduard Rovira, Isadora Monteiro

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

Duration of the project:
2003-2010

1.3 Malaria Immunology

Malaria Immunopathology Programme

In 2009 this programme mainly focused on the development of immune assays to measure antibody responses to *Plasmodium falciparum*. The assays under development have the following characteristics:

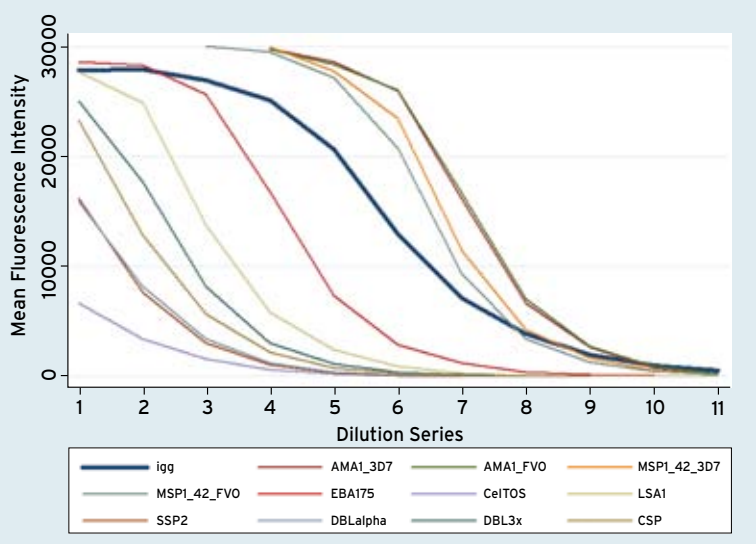
- Multiplex: a wide panel of proteins, including different genotypes, are coupled to Bio-Plex beads to analyse simultaneously the levels of antibodies to multiple parasite antigens (PfMSP-1$_{19}$, PfAMA-1, PfEBA-175, PfMSP-1$_{42}$-3D7, PfMSP-1$_{42}$-FVO) obtained from collaborators in India and USA, using the Luminex platform.
- Miniaturized: due to the low amounts of blood obtained from children, assays that use a small volume of serum or plasma are prioritized.
- High-throughput: due to the large number of samples analysed in our immuno-epidemiological studies and vaccine trials, these techniques favour the processing of many samples in a short time.
- Functional assays: we are establishing techniques that can assess the functional capacity of the antibodies, like the growth inhibition assay (GIA).

We have compared the performance of GIA protocols obtained through collaborations and publications that use different procedures for quantifying the growth of *P. falciparum* in vitro (spectrophotometry with the enzyme pLDH [NIH, US Army], fluorescense-activated cell sorting (FACS) with hydroethidine staining [US Army], and FACS with fluorescent parasites D10-GFP [Melbourne]), with the objective of choosing the best method to be applied in the other studies in the area of malaria immunology.

In addition, we are developing cellular immune assays to understand the biology of B cells in malaria-endemic areas, as B cells are responsible for producing the antibodies. Specifically, we aim to:

- Describe the induction and longevity of the specific memory B cell (MBC) response.
- Explore the extent to which vaccination might "boost" blood stage-specific MBC.
- Explore the phenotype of B-cell populations in vaccinated and control-vaccinated individuals.

Peripheral blood mononuclear cells have been collected in children participating in the phase III vaccine trial of RTS,S/AS01E in Mozambique in order to assess the B-cell responses.



Development of a multiplex panel of *P. falciparum* antigens using a standard protocol for absolute quantification of IgG antibody (mcg/mL) in serum or plasma

Principal investigator:
Pedro L. Alonso

Co-investigators:
Carlota Dobaño, Joseph J. Campo, Gemma Moncunill

Laboratory technician:
Pau Cisteró

Funder:
Fundación Ramón Areces, Madrid (Spain).

Duration of the project:
2008-2012

1.3 Malaria Immunology

AgeMal: Exposure to *Plasmodium falciparum* and development of immunity against malaria in children under one year

The overall objective of this project was to evaluate the effect of exposure to *Plasmodium falciparum* erythrocytic stage antigens during different periods of infancy on the development of naturally acquired immunity (NAI). In order to explore the effect of age in the build-up of NAI, a three-arm randomized double-blind placebo-controlled trial was conducted in an endemic area of southern Mozambique in which we selectively controlled exposure to *P. falciparum* at different periods during infancy (2.5–5.5 months, 5.5–10.5 months or none) with monthly chemoprophylaxis with sulphadoxine-pyrimethamine+artesunate.

Infants were enrolled at birth or when aged less than 2 months from HIV-negative women and allocated to one of three cohorts of 98 children each. Participants were followed up by active and passive case detection until 11 months of age and by passive case detection from 11 to 24 months. Five cross-sectional surveys were conducted to obtain blood samples.

We compared the risk of clinical malaria and anaemia during the second year of life between cohorts and studied its correlation with the type and quality of immune responses (antibodies to several *P. falciparum* antigens, cytokines), oxidative stress markers and host genetic factors.

The statistical analyses of the field study were completed in 2009. No significant differences were found in the risk of clinical malaria during the second year of life in children who had been exposed to malaria for the first time at different ages during the first year. This suggested that exposure to *P. falciparum* between 0 and 5.5 months of age contributes to NAI, indicating that malaria vaccines administered very early

AgeMal working group

in life would have an effect on the infant immune system. This is consistent with recent evidence of significant vaccine efficacy of a malaria vaccine candidate given to infants through the Expanded Programme on Immunization scheme.

The statistical analysis of **maternal immune responses** was completed in 2009. Antibody responses to PfMSP-1$_{19}$, PfAMA-1, PfF2-EBA-175, R29varR+-PfDBLα and parasite lysate (crude hemozoin extracted using digitonin) were checked by enzyme-linked immunosorbent assay. IgG to variant surface antigens (VSA) using CS2-CSA laboratory strain, two placental isolates and three isolates from children were measured by fluorescense-activated cell sorting in 299 peripheral plasmas and 247 cord plasmas. Cytokine concentrations (IL-12p70, IFNγ, IL-2, IL-10, IL-8, IL-6, IL-4, IL-5, IL-1β, TNF, TNFβ) after 24 h stimulation with schizont lysate were determined in culture supernatants of 251 peripheral blood mononuclear cells (PBMCs) and 213 cord blood mononuclear cells (CBMCs) using the Bender MedSystems Multiplex kit; cytokine messenger RNA levels (IL-2, IL-4, IL-6, IL-10, IFN-γ TNF, IL-13, normalized to the housekeeping gene RPL13a) were measured in a subsample by real-time quantitative polymerase chain reaction (RTqPCR). Parasite infection was assessed in placentas by histology and in peripheral and cord blood by microscopy and/or RTqPCR. Preliminary results are:

- Maternal infection boosted maternal antibodies, transplacental IgGs, foetal IgMs and cytokines (IL-5 and IL-8).
- Levels of maternal IgGs, transplacental IgGs and maternal cytokines (IL-12, IL-2) increased with parity in pregnant women.
- Low birth weight tended to be associated with high maternal IgG/IgM and production of cytokines (IL-12, IL-8, TNF) by PBMCs.
- Anaemia was associated with high maternal IgMs and cord cytokines (IL-12, 10, 8 and 4).
- In utero exposure depended on maternal and placental infection and had a negative effect on the child's immunity.

The statistical analysis of **immune responses in children** will be completed in 2010, but preliminary analyses of antibodies indicate that:

- There was an important contribution of passively transferred maternal IgG antibodies recognizing *P. falciparum* antigens in early infancy that declined during the first year of life.
- IgG and IgM antibodies were acquired with age and exposure to parasite infection.
- IgG subclasses recognizing parasite extract, MSP-1$_{19}$, AMA-1 and EBA-175 were of the cytophylic isotypes IgG1 and IgG3.
- IgG responses to VSA were very low during the first year of life.
- IgG increased significantly between acute episode and convalescence, whereas IgM levels were higher in acute episode than convalescence.

Preliminary analyses of cellular immune responses indicate that cytokine responses to *P. falciparum* lysate (measured as messenger RNA in PBMC pellets or protein concentrations in culture supernatants) appeared to increase with age during the first year of life, and were higher during acute clinical episodes, declining at convalescence.

The statistical analysis of **oxidative stress**

1.3 Malaria Immunology

in children will also be completed in 2010, but preliminary analyses indicate that:

- Hydroxynonenal (HNE) adducts on the erythrocyte surface but not reduced glutathione (GSH) (markers of oxidative stress damage) increased in the acute and convalescent states compared with non-infected.
- Oxidized low-density lipoprotein (LDL) increased with age, although with strong individual heterogeneity.
- Extracellular plasma thiols seemed to correlate negatively with HNE.
- There was no correlation between HNE-adducts, GSH or plasma oxidized LDL.

As markers of anti-oxidative stress response, there was an increase in the anti-oxidant enzymes glutathione peroxidase and glutathione reductase in the acute and convalescent compared with the non-infected samples.

The statistical analysis of the **genetic factors of the host** will be completed in 2010, but a number of single-nucleotide polymorphisms (SNPs) were identified in preliminary analyses in candidate genes IL-4, IL-13, IFN-γ and CD14 that appear to be associated with susceptibility to clinical malaria. The genetic markers examined were 9 TH1/TH2 genes, 19 inflammatory and immune response genes, 9 oxidative stress genes, and 4 haematological genes (a total of 41 genotypes). The automated Qiagen M48 chemistry was adopted to isolate human DNA from the PBMC samples and the Sequenom MassARRAY system iPlex gold was used for SNP genotyping of the samples.

Principal investigator:
Pedro L. Alonso

Co-principal investigator:
Carlota Dobaño

Co-investigators:
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Laboratory technicians:
Alfons Jiménez, Pau Cisteró

Project manager:
Patricia García

In collaboration with:
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- Evelin Schwarzer. Università di Torino, Torino (Italy).
- Peter Le Souef. University of Western Australia, Perth (Australia).
- Louis Schofield. Walter & Eliza Hall Institute of Medical Research, Melbourne (Australia).
- Chetan Chitnis. International Centre for Genetic Engineering and Biotechnology, New Delhi (India).
- Denise Doolan. Queensland Institute of Medical Research, Brisbane (Australia).

Funders:
FP6 programme, European Union.
Instituto de Salud Carlos III (ISCIII), Madrid (Spain).
Ministerio de Ciencia e Innovación, Madrid (Spain).

Duration of the project:
2005-2010

CYTOMAL: Study of immunity and susceptibility markers to malaria in individuals exposed to *Plasmodium falciparum* infection

The aim of this research is to identify cellular immune responses against *Plasmodium falciparum* which could be used as markers of immunity and/or susceptibility to malaria in individuals naturally exposed to the infection. The specific objectives are:

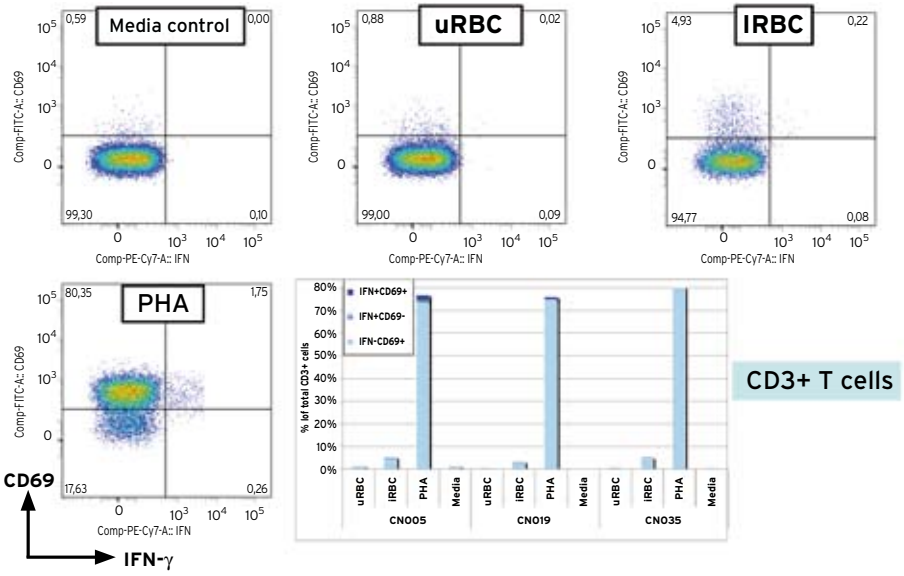
1) To characterize immunopathological markers of severe malaria in children.

2) To characterize markers of clinical immunity in children and adults.

3) To characterize immunopathological markers of placental malaria in pregnant women.

The main activity has been the establishment of a number of cellular immunology techniques to measure cytokine responses in humans.

- Measurement of the concentration of TH1 and TH2 cytokines in culture supernatants after in vitro stimulation with *P. falciparum*, including schizont lysates, live parasites, positive (phytohaemagglutinin mitogen) and negative controls. Multiplex cytokine profiling systems are useful tools for investigating correlates of protective immunity. Several Luminex and flow cytometry methods are commercially available but there is limited information on the relative performance of different kits. Microsphere suspension array technologies tested differed in sensitivity and reproducibility and in the sample volume, the number of cytokines measured, and the time and cost of the assays. Absolute values of cytokine detected with different methods could not be compared. In our system, the flow cytometry methods



Measurement of intracellular IFN-γ in activated T cells after stimulation with *P. falciparum* antigens and controls by flow cytometry

1.3 Malaria Immunology

tested appeared to perform better than the Luminex methods, and were chosen for subsequent studies.

- Measurement of cytokines (mainly IFN-γ) secreted by leucocytes after stimulation with *P. falciparum* antigens by *ex vivo* ELISpot.

- Measurement of intracellular cytokines after stimulation with *P. falciparum* antigens by flow cytometry. We have established two 6-color panels and one 8-color panel to measure IFN-γ and IL-2 production in CD4 and CD8 cells, γδ T cells and natural killer cells.

A second activity was the measurement of cytokine responses (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IFN-γ, TNF, TNFβ) in different groups of malaria patients and controls. The patterns of cytokine responses are being analysed in relation to these variables: age (children vs. adult), exposure (*naive* vs. first vs. few vs. chronic), disease status (asymptomatic vs. mild vs. severe), infection status (infected vs. non-infected), ethnicity (African vs. European), pregnancy status and sex.

Principal investigator:
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Co-investigators:
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Laboratory technician:
Alfons Jiménez

Funder:
Plan Nacional de I+D, Ministerio de Ciencia e Innovación, Madrid (Spain).

Duration of the project:
2008-2011

ExtMal039: Study of the asexual blood-stage immunity markers associated with prolonged protection in children vaccinated with RTS,S/AS02A

A previous phase IIb efficacy trial of the RTS,S malaria vaccine candidate formulated in the AS02A adjuvant (RTS,S/AS02A) in children in Manhiça, Mozambique, showed significant and sustained protective efficacy in vaccinated children up to 45 months after first vaccination (Sacarlal *et al.*, 2009). Antibody titres to the pre-erythrocytic stage vaccine antigen declined rapidly and showed no correlation with protection against clinical malaria, although IgG levels remained higher in the vaccine group. To date, mechanisms of protracted protection of RTS,S/AS02A remain unclear.

This study aims to test the hypothesis that RTS,S vaccination leads to a partially protective vaccine-induced pre-erythrocytic response that limits sporozoite development to the blood stage, resulting in prolonged exposure to low-dose asexual blood-stage parasites and enhancement of long-lasting blood-stage immunity (Guinovart *et al.*, 2009).

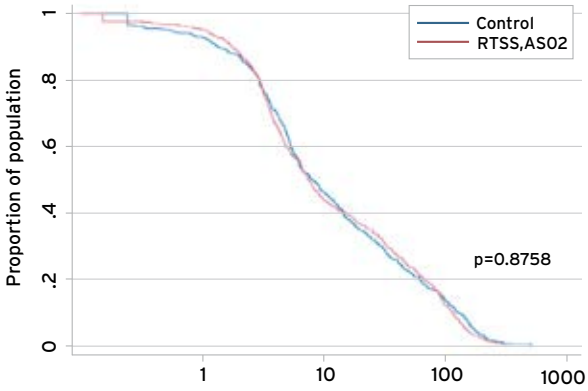
Thus, five years after vaccination, a cross-sectional survey of both vaccine cohorts was conducted in which parasitaemia was measured by microscopy and blood was collected from the participants

to investigate the blood-stage immunogenicity. We found no significant differences in the prevalence of infection between vaccine and control groups at 60 months after first immunization, indicating that RTS,S/AS02A protection lasted for four years in these Mozambican children.

We then assessed antibody-based immune response to asexual erythrocytic antigens in a randomized subsample of RTS,S/AS02A-vaccinated and control children from both study cohorts at study month 8.5. Specifically, in 2009 we measured:

- Level of IgG antibodies against a panel of blood-stage antigens by multiplexed bead assay (luminex) in 645 children. Antigens that were included in the panel as markers of blood-stage immunity were PfMSP-1$_{42}$ (3D7 and FVO types), PfAMA-1 (3D7 and FVO types) and PfEBA175.
- Level of IgG antibodies against variant surface antigens (VSA) expressed on R29 *P. falciparum* parasites in 869 children by flow cytometry.

After preliminary univariate crude analyses, we found no difference in antibody responses between vaccinated and control children six months post-vaccination with these blood-stage immune markers and thus no support for the hypothesis. More comprehensive and multivariate analyses, as well as assessment at further time points, are needed to determine the effect of RTS,S vaccination on blood-stage immune acquisition. These data were presented at the 5th MIM Pan-African Malaria Conference in Nairobi, Kenya in November 2009.



Levels of IgG against *P. falciparum* variant surface antigens represented as reverse cumulative distribution of MFI values measured by flow cytometry. No significant differences in antibody responses were found between vaccinated and control children (N=869).

Principal investigators:
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Co-principal investigators:
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Co-investigators:
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In collaboration with:
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Funder:
The PATH Malaria Vaccine Initiative (MVI), Bethesda (USA).

Duration of the project:
2008-2010

Publications:

- Sacarlal J, Aide P, Aponte JJ, Renom M, Leach A, Mandomando I, Lievens M, Bassat Q, Lafuente S, Macete E, Vekemans J, Guinovart C, Sigaúque B, Sillman M, Milman J, Dubois MC, Demoitié MA, Thonnard J, Menéndez C, Ballou WR, Cohen J, Alonso PL. Long-term safety and efficacy of the RTS,S/AS02A malaria vaccine in Mozambican children. **J Infect Dis**. 2009 Aug 1;200(3):329-36.
- Guinovart C, Aponte JJ, Sacarlal J, Aide P, Leach A, Bassat Q, Macete E, Dobaño C, Lievens M, Loucq C, Ballou WR, Cohen J, Alonso PL. Insights into long-lasting protection induced by RTS,S/AS02A malaria vaccine: further results from a phase IIb trial in Mozambican children. **PLoS ONE.** 2009;4(4):e5165.

1.3 Malaria Immunology

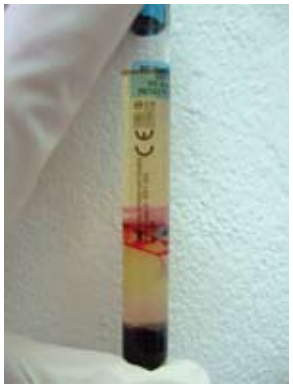
Mal055-Immuno: Study of immune correlates of protection against malaria after vaccination with RTS,S/AS01E. A comprehensive immunological arm of a phase III double-blind, randomized, controlled multi-centre trial

As the defining study for RTS,S vaccine formulated in the AS01E adjuvant (RTS,S/AS01E) licensure, and probably the last trial in which there will be an unvaccinated control group, the phase III trial Mal055 offers the best opportunity to understand the mechanisms of vaccine action and immune correlates of vaccine-induced protection. We propose to conduct a single multi-centre co-operative study—ancillary to the phase III double-blind, randomized, controlled trial—which presents the required expertise, rigour and power to investigate the immunological basis of RTS,S-induced immunity. Our proposal is to go beyond the current measurement of the vaccine-induced antibody response, that of acquired anti-circumsporozoite protein antibody titres, and to include assessment of the isotype (subclasses), quality (affinity/avidity) and functionality (invasion/development inhibition/sporozoite migration) of the IgG antibody responses against pre-erythrocytic antigens. In addition, the aim is to measure cellular immune responses (B and T cells) induced after vaccination with RTS,S/AS01E in a subset of study children at the screening and at cross-sectional visits. Finally, we propose to measure the induction of antibody and cellular immune responses against the blood stage of *Plasmodium falciparum* to further investigate potential mechanisms of RTS,S-induced long-term protection.

The specific objectives of the study are:

1) To describe induction of antibody and cellular immune responses against pre-

Automatic count of peripheral blood mononuclear cells isolated from children participating in the RTS,S phase III vaccine trial that will be used to assess cytokine responses after vaccination.



Separation of blood into erythrocytes, leukocytes and plasma phases after centrifugation using commercially available vacutainers pre-filled with a density gradient reagent. Venous blood was obtained from children participating inthe RTS,S phase III clinical trial to evaluate immune responses to the malaria candidate vaccine.

erythrocytic and asexual erythrocytic stage *P. falciparum* antigens after vaccination with RTS,S/AS01E.

2) To compare antibody and cellular immune responses induced by RTS,S/AS01E between age cohorts (infants 6-12 weeks of age vs. children 5-17 months of age).

3) To assess the effect on antibody and cellular immune responses of a fourth "booster" dose of RTS,S/AS01E vaccine.

4) To compare antibody and cellular immune responses induced by RTS,S/AS01E between geographical areas of different malaria transmission intensities.

5) To describe immune correlates associated with vaccine and naturally acquired protection.

The immunology study is being conducted at 8 of the 11 sites participating in the RTS,S/AS01E vaccine trial and includes two age cohorts (infants 6-12 weeks of age and children 5-17 months of age).

During 2009, in Manhiça blood samples were collected, processed and stored at screening and one month after the third vaccination from a subsample of around 300 children for future immunology studies.

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Project manager:
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In collaboration with:
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- Claudia Daubenberger. Swiss Tropical Institute, Basel (Switzerland).
- Maximillian Mpina. Ifakara Health Institute, Research and Training Centre Bagamoyo (Tanzania).
- Eleanor Riley. London School of Hygiene and Tropical Medicine, London (UK).
- Mwanaidi Kafuye. Joint Malaria Programme, Korogwe (Tanzania).
- Maxime Agnandji. Medical Research Unit, Albert Schweitzer Hospital, Lambaréné (Gabon).
- Ben A. Gyan and David Dosoo. Kintampo Health Research Centre, Ghana/Noguchi Memorial Institute for Medical Research, Kintampo (Ghana).
- Hermann Sorgho. Institut de Recherche en Sciences de la Santé (IRSS)/Centre Muraz, Nanoro (Burkina Faso).
- Simon Kariuki. Kenya Medical Research Institute (KEMRI/CDC), Kisumu (Kenya).
- John Waitumbi. Kenya Medical Research Institute (KEMRI)/Walter Reed Project, Kisumu (Kenya).
- GlaxoSmithKline Biologicals, Rixensart (Belgium).

Funder:
The PATH Malaria Vaccine Initiative (MVI), Bethesda (USA).

Duration of the project:
2009–2013

Publications:

- Dobaño C, Campo JJ. Understanding protective immune mechanisms induced by malaria vaccines in the context of clinical trials. **Hum Vaccin**. 2009 Aug;5(8):562-5.

1.3 Malaria Immunology

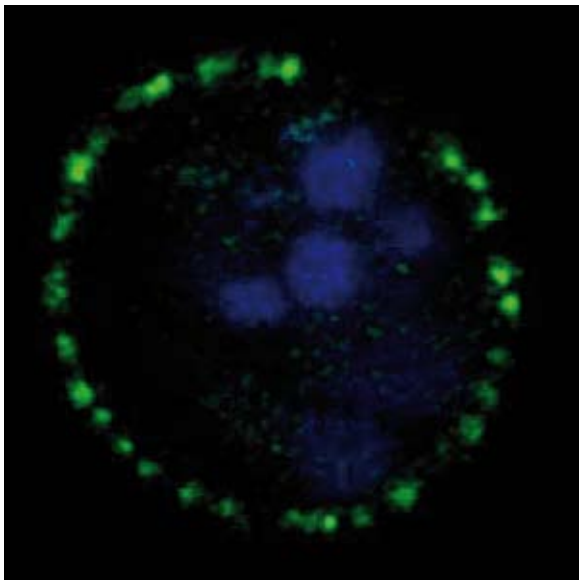
TIMNET-Immuno: Effect of intermittent preventive treatment with sulphadoxine-pyrimethamine in Mozambican pregnant women on acquisition of natural immunity to malaria in mothers and their infants

Intermittent preventive treatment with sulphadoxine-pyrimethamine (IPTp-SP) is recommended for malaria prevention during pregnancy in sub-Saharan Africa. However, little is known about its effect on malaria-specific immunity of mothers and infants.

Plasma from 302 pregnant women participating in an IPTp-SP placebo-controlled trial was analysed for presence of antibodies against merozoite antigens, whole asexual parasites and variant surface antigens (VSAs) from chondroitin sulphate A-binding and non-binding lines. Antibody levels were compared between intervention groups, and their association with morbidity outcomes was assessed.

HIV-positive mothers receiving SP had lower peripheral antibodies against apical membrane antigen-1 and VSAs, and lower cord antibodies against erythrocyte-binding antigen-175 and parasite lysate than HIV-positive placebo recipients. No difference between intervention groups was observed among HIV-negative mothers. High antibody levels were associated with maternal infection and infants' increased risk of a first malaria episode. Antibody responses were not consistently associated with reduced maternal anaemia, prematurity or low birthweight.

The IPTp-associated reduction of antibodies in HIV-infected women, but not among



Surface antigens on *Plasmodium falciparum*-infected erythrocytes (immunofluorescence).

the HIV-uninfected, may reflect a higher efficacy of the intervention in preventing malaria infection among HIV-positive mothers. This reduction did not translate into enhanced risk of malaria-associated morbidity in mothers and infants. These data were presented at the 5th MIM Pan-African Malaria Conference in Nairobi, Kenya in November 2009.

Another objective of the study was to evaluate the effect of IPTp with SP in the development of naturally acquired antibody responses in their children during the first year of life. The laboratory determinations in the infants at ages 3, 9 and 12 months were completed during this year, and will be analysed statistically in 2010. The determinations include:

- Levels of IgG, IgG1, IgG2, IgG3, IgG4 and IgM antibodies against *Plasmodium falciparum* antigens PfMSP-1$_{19}$, PfAMA-1 and PfEBA-175 by enzyme-linked immunosorbent assay (ELISA).
- Levels of IgG and IgM antibodies against *Plasmodium falciparum* blood-stage lysate by ELISA.
- Levels of IgG antibodies against *P. falciparum* VSA by flow cytometry.
- Submicroscopic infections by real-time quantitative polymerase chain reaction.

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Co-principal investigator:
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Funders:
Bill & Melinda Gates Foundation, Seattle (USA).
Banco Bilbao Vizcaya Argentaria, Fundación BBVA, Bilbao (Spain).

Duration of the project:
2007-2010

Publications:

- Mayor A, Serra-Casas E, Bardají A, Sanz S, Puyol L, Cisteró P, Sigauque B, Mandomando I, Aponte JJ, Alonso PL, Menéndez C. Sub-microscopic infections and long-term recrudescence of *Plasmodium falciparum* in Mozambican pregnant women. **Malar J.** 2009; 8:9.
- Sikora M, Ferrer-Admetlla A, Laayouni H, Menendez C, Mayor A, Bardaji A, Sigauque B, Mandomando I, Alonso PL, Bertranpetit J, Casals F. A variant in the gene *FUT9* is associated with susceptibility to placental malaria infection. **Hum Mol Genet.** 2009 Aug 15;18(16):3136-44.

1.4 Evaluation of Interventions: Vaccines and Drugs

Molecular markers of *Plasmodium falciparum* drug resistance in the context of the intermittent preventive treatment trials for malaria in pregnant women

Many factors are likely to influence the efficacy of intermittent preventive treatment during pregnancy (IPTp). One of the most relevant factors is probably the sensitivity of *Plasmodium falciparum* to the antimalarial drug(s) used for IPTp. As with any drug-based intervention strategy, there is also a need to evaluate the impact of IPTp on selection for drug resistance in the field setting.

In order to put the results of individual IPTp trials into context, and to provide information for policy makers regarding the predicted efficacy of IPTp in additional areas, we were collecting information on the prevalence of mutant parasites at each study site during the time that the trial was being conducted. We were also estimating the change in the prevalence of drug resistance markers in women who receive IPTp with sulphadoxine-pyrimethamine, mefloquine and cotrimoxazole. This is currently being done in two IPTp studies carried out in Manhiça.

Preliminary data from the first trial shows that IPTp appears to be associated with some changes in the prevalence of genotypes involved in SP resistance. However, this effect is only observed immediately after the last IPTp dose, and prevalences return to normal levels two months after the last dose. By improving our understanding of the conditions in which IPTp will be most effective, the results of this study will help to ensure that IPTp is used to maximum effect in preventing morbidity from malaria in pregnant women and their infants.

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In collaboration with:
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Funders:
Bill & Melinda Gates Foundation, Seattle (USA).
Fondo de Investigación Sanitaria (FIS), Instituto de Salud Carlos III (ISCIII), Madrid (Spain).

Duration of the project:
2008-2012

Evaluation of four artemisinin-based combinations for treating uncomplicated malaria in African children

The overall goal of the project was to compare the efficacy and safety of four artemisinin-based combination therapies (amodiaquine-artesunate, dihydroartemisinin-piperaquine, artemether-lumefantrine and chlorproguanil-dapsone-artesunate) for the treatment of uncomplicated malaria in children. It was a multi-centre, phase IV, randomized, open trial with three arms that is took place at 10 research sites in five African countries (Burkina Faso, Nigeria, Zambia, Gabon and Mozambique), with the East African Network for Monitoring of Antimalarial Treatment (EANMAT), an active network present in Kenya, Tanzania, Uganda, Rwanda and Burundi and five European partners (Belgium, Germany, France, United Kingdom and Spain).

The objectives were to establish the safety and efficacy of these new combination therapies during the 28 days post treatment and to determine the rate of re-treatment needed for each regimen during the following six months after treatment.

At the Centro de Investigação em Saúde de Manhiça (CISM), 511 children with uncomplicated malaria were randomized to three of the four ACTs. The total trial sample size was 5100, to be achieved among 10 sites in 7 sub-Saharan countries. Follow-up of study children was accomplished in 2009. Data cleaning and analysis will be done in 2010.

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- Bruno Gryseels. Institute of Tropical Medicine, Antwerp, Belgium.
- Emmanuel Ezedinachi. Institute for Tropical Diseases Research & Prevention, University of Calabar Teaching Hospital, Calabar, Nigeria.
- Serge Potiandi Diagbouga. Centre Muraz, Bobo-Dioulasso, Burkina Faso.
- Vincent Brown. Epicentre, Paris, France.
- Saadou Issifou. Medical Research Unit,
- Lambaréné, Gabon.
- Janet Hemingway. The Liverpool School of Tropical Medicine, Liverpool, U.K.
- Drummond Bone. The University of Liverpool, Liverpool, UK.
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- Peter Kremsner. Institute for Tropical Medicine, University of Tubingen, Tubingen, Germany.
- Ambrose O. Talisuna, Moses Kamya. Uganda Malaria Surveillance Project, Mulago Hospital Complex, Kampala, Uganda.
- Emmanuel Kafwembe. Tropical Diseases Research Centre, Ndola, Zambia.
- Daniel Ngamije, National Malaria Control Programme Rwanda, Kigali, Rwanda.

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

Duration of the project:
2007–2010

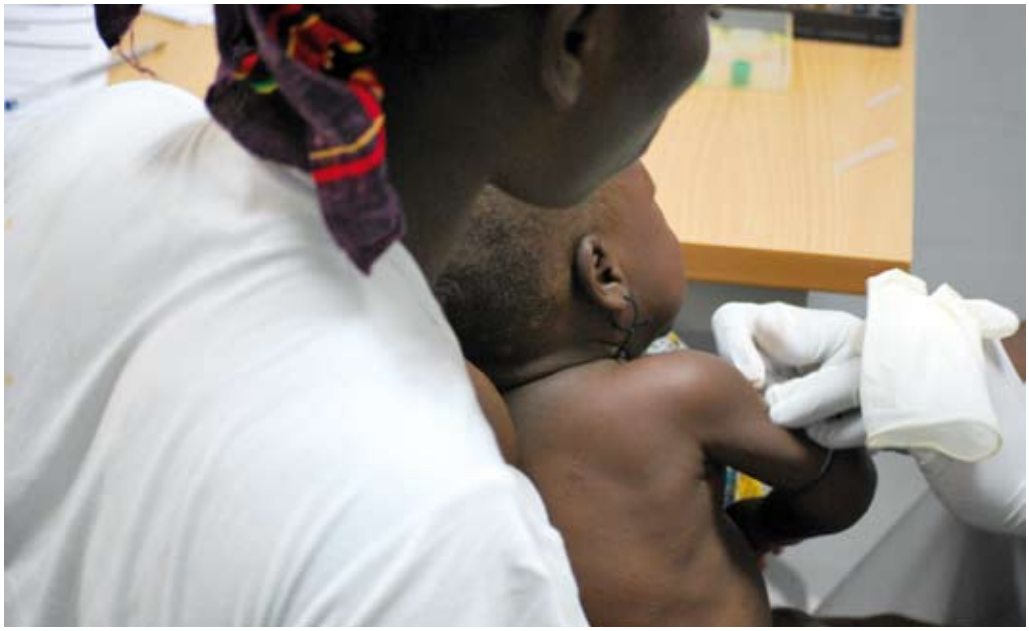
1.4 Evaluation of Interventions: Vaccines and Drugs

MalO55: A phase III, double-blind (observer-blind), randomized, controlled multi-centre study to evaluate, in infants and children, the efficacy of the RTS,S/ASO1E candidate vaccine against malaria disease caused by *Plasmodium falciparum* infection across diverse malaria transmission settings in Africa

In 2009 RTS,S was the most advanced malaria vaccine candidate and the first to demonstrate in clinical trials that it can protect young children living in malaria endemic areas against infection and clinical disease caused by *Plasmodium falciparum*. This vaccine was created in 1987 by GlaxoSmithKline, and CRESIB has been working with the Centro de Investigação em Saúde de Manhiça (CISM) since 2002 in the clinical development of the vaccine, in collaboration with the PATH Malaria Vaccine Initiative (MVI) and GlaxoSmithKline Biologicals (GSK).

In 2003, the first phase IIb trial in Mozambican children aged 1 to 4 years old was initiated and has proven that RTS,S reduces the incidence of clinical malaria episodes (35.3%) and severe malaria (48.6%) for a period of 18 months post vaccination.

CRESIB and CISM carried out the first I/IIb clinical trials in infants to evaluate safety, efficacy and immunogenicity. The results were published in 2007, proving that the vaccine is safe, well-tolerated and efficacious against new infections



(65.9%) in this age group.

In May 2009 GSK, PATH MVI and 11 leading African research centres launched a phase III trial of GSK's RTS,S malaria vaccine candidate, known as the MAL055 study, in Mozambique and six other African countries, with an enrolment target of 16,000 children and infants.

On 6 August 2009, CISM began the phase III trial of RTS,S malaria vaccine, having enrolled and vaccinated 1002 children aged 5 to 17 months. Manhiça is planning to start the enrolment and vaccination of infants aged 6 to 12 months in 2010.

The main objectives of this study were to continue evaluating the safety, efficacy and immunogenicity of the candidate vaccine, this time in different conditions of malaria transmission. If the results are favourable, they will allow it to be licensed in the near future.

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Co-investigators:
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Project manager:
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In collaboration with:
- Eusébio Macete and Sónia Machevo. Centro de Investigação em Saúde de Manhiça (CISM), Manhiça (Mozambique).

Funders:
The PATH Malaria Vaccine Initiative (MVI), Bethesda (USA).
GlaxoSmithKline Biologicals, Rixensart (Belgium)

Duration of the project:
2009-2013

1.5 From Tools to Policy

Economic burden of malaria in infants and young children in the malaria vaccine early adopter sub-Saharan African countries

While several studies document the epidemiological and clinical burden of malaria, there is still a lack of information on the economic burden of the disease. In a context of scarce resources it is fundamental to know the costs that malaria involves for families, for the health system and for the economy of a country as a whole. This information can guide future preventive and treatment interventions and help to design health policy choices at both a local and a national level.

Though some studies have focused on local estimates of how much malaria costs to families and to the health system, there is still a lack of information and of simple models that could help to extend malaria cost estimates to the country context, making it possible to generalize

1.5 From Tools to Policy

data on small areas to the whole national population. This study aimed to fill these gaps, at least for three sub-Saharan African countries, namely Ghana, Kenya and Tanzania, by accomplishing the objectives set out and described below.

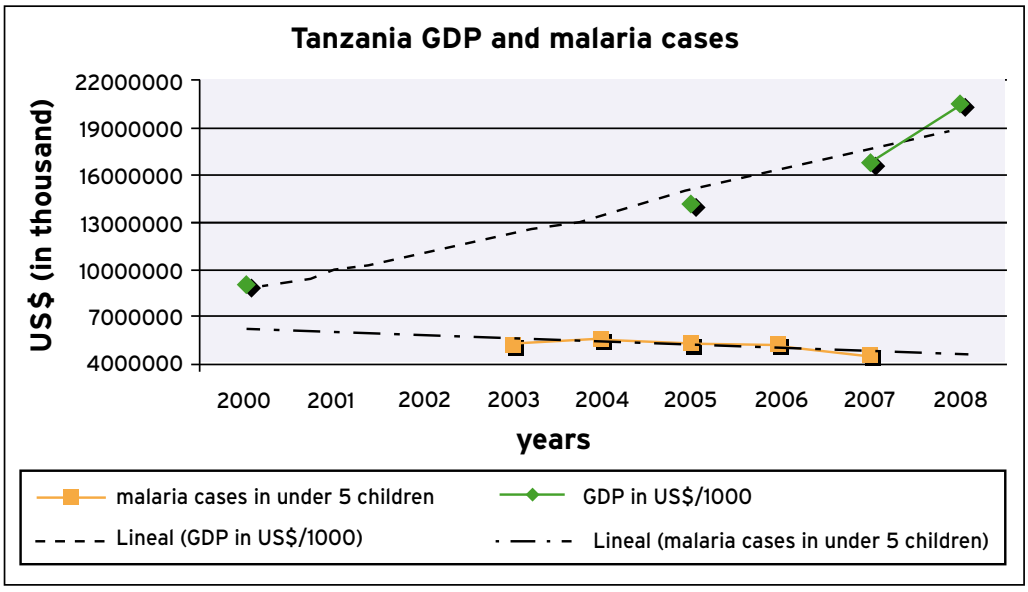
The main objective of this study was to estimate the economi c burden of malaria in infants and children in Ghana, Tanzania and Kenya. This burden included:

- Direct costs of prevention and treatment of malaria from a societal point of view.
- Impact of malaria on productivity.
- Impact of malaria on economic well-being.

Other objectives per country were:

- To understand/document standard-of-care in infants and young children.
- To understand/document resource use and associated cost for prevention strategies and treatments in infants and young children.
- To understand/document household costs for the prevention and treatment of infants and young children.
- To build a central cost database that could be used to populate health economic models.
- To assess the impact that malaria has on productivity at the individual and national level.
- To assess the impact that malaria has on economic well-being at national level.
- To assess the impact of malaria on impairment development in infants and young children at an individual and national level.

During the last few months of the 2009



Trends of malaria cases in under 5 children and Gross Domestic Product (GDP) in Tanzania

the protocol was written and a thorough literature review was undertaken on costs and on epidemiological and clinical data. Contacts were found in the three countries to organize data collection on site and decision trees were constructed for estimating of costs of a malaria episode in three countries.

Principal investigator:
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Co-investigator:
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- Xavier Badia. IMS Health, Barcelona (Spain).

Funder:
GlaxoSmithKline Biologicals, Rixensart (Belgium), through IMS Health, Barcelona (Spain).

Duration of the project:
2009-2010

Understanding the implementation and reception of indoor residual spraying in Manhiça, Mozambique

This anthropological study examined the social, cultural and historical factors that play a role in the reintroduction and reception of indoor residual spraying (IRS) for malaria prevention in Mozambique. The study went beyond "acceptability" issues and aimed to understand the more informal processes and practices through which health interventions such as IRS were implemented and received. This understanding is essential to the long-term success of any new health intervention. Beyond this immediate context, the acceptance (or rejection) of interventions such as IRS cannot be adequately understood in isolation from the implementation process, and this in turn cannot be understood independently of the wider context of policy debates, media coverage, local and regional politics and historical processes. Understanding IRS policy and implementation in Mozambique and the southern African Region more generally therefore requires a historical analysis of the political, social and cultural factors involved in the previous attempt (and failure) to control malaria through spraying with DDT, and of the *discourse* through which current IRS programmes are presented and the *rhetorical strategies* that are used to justify them.

The project involved studying the implementation of indoor spraying and its reception on the ground in Mozambique, using participant observation, in-depth interviews and focus group discussions.

1.5 From Tools to Policy

It also involved interviews with national policy makers and stakeholders and international experts.
In 2009 the fieldwork in Mozambique was completed and the policy and international expert interviews were carried out.

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 (UK).
- Samuel Mabunda. National Malaria Control Programme, Ministry of Health, Maputo (Mozambique).

Funder:
Bill & Melinda Gates Foundation, through the London School of Hygiene and Tropical Medicine, London (UK).

Duration of the project:
2007-2009

MalERA: Malaria eradication research agenda

Recent years have witnessed a renewed impetus for malaria control and the long-term goal of malaria eradication has been established. However, there is general consensus that with currently available tools malaria can be better controlled and eliminated in some areas, but worldwide eradication will not be achievable. Therefore, research and development (R&D) forms a crucial part of the global strategy to control, eliminate, and ultimately eradicate malaria.

The malERA initiative complements the 2008 Global Malaria Action Plan with R&D issues. It consisted of a rigorous scientific consultative process to identify current knowledge gaps and new tools needed for malaria eradication. The goal was to develop a multidisciplinary global R&D agenda that can be implemented by research and public health agencies and sponsors.

This consultation process was organized in seven consultative groups (vaccines; drugs; vector control; mathematical modelling; health systems, operational research and diagnostics; monitoring & evaluation and surveillance; and integration strategies) and will culminate in the production of a White Paper that outlines the proposed R&D agenda and will be published to ensure open access.

Over the course of one and a half years, more than 200 experts from 37 countries participated in 17 malERA meetings around the world. As a result of this process, each consultative group produced a scientific paper which will be the basis for the White Paper to be consolidated during the Zenith Week meeting in March 2010 with broader input from the R&D community.

In 2009, key outputs of the malERA process were shared with the broader scientific and health policy communities at the 5th MIM Pan-African Malaria Conference in Nairobi, Kenya and at the

American Society of Tropical Medicine and Hygiene (ASTMH) conference in Washington D.C.

The Steering Committee and Consultative Groups were composed of independent scientists with proven expertise in malaria and other infectious diseases, coordinated by Dr. Pedro L. Alonso. Continuity and cross-sector communication within the different programme elements was provided by a Secretariat based at CRESIB.

malERA
Malaria Eradication Research Agenda

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Funder:
Bill & Melinda Gates Foundation, Seattle (USA).

Duration of the project:
2008-2010

iVAX: Interdisciplinary *Plasmodium vivax* research consortium planning grant

The overall goal of this project is to establish an interdisciplinary *Plasmodium vivax* research network, define a priority research agenda for *P. vivax* and develop a proposal for an interdisciplinary P. vivax research consortium (iVAX) that will aim to contribute to the development of new tools targeting *P. vivax* by addressing the most important gap in the knowledge of this parasite.

The project started on 30 September 2009 and its kick-off meeting was held in Barcelona on 29-30 October 2009. It brought together 11 experts from Australia, Brazil, Colombia, India, Papua New Guinea, Thailand and the United States as well as from CRESIB with the aim of establishing the main structure and the priority research lines. During this meeting it was decided to focus the proposal on the

1.5 From Tools to Policy

host-parasite biology and to develop the following consultative groups:

- Reticulocytes
- Hypnozoites
- Immunology
- Pathophysiology
- Vector Biology
- Epidemiology

A second meeting was scheduled to be held in Atlanta in February 2010, inviting further investigators to discuss each research priority in depth.

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Funder:
Bill & Melinda Gates Foundation, Seattle (USA).

Duration of the project:
2009-2010

Publications:
- Mueller I, Galinski MR, Baird JK, Carlton JM, Kochar DK, Alonso PL, del Portillo HA. Key gaps in the knowledge of *Plasmodium vivax*, a neglected human malaria parasite. **Lancet Infect Dis.** 2009 Sep;9(9):555-66.

1.6 The *Plasmodium vivax* Consortium

The *Plasmodium vivax* Consortium

Plasmodium vivax is geographically the most widely distributed cause of malaria in people, with up to 2500 million people at risk and an estimated 80 million to 300 million clinical cases every year, including severe disease and death. Despite this large burden of disease, *P. vivax* is overlooked and left in the shadow of the enormous problem caused by *Plasmodium falciparum* in sub-Saharan Africa. As a consequence, there are substantial gaps of knowledge on the clinics, epidemiology and physiopathology of the infection caused by *P. vivax*.

The *Plasmodium vivax* Consortium is a four-year programme coordinated by CRESIB and funded by the Fundació Cellex with the objective of improving knowledge on *P. vivax* malaria and accelerating the development of new control tools, especially vaccines. The consortium is formed by six institutions from five different countries (Papua New Guinea, India, Brazil, Colombia and Spain) and has five specific objectives:

1) To carry out prospective longitudinal studies on the epidemiology of *P. vivax* malaria in two regions with different transmissions: Brazil and Papua New Guinea.

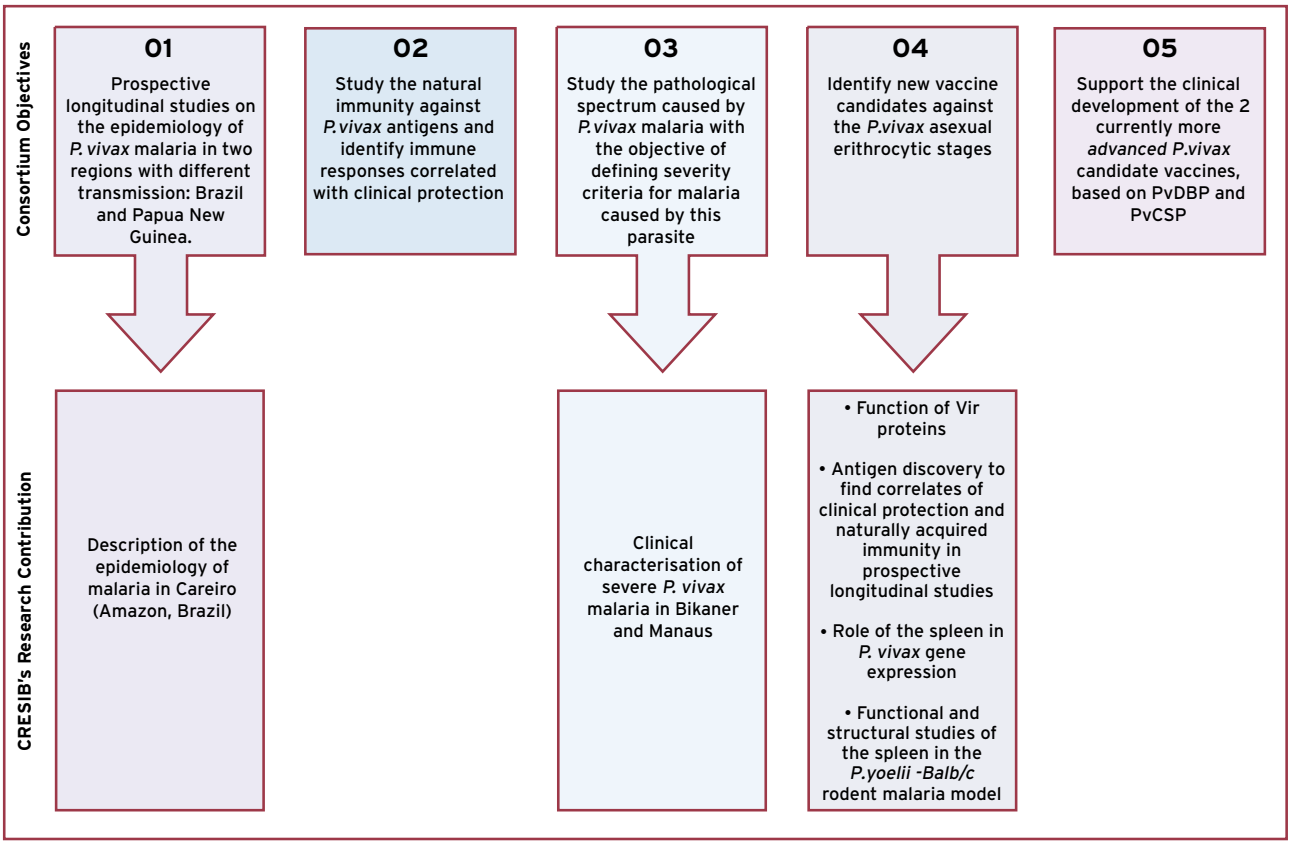
2) To study the natural immunity against *P. vivax* antigens and to identify immune responses correlated with clinical protection.

3) To study the pathological spectrum caused by *P. vivax* malaria with the objective of defining severity criteria for malaria caused by this parasite.

4) To identify new vaccine candidates against the *P. vivax* asexual erithrocytic stages.

5) To support the clinical development of the two currently most advanced *P. vivax* candidate vaccines, based on the Duffy binding protein (PvDBP) and the circumsporozoite protein (PvCSP).

The *Plasmodium vivax* Consortium objectives and CRESIB's research contribution to them:



1.6 The *Plasmodium vivax* Consortium

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.

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.

By the end of 2009, three of the four cross-sectional studies had been conducted and the last one has been scheduled for spring 2010. Passive case detection surveillance has been ongoing with good compliance by the censed population, and malaria cases have been captured at the different health posts covering the study area.
Data has been routinely transcribed into electronic databases and analyses will be performed as soon as the last cross-sectional visit has concluded.

Study field workers and laboratory technicians in Careiro's Health Centre, Amazon, Brazil

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Funder:
Fundació Cellex, Barcelona (Spain).

Duration of the project:
2008–2010

Clinical characterization of severe *Plasmodium vivax* malaria in Bikaner (India) and Manaus (Brazil)

In recent years, the paradigm that viewed *Plasmodium 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 the above, the World Health Organization 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 characterize 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 hospitalizations 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.

The project has started at both sites, and in Manaus more than 250 patients have already been recruited. Progress in Bikaner has been slower, due to a particularly low malaria season and the high seasonality of malaria transmission in the area. We aim to continue patient recruitment until the end of 2010.

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Funder:
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Duration of the project:
2007-2010

Function of the Vir proteins

Sequence analysis of the entire *vir* gene repertoire from the Salvador I strain showed that only 171 *vir* genes contain predicted transmembrane (TM) domains and only four *vir* genes, all pertaining to subfamily D, possess the exact PEXEL/HT motif, whereas 160 deduced Vir proteins possess a PEXEL-like motif. Based on laser confocal images obtained from smears of a single wild isolate and using immune serum raised against a conserved peptide sequence from subfamily D, Vir proteins were originally thought to be exclusively located at the surface of infected reticulocytes. However, *in silico* analysis of protein domains and

1.6 The *Plasmodium vivax* Consortium

secondary structures from sequences of parasites obtained directly from patients revealed that subfamily A is related to the SURFIN subtelomeric *Plasmodium falciparum* multigene family and that Vir Subfamily D contains 2TM domains similar to the *Pfmc-2tm* multigene family. This data indicates that Vir proteins might have subcellular localizations other than the surface membrane of infected reticulocytes. Unfortunately, in the absence of a continuous *in vitro Plasmodium vivax* culture system to obtain unlimited material, these results cannot be considered unequivocal. As an alternative approach, heterologous transfection and expression of *vir* genes in *P. falciparum* can be exploited.

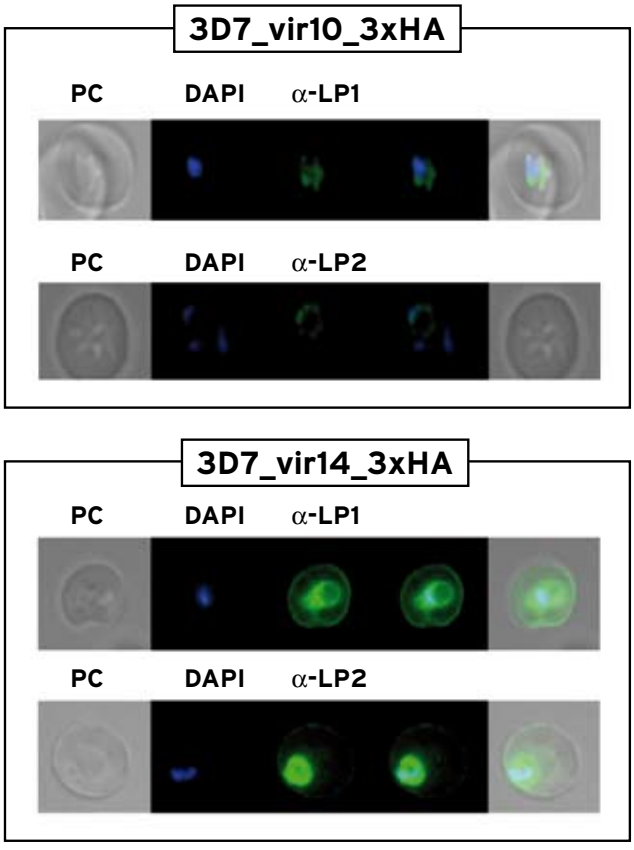
The main objective of this project is to determine the subcellular localization of Vir proteins, and the specific objectives are:

1) To construct transgenic lines of *P. falciparum* expressing in trans different Vir proteins.

2) To determine their subcellular localization through confocal laser microscopy.

3) To establish a short-term *in vitro* culture of *P. vivax*.

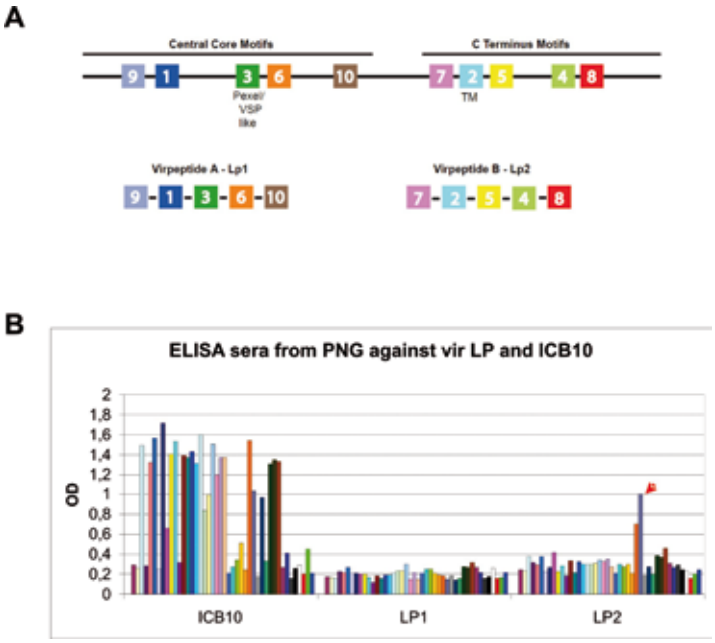
4) To validate the findings obtained in heterologous transfections.



Confocal laser microscopy of transgenic lines of *P. falciparum* expressing Vir proteins. Identification of proteins Vir10 and Vir14 in the transgenic lines was achieved by using guinea pig polyclonal antibodies that recognize different Vir peptides. Parasites were incubated with DAPI (nucleus marker, in blue) and anti-LP1 or LP2 (marker of *P. vivax* specific Vir proteins, in green).

We have constructed several *P. falciparum* transgenic lines expressing Vir proteins with different predicted protein domains and subcellular localizations. The results indeed demonstrated that Vir proteins have different subcellular localizations.

To validate these results, we synthesized long peptides (90-100 aa) and generated polyclonal mono-specific anti-vir LP1 and LP2 antibodies in guinea pigs. The peptides are recognized by immune sera of *P. vivax*-infected patients and the antibodies recognized Vir proteins expressed in transgenic lines of *P. falciparum*.



A. Schematic representation of the Vir multigenic family conserved motifs present in the long peptides 1 and 2 (Lp1 and Lp2). Motif 2: transmembrane domain (TM); motif 3: PEXEL/VSP-like

B. ELISA results of the LP1 and LP2 peptides against 32 sera from *P. vivax* patients from PNG (in red, those sera that recognize the LP2). The recombinant protein ICB10, representing the C-terminal region of PvMSP1, was used as positive control as this protein is highly immunogenic in natural infections

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Duration of the project:
2007-2011

Publications:
- Fernandez-Becerra C, Yamamoto MM, Vêncio RZN, Lacerda M, Rosanas-Urgell A, del Portillo HA. *Plasmodium vivax* and the importance of the subtelomeric multigene *vir* superfamily. **Trends Parasitol.** 2009 Jan;25(1):44-51.

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Antigen discovery to find correlates of clinical protection and naturally acquired immunity in prospective longitudinal studies

Computational biology is rapidly accelerating the discovery of new targets for intervention. We are proposing to use different algorithms to predict targets for vaccine development in *P. vivax*. Antigens will be expressed using *in vitro* cell-free protein expression systems. These offer an alternative to the *E. coli* cell-based systems and have a number of potential advantages, including successful production of proteins that undergo proteolysis or accumulate in inclusion bodies. The new antigens will be used in immune-epidemiological studies using the Bio-Plex system. Suspension array technologies with high-throughput capacity to simultaneously analyse several proteins with a minimal amount of immune sera have been developed.

The main objective of this project is to discover new antigens for *P. vivax* vaccine development by finding correlates of clinical protection and naturally acquired humoral immune responses in prospective longitudinal studies. Specific aims are:

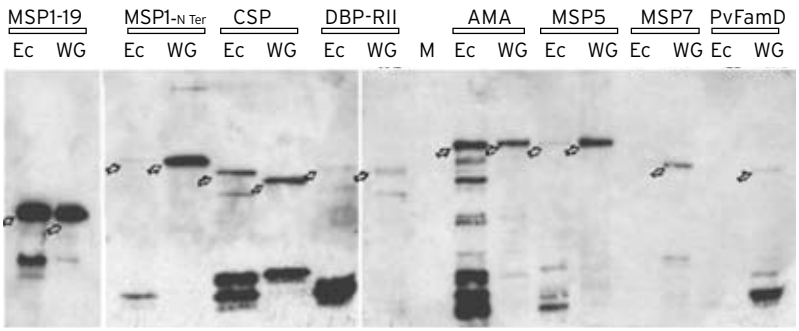
1) To use the cell-free wheat germ expression systems to express malarial proteins.

2) To validate this system compared with *E. coli* expression systems.

3) To implement and validate the Bio-Plex and protein array technologies for *P. vivax* antigens.

4) To find correlates of clinical protection and naturally acquired immunity in children of 1-5 years of the prospective longitudinal cohort Cellex study conducted in Papua New Guinea.

We have constructed several expression recombinant vectors for malaria proteins. Recombinant clones sent to our partner in Japan were not expressing our own constructs credibly. We have now surrogated the cell-free expression system of wheat germ as well as *E. coli* and have successfully expressed 20 *P. vivax* antigens, including seven lead vaccine candidates.



WB - anti GST 1:5000

Western-blot showing the expression of 8 proteins of *P. vivax* fused to GST and expressed in the soluble fraction of cell-free systems from *E. coli* (Ec) and wheat germ (WG). Proteins were detected using a rabbit anti-GST polyclonal antiserum at a 1:5000 dilution.

The Bio-Plex system for antigen discovery in *P. vivax* has been enabled and validated in our group using the PvMSP1 protein as a molecular marker. Seven antigens have been coupled to Bio-Plex beads and the "proof-of-concept" of their immunogenicity using Bio-Plex has been tested using immune sera.

In addition, the prospective longitudinal study in Papua New Guinea has been finished and real-time polymerase chain reaction analysis has been performed in all samples (9000) for *P. falciparum* and *P. vivax*.

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Funder:
Fundació Cellex, Barcelona (Spain).

Duration of the project:
2007-2011

Role of the spleen in *Plasmodium vivax* gene expression

Plasmodium vivax invades predominantly—if not but exclusively—reticulocytes, and it is widely accepted that it must necessarily pass through the spleen. The spleen is a complex organ that is perfectly adapted to selectively filtering and destroying senescent red blood cells (RBCs), infectious microorganisms that escape the epithelial barriers, and *Plasmodium*-infected RBCs.

Experimental malaria infections in splenectomized animals have demonstrated the essential role of the spleen in the expression of variant antigens implicated in cytoadherence and sequestration. *Plasmodium knowlesi* parasites expressing variant proteins at the surface of infected red blood cells (iRBCs) rapidly lost this expression and serum agglutination capacities of iRBCs if transferred into splenectomized rhesus monkeys and recovered them again if transferred back to normal animals. In studies of *Plasmodium falciparum* infections in splenectomized Saimiri monkeys, a slightly different result was observed: iRBC lost their cytoadherence capacity though a new set of variant antigens seemed to be expressed. Together, these data demonstrate the importance of the spleen in expression of variant antigens

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associated with cytoadherence.

Since it is widely accepted that *P. vivax* does not cytoadhere, it would be interesting to address the role of the spleen in the expression of the vir variant proteins. Moreover, since the complete genome sequence of *P. vivax* is publicly available, this transcription analysis can be performed globally.

The main goal of this proposal was to identify genes of *P. vivax* whose expression is dependent on the spleen in the experimental monkey model of *Aotus lemurinus griseimebra* infected with the *P. vivax* Salvador-1 strain and to use this information in furthering vaccine development.

New bioinformatics analysis of data from the microarray containing all *P. vivax* coding sequences revealed 24 proteins whose expression is spleen-dependent. We have designed specific primers to amplify, from gDNA or cDNA, the complete genes encoding these 24 proteins. During 2009 we were expressing these proteins in the wheat germ cell-free system to validate the transcriptional results at the protein level (i.e., antigen discovery).

Microarray results

Microarray showing a differential pattern of expression in the presence (green) or absence (red) of the spleen.

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Funder:
Fundació Cellex, Barcelona (Spain).

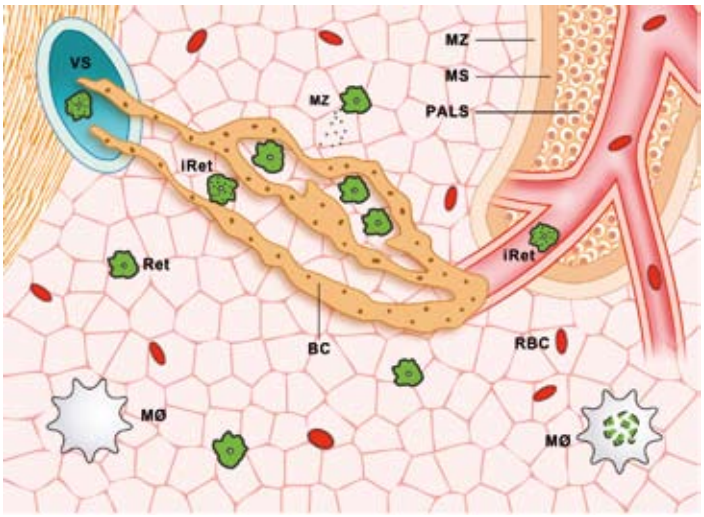
Duration of the project:
2007-2011

Functional and structural studies of the spleen in the *Plasmodium yoelii-Balb/c* rodent malaria mode

The spleen is a complex organ that is perfectly adapted to selectively filtering and destroying senescent red blood cells (RBCs), infectious microorganisms that escape the epithelial barriers, and *Plasmodium*-infected RBCs. This filtering capacity is related to its complex structure and blood microcirculation. The spleen consists of a trabecular system in which the white pulp (WP), lymphoid tissue where most immune effector cells reside, the red pulp (RP), a reticular meshwork where destruction of senescent and aberrant RBCs occurs, and the marginal zone, lying between the WP and the RP where inert particles, bacteria and viruses are eliminated, form a complex structure. In addition, blood enters the spleen through a central artery which branches into capillaries, the majority of which empty into the filtration beds of the red pulp before reaching the venous system in a so called "open system". The spleen is therefore a complex organ whose 3D structure consists of distinct microanatomical zones exquisitely adapted to perform different functions.

The main objective of this project was to determine the mechanism by which the *Plasmodium yoelii* non-lethal strain is able to escape spleen clearance and the role of barrier cells in this escape. The specific objectives were:

1) To refine analysis of the structural changes displayed by the spleen of mice experimentally inoculated with lethal and non-lethal *P. yoelii* using novel probes/reagents generated for histological analysis.



Model of spleen-clearance evasion mechanism in reticulocyte-prone non-lethal malaria. *P. yoelii* 17X induces spleen structural remodeling, including the formation of barrier cells to which infected reticulocytes specifically cytoadhere, protecting themselves from macrophage spleen clearance. Abbreviations: VS, venous sinus lumen; RBC, red blood cells; Ret, reticulocytes; iRet, infected reticulocytes; mz, merozoites; PALS, periarteriolar lymphoid tissue; MS, marginal sinus; MZ, marginal zone; MØ, macrophages; BC, barrier cells.

1.6 The *Plasmodium vivax* Consortium

2) To perform *in vivo* quantitative imaging of the spleen of mice experimentally inoculated with lethal and non-lethal *P. yoelii*-harbouring reticulocytes.

3) To search for molecular markers of "barrier cells" in experimental infections of BALB/c mice infected with *P. yoelii* through global transcription analysis.

4) To construct conditional gene knockdown mice to demonstrate functionally when, where and how the *P. yoelii*-harbouring reticulocytes interact with the barrier cells.

After two years of investing in enabling technologies in the mouse model, we have implemented intravital imaging and magnetic resonance imaging of the mouse spleen and have discovered a new spleen-evasion mechanism in reticulocyte-prone non-lethal malaria parasites, resembling *P. vivax*. A manuscript has been submitted to PLoS Pathogens. Furthermore, a patent on the use of reticulocytes-derived exosomes has been registered: Reticulocyte-derived exosomes, a method for their isolation and usage (P200931275) and a protocol for studying the human spleen in malaria patients presenting to the Hospital Clínic de Barcelona has been approved.

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- María Calvo. Hospital Clínic de Barcelona, Barcelona (Spain).
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Funders:
Fundació Cellex, Barcelona (Spain).
Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR), Barcelona (Spain).

Duration of the project:
2007-2011

The IPTi Consortium

Intermittent Preventive Treatment in infants (IPTi) is the administration of an antimalarial drug at the time of childhood vaccinations in the first year of life and takes advantage of delivery alongside the well-functioning Expanded Programme on Immunization (EPI) of the World Health Organization (WHO). Under this approach, infants receive an antimalarial drug two or three times during the first year of life whether or not they have malaria and are provided with prophylactic protection for the period in which the antimalarial drug is present in the blood.

The IPTi Consortium was established in 2003 and funded by the Bill & Melinda Gates Foundation. CRESIB has coordinated the secretariat of the Consortium, which comprised 19 institutions working together to rapidly resolve the outstanding scientific questions needed to be addressed before IPTi could be recommended policy for malaria control and prevention.

The Consortium conducted randomized, double-blinded, placebo-controlled efficacy trials of IPTi with sulphadoxine-pyrimethamine, SP (IPTi-SP) in Mozambique and Gabon, and with alternative medicines and combinations in Kenya, Tanzania and Papua New Guinea using artesunate plus SP and artesunate plus amodiaquine and chlorproguanil-dapsone (lapdap) in Kenya, mefloquine and chloproguanil-dapsone (lapdap) in Tanzania, and artesunate plus SP and amodiaquine plus SP in Papua New Guinea.

Through two sets of implementation studies of IPTi-SP, conducted in parallel to the efficacy trials, the Consortium generated information on operational issues, acceptability, and the costs of implementation in a variety of countries and different health systems.

It was the first time that a potential malaria control tool had undergone such a thorough and robust evaluation, taking promising research results through a full evaluation to implementation and public health impact. It is also likely to become a model of international collaboration and partnership in the development and evaluation of new control tools.

IPTi has undergone reviews by two expert committees: the Technical Expert Group (TEG) convened by the WHO, and a group of experts convened by the US Institute of Medicine (IOM). Both committees recommended (IOM in July 2008 and TEG in April 2009) that IPTi should be implemented in areas of moderate to high malaria transmission, and with SP in areas where there is not very high-level resistance to SP.

IPTi is delivered alongside EPI vaccines and research has demonstrated that this practice does not have a negative effect on responses to EPI vaccines or on people's attitudes to EPI. Therefore, in October 2009 the Strategic Advisory Group of Experts –the policy advisory body for EPI– endorsed the delivery of IPTi alongside EPI vaccines. The WHO Global Malaria Programme (GMP) is now taking the lead to develop a joint statement from the WHO (GMP, AFRO and EPI) and UNICEF recommending the use of IPTi.

These are the projects that the Consortium has undertaken:

1.7 The Intermittent Preventive Treatment in infants (IPTi) Consortium

- Pooled analysis of the efficacy of IPTi-SP
- Pooled analysis of the safety of IPTi-SP
- Effect of IPTi-SP on immune responses to EPI vaccines
- Effect of IPTi-SP on the development of naturally acquired immunity to malaria
- Effect of SP drug resistance on efficacy of IPTi-SP
- Alternative drugs and combinations for IPTi
- Effectiveness of IPTi delivered through the existing health system
- UNICEF pilot implementation of IPTi in six African countries
- Cost-effectiveness of IPTi
- Acceptability of IPTi
- The age pattern of malaria and the applicability of IPTi and a web-based decision-support tool on where to implement IPTi
- Modelling the impact of IPTi

In purple, projects in which CRESIB researchers have participated during 2009. The efficacy and safety of IPTi with SP results have been published in:

- Aponte JJ, Schellenberg D, Egan A, Breckenridge A, Carneiro I, Critchley J, Danquah I, Dodoo A, Kobbe R, Lell B, May J, Premji Z, Sanz S, Sevene E, Soulaymani-Becheikh R, Winstanley P, Adjei S, Anemana S, Chandramohan D, Issifou S, Mockenhaupt F, Owusu-Agyei S, Greenwood B, Grobusch MP, Kremsner PG, Macete E, Mshinda H, Newman RD, Slutsker L, Tanner M, Alonso P, Menendez C. Efficacy and safety of intermittent preventive treatment with sulfadoxine-pyrimethamine for malaria in African infants: a pooled analysis of six randomised, placebo-controlled trials. **Lancet**. 2009 Oct 31;374(9700):1533-42.

IPTi Immuno: Impact of intermittent preventive treatment on the development of naturally acquired immunity in Mozambican infants

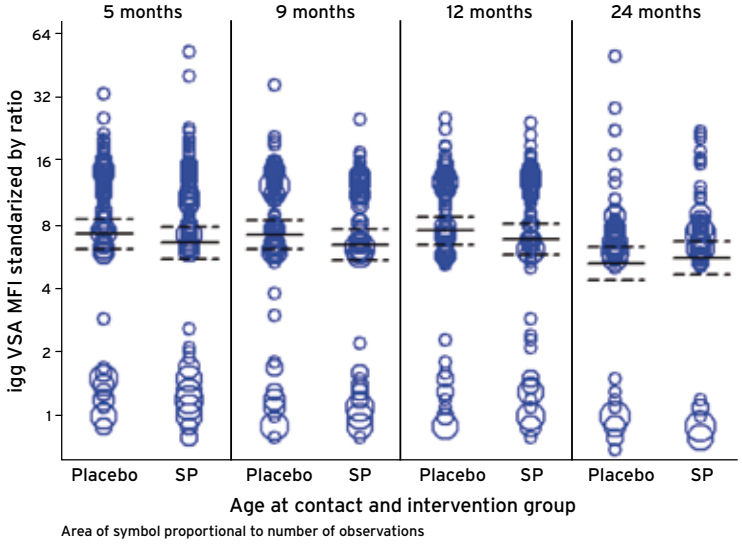
This study aimed to investigate the impact of intermittent preventive treatment in infants (IPTi) on the development of naturally acquired immunity (NAI). This intervention in children may interfere with the acquisition of immunity to malaria later in life and could result in negative consequences, such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

To investigate the possible interference to NAI, blood samples were collected from children who had received IPTi with sulphadoxine-pyrimethamine or a placebo treatment to measure the pattern of immune responses to *Plasmodium 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 IPTi might have on the risk of clinical malaria.

During 2009, statistical analyses of antibody responses to variant surface antigens (VSA) expressed on *P. falciparum* by a flow cytometry assay (fluorescence-activated cell sorting) was completed and measured at ages 5, 9, 12 and 24 months, and the statistical analyses of antibodies that inhibit *in vitro* growth of *P. falciparum* at ages 12 and 24 months was performed.

Data showed that IPTi does not affect the levels of VSA antibodies or the frequency of growth inhibitory antibodies during the first two years of life. These functional antibodies are not associated with future risk of malaria.

The results were presented at the Jornadas de Saúde (Maputo, Mozambique, September 2009) and at the 5th MIM Pan-African Malaria Conference in Nairobi, Kenya in November 2009.



There is no significant difference in the levels of anti-VSA IgG antibodies between children receiving SP or placebo. IgG levels significantly decreased during the first 2 years of life

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- 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).

Funder:
Bill & Melinda Gates Foundation, Seattle (USA).

Duration of the project:
2003-2009

1.7 The Intermittent Preventive Treatment in infants (IPTi) Consortium

IPTi acceptability: Acceptability of intermittent preventive treatment in infants for the control of malaria and anaemia

The overall goal of the project is to facilitate the implementation and long-term Acceptability of Intermittent Preventive Treatment of malaria in infants (IPTi) linked to the Expanded Programme on Immunization (EPI) in Africa by identifying and understanding actual and potential impediments and facilitating factors, and to make recommendations for overcoming impediments and developing and strengthening facilitating factors, thus helping to reduce the burden of malaria and anaemia in infants.

The general approach and design of this study was anthropological. This entails directly studying people's actions and reactions associated with IPTi, EPI and other related issues, and placing these in the wider context of both local culture and broader social, political, historical and economic processes. It also entails comparing these actions and contexts across different settings.

The study is linked to the IPTi efficacy studies being carried out under the auspices of the IPTi Consortium in Lambaréné (Gabon), Kisumu (Kenya), Kilimanjaro (Tanzania), Madang (Papua New Guinea) and two UNICEF-led implementation studies in Lilongwe (Malawi) and Navrongo (Ghana).

In early 2009 data collection was completed at all sites except Papua New Guinea, where data collection continued until mid-2009. In August 2009 the results of the acceptability studies undertaken in

sub-Saharan Africa were published, and the following conclusions were drawn:

- IPTi delivered through EPI fits well with local health cultures and appears to become easily routinized.
- There is little evidence that IPTi has any negative impact on attitudes to EPI or EPI adherence.
- There is also little evidence that the concurrent delivery of IPTi and immunization has led to people perceiving IPTi as immunization and changing existing health-seeking behaviour detrimentally.
- Local understanding of new drugs and regimens (and existing ones) is patchy and often inaccurate.
- It is important that an infant formulation is developed, and this should ideally be a single dose administered in the clinic as part of EPI.

Principal investigator:
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Co-investigators:
Marjolein Gysels, Christopher Pell

Project manager:
Marjolein Gysels

In collaboration with:
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- 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).

Funder:
Bill & Melinda Gates Foundation, Seattle (USA).

Duration of the project:
2007-2009

Publications:
- Gysels M, Pell C, Mathanga DP, Adongo P, Odhiambo F, Gosling R, Akweongo P, Mwangi R, Okello G, Mangesho P, Slutsker L, Kremsner PG, Grobusch MP, Hamel MJ, Newman RD, Pool R. Community response to intermittent preventive treatment of malaria in infants (IPTi) delivered through the expanded programme of immunization in five African settings. **Malar J.** 2009;8:191.
- Schwarz NG, Gysels M, Pell C, Gabor J, Schlie M, Issifou S, Lell B, Kremsner PG, Grobusch MP, Pool R. Reasons for non-adherence to vaccination at mother and child care clinics (MCCs) in Lambaréné, Gabon. **Vaccine.** 2009 Aug 27;27(39):5371-75.

1.7 The Intermittent Preventive Treatment in infants (IPTi) Consortium

IPTi cost-effectiveness working group

Intermittent preventive treatment of malaria in infants (IPTi) has been shown to decrease clinical malaria by approximately 30% in the first year of life and is a promising malaria control strategy for sub-Saharan Africa which can be delivered alongside the Expanded Programme on Immunization (EPI). To date, there have been limited data on the cost-effectiveness of this strategy using sulphadoxine-pyrimethamine (SP) and no published data on cost-effectiveness using other antimalarials.

The cost-effectiveness working group analysed data from five countries in sub-Saharan Africa using a total of five different IPTi drug regimens: SP, mefloquine (MQ), 3 days of chlorproguanil-dapsone, SP plus 3 days of artesunate (SP-AS3) and 3 days of amodiaquine-artesunate (AQ3-AS3). The cost per malaria episode averted and cost per disability-adjusted life-year (DALY) were modelled using both trial-specific protective efficacy for all IPTi drugs and a pooled protective efficacy which included IPTi with SP, malaria incidence, an estimated malaria case fatality rate of 1.57%, IPTi delivery costs and country-specific provider and household malaria treatment costs.

At sites where IPTi had a significant effect on reducing malaria, the cost per episode averted for IPTi-SP was very low: USD 1.36–4.03 based on trial specific data and USD 0.68–2.27 based on the pooled analysis. For IPTi using alternative antimalarials, the lowest cost per case averted was for AQ3-AS3 in western Kenya (USD 4.62) and the highest was for MQ in Korowge, Tanzania (USD 18.56). Where efficacious, based only on intervention costs IPTi was shown to be cost-effective at all the sites and highly cost-effective at all but one of the sites, ranging from USD 2.90 (Ifakara, Tanzania

IPTi Cost-Efectiveness Working Group

with SP) to USD 39.63 (Korogwe, Tanzania with MQ) per DALY averted. In addition, IPTi reduced health system costs and showed significant savings to households from malaria cases averted. A threshold analysis showed that there is room for the IPTi efficacy to fall and still remain highly cost-effective at all sites where IPTi had a statistically significant effect on clinical malaria.

IPTi delivered alongside EPI is a highly cost-effective intervention against clinical malaria with a range of drugs in different malaria transmission settings. Where IPTi did not have a statistically significant impact on malaria, generally at low transmission sites, it was not cost-effective.

Study Site		Cut-off level of CFR for ICERs to reach USD36
Ifakara SP	Trial	0.10%
Ifakara SP	Pooled	0.19%
Navrongo SP	Trial	0.10%
Navrongo SP	Pooled	0.08%
Manhiça SP	Trial	0.28%
Manhiça SP	Pooled	0.13%
Kumasi SP	Trial	0.11%
Kumasi SP	Pooled	0.06%
Tamale SP	Trial	0.10%
Tamale SP	Pooled	0.05%
Lambaréné SP	Pooled	0.65%
Western Kenya SP+Art	Trial	0.28%
Western Kenya AQ+Art	Trial	0.35%
Korogwe MQ	Trial	1.57%

Principal investigator:
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In collaboration with:
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- Peter Otieno. CDC/KEMRI Research Station, Kisumu (Kenya).
- Fred Matovu. Consultant, Makerere (Uganda).
- Paul Masika. National Institute for Medical Research, Tanga (Tanzania).
- Prosper Biao. Consultant, Lambaréné (Gabon).
- Fatuma Manzi. Ifakara Health Research and Development Centre (Tanzania).
- Carol Davy. Papua New Guinea Institute of Medical Research, Goroka (Papua New Guinea).

Funder:
Bill & Melinda Gates Foundation, Seattle (USA).

Duration of the project:
2006-2009

1.8 MiP: The Malaria in Pregnancy Consortium

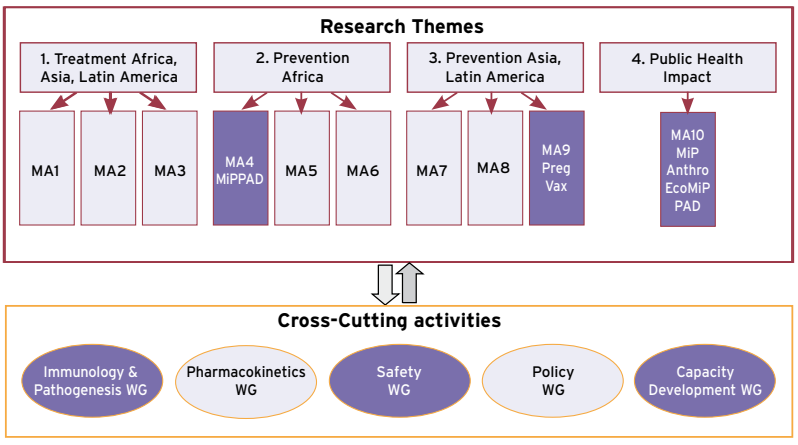
The Malaria in Pregnancy (MiP) Consortium

Malaria in pregnancy (MiP) affects up to 50 million women around the world every year and can result in pregnancy loss, maternal death, severe maternal anaemia and low infant birth weight that greatly increases the risk of death. MiP is responsible for as many as 100,000 child deaths every year and in sub-Saharan Africa, where severe malaria accounts for approximately 10% of maternal deaths, an estimated 25,000 maternal deaths could be prevented each year by improved control of malaria in pregnancy.

The MiP Consortium is a five-year programme of research to evaluate new and improved interventions for the prevention and treatment of malaria in pregnancy. The Consortium, coordinated by the Liverpool School of Tropical Medicine and constituted by 43 partner institutions in 29 countries around the world, is supported by the Bill & Melinda Gates Foundation and the European Union. The mission of the Consortium is to improve the control of malaria in pregnancy in Africa, Asia and Latin America.

Ten major activities (MAs) govern research in four key areas of malaria in pregnancy: prevention, treatment, burden assessment and how best to scale up existing strategies and interventions. CRESIB is involved in the coordination of three of these MAs:

- MA4: New drugs for IPTp (MiPPAD)
- MA9: Prevention strategy in Latin America (PregVax)
- MA10: Public Health Impact (MiPAnthro and EcoMiPPAD)



MiP Consortium organization. In purple activities and working groups in which CRESIB researchers are participating. MA: Major Activity, WG: Working Group

MiPPAD: Evaluation of alternative antimalarial drugs to sulphadoxine-pyrimethamine for intermittent preventive treatment in pregnancy (IPTp) in the context of insecticide-treated nets

Malaria in pregnancy (MiP) is one of the most important preventable causes of low birth weight deliveries worldwide and a major cause of severe maternal anaemia contributing to maternal mortality. Finding effective preventive interventions to reduce the incidence and consequences of malaria infection in pregnant women is a priority in endemic countries. As part of the MiP Consortium, this project aimed not only to develop new anti-malaria in pregnancy prevention but also to promote European and African research collaboration and to strengthen the capacity of African institutions to conduct clinical research.

MiPPAD aims to contribute to the development of new clinical interventions to fight malaria in pregnancy by evaluating different alternatives to antimalarial drugs used for intermittent preventive treatment in pregnancy (IPTp) in the context of insecticide-treated mosquito nets. The safety and efficacy of sulphadoxine-pyrimethamine, recommended by the World Health Organization for IPTp, was compared with that of mefloquine in 4716 women in four sub-Saharan African countries: Benin, Gabon, Mozambique and Tanzania. To gain a better understanding of the control tools for malaria during pregnancy in

Pregnant woman showing deployed ITN during a household visit (Manhiça, Mozambique)

1.8 MiP: The Malaria in Pregnancy Consortium

HIV-infected populations, this study also includes 1070 HIV-infected pregnant women recruited in Kenya, Mozambique and Tanzania.

In addition to the African countries mentioned, the participation of Austria, France, Germany and Spain strengthens coordination and networking between sites.

CRESIB coordinates the timely implementation and progress of the project, with the support of the MiPPAD Executive Committee constituted by the principal investigators of all partner sites. This includes the preparation and/or conditioning of all the study tools, clinical monitoring, data collection at the central database at the *Centro de Investigação em Saúde de Manhiça (CISM)* in Mozambique, and safety reporting with the support of an independent data safety monitoring board. Networking and training are actively promoted between sites in addition to a panel of trial-related courses, and a master's and a PhD scholarship. As an example, the MiPPAD Statistics Working Group is composed of one senior and one junior member from each site for the analysis of the global data.

In 2009, the study coordination infrastructure was established and trials were initiated at two of the five African partner sites.

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Funders:
European & Developing Countries Clinical Trial Partnership (EDCTP), European Union.
Malaria in Pregnancy Consortium (MiPc), Liverpool School of Tropical Medicine (LSTM), Liverpool (UK).
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

ImmunoMiPPAD: Role of maternal immunity in the clinical outcomes of malaria in pregnancy

Women are at higher risk of infection and disease when pregnant. Since parity-increasing levels of IgGs against the surface of infected erythrocytes isolated from the placenta mirror the increasing resistance to malaria over successive pregnancies, they have been causally related to the acquisition of protection against malaria during pregnancy. However, increasing levels of antibodies with parity have been found for a broad range of *Plasmodium falciparum* antigens, suggesting the possibility of a generalized effect of the physiology of pregnancy on maternal immunity.

The increased susceptibility of *primigravidae* women to malaria may be explained by pregnancy-associated physiologic factors conferring advantage on malaria infection, rather than just a lack of immunity against placental parasites. The objective of the study was to determine the role of maternal immuno-endocrine factors measured at different time points during pregnancy in protection against poor pregnancy outcomes in malaria-exposed pregnant women.

Five-hundred and thirty pregnant women will be enrolled for an open, randomized superiority trial in the context of the Malaria in Pregnancy Prevention Alternative Drugs (MiPPAD) project. Blood samples will be collected before the women receive intermittent preventive treatment in pregnancy doses (first at least 13 weeks into gestation and second at least one month after the previous dose) and at delivery. Antibody-mediated immunity (malaria-specific and general), cellular immunity (effector and memory) and hormonal levels will be determined and associated with pregnancy outcomes.

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Funders:
Fondo de Investigación Sanitaria (FIS), Instituto de Salud Carlos III (ISCIII), Madrid (Spain).
Malaria in Pregnancy Consortium (MiPc), Liverpool School of Tropical Medicine (LSTM), Liverpool (UK).

Duration of the project:
2009-2012

1.8 MiP: The Malaria in Pregnancy Consortium

Eco-MiPPAD: Economic Evaluation of malaria in pregnancy preventive alternative drugs

The economic evaluation of alternative drugs for Intermittent Preventive Treatment in pregnancy (IPTp) is part of a general project of cost-effectiveness of all the preventive interventions of Malaria in Pregnancy within the Malaria in Pregnancy Consortium.

Data collection for this study will be undertaken in Tanzania. However, the overall cost-effectiveness of alternative drugs for IPTp will include all the countries where the MiPPAD trials were taking place (Tanzania, Mozambique, Kenya, Benin and Gabon).

The primary objective of the study was to calculate the incremental cost-effectiveness of mefloquine compared with sulphadoxine-pyrimethamine as a treatment for the prevention of malaria in pregnancy. In the short term, we will consider the cost-effectiveness analysis of the two trials in relation to the intervention's efficacy on mothers' health (in particular on malaria and anaemia during pregnancy) and on neonatal health (birth weight, neonatal mortality and morbidity). In the medium term, the cost-effectiveness analysis of the two trials in relation to the intervention's efficacy on infants' health will be done in terms of the interventions' direct influence on morbidity and mortality reduction during the first year of life, and on the morbidity and mortality reduction as a consequence of a lower prevalence of low birth weight.

The secondary objectives of the project included:

1) Constructing a cost-effectiveness model for the economic evaluation of IPTp, which can provide policy makers with evaluation tools that can be used if changes in the intervention need to be considered in the future.

2) Testing to determine the variables that have the greatest effect on the cost-effectiveness in the estimated model.

3) Determining the levels of several important variables (incidence of malaria, protective efficacy, babies' weight at birth, etc.) beyond which the intervention ceases to be cost-effective.

In 2009 the protocol for the study was written and on-site investigators were recruited. Afterwards, the protocol was submitted to the Institutional Review Board for approval at the Ifakara Health Institute.

Principal investigator:
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Funder:
Malaria in Pregnancy Consortium (MiPc), Liverpool School of Tropical Medicine (LSTM), Liverpool (UK).

Duration of the project:
2009-2014

PregVax: *Plasmodium vivax* infection in pregnancy

The impact of *Plasmodium vivax* infection during pregnancy is studied because approximately 25 million women live in areas where this parasite infection is endemic. Studies try to shed some light on the matter and characterize the effects of *P. vivax* malaria in pregnancy, as was done previously with *Plasmodium falciparum* malaria.

The PregVax research programme is an observational cohort study carried out in five countries where *P. vivax* is endemic: Brazil, Colombia, Guatemala, India and Papua New Guinea. These countries are representative of the majority of infections of this parasite in the world.

Clinical and epidemiological studies and immunological analyses were included in the PregVax research programme in order to:

- Study the prevalence of *P. vivax* infection.
- Study the impact of *P. vivax* malaria on birth weight, premature births and maternal anaemia.
- Study naturally acquired antigen-specific as well as innate non-specific immune responses in pregnant women infected with *P. vivax* parasites, and their association with pregnancy outcomes.

After initial steps had been taken in 2008, the project was definitively launched in 2009. Working tools previously developed were applied in a standardized way for all the participating institutions. Furthermore, recruitment and follow-up of pregnant women participating in the study has been running actively, allowing the planning of the clinical and epidemiological studies, immunological analyses and other pathophysiological components of the project.

2009 reports showed good progress in project development, following the initially proposed timeline. CRESIB's team has monitored the recruitment and the application of working tools with field visits. Moreover, quality studies on recruitment and initial analyses have started to be carried out. As part of the immunology studies, the standard

1.8 MiP: The Malaria in Pregnancy Consortium

operating procedures for the collection, storage and quantification of blood samples have been established, and a selection of *P. vivax* Vir proteins have been cloned and expressed for use in future immunoassays. Furthermore, studies of adhesion of parasites to placental receptors have already begun in Papua New Guinea.

In June 2009, CRESIB's team took part in the 2nd annual meeting of the Malaria in Pregnancy Consortium (MiPc) in Dakar (Senegal), presenting a review of the project status.

Principal investigator:
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Project manager:
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Project assistant:
Yolanda Antín (until August 2009), Cecilia Olmos (from November 2009).

In collaboration with:
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- Carlo Severini. Instituto Superiore di Sanità, Rome (Italy).
- Ivo Müeller. Papua New Guinea Institute of Medical Research, Goroka (Papua New Guinea).
- Swati Kochar. S.P. 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 (Guatemala).
- Myriam Arévalo and Sócrates Herrera. Instituto de Inmunología, Cali (Colombia).
- Meghna Desai. Centers for Disease Control and Prevention, Atlanta (USA).
- Stephen Rogerson. University of Melbourne, Melbourne (Australia).

Funders:
FP7 Programme, European Union.
Malaria in Pregnancy Consortium (MiPc), Liverpool School of Tropical Medicine (LSTM), Liverpool (UK).
Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR), Barcelona (Spain).
Ministerio de Ciencia e Innovación, Madrid (Spain).

Duration of the project:
2008–2012

MiPAnthro: Malaria in Pregnancy Consortium. Public health impact. Anthropology component

The anthropological component of the Malaria in Pregnancy Consortium (MiPc), part of the Public Health Impact research activities, aimed to describe the broader social and cultural context of malaria in pregnancy and its influence on the acceptability and implementation of different MiP treatment and prevention strategies.

This study took an ethnographic approach using narrative and observational qualitative techniques to collect data, and grounded theory methodology to analyse

these data. Data collection involved five sites (four in Africa and one in Papua New Guinea) where different MiPc clinical trials are underway.

The following milestones were reached in 2009:

- Ethical Approvals were obtained from the five sites and from the MiPc Executive Committee.
- Recruitment of personnel was concluded for Malawi, Navrongo, Kisumu and Kumasi.
- Research assistants were trained in the use of the tools designed for the project.
- Data collection tools were adapted to the different contexts and piloted.
- Data collection started at the African sites.

Principal investigator:
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- Ivo Müller. Papua New Guinea Institute of Medical Research, Goroka (Papua New Guinea).

Funder:
Malaria in Pregnancy Consortium (MiPc), Liverpool School of Tropical Medicine (LSTM), Liverpool (UK).

Duration of the project:
2007-2011

Research

- **Malaria**
- Imported Diseases
- HIV/AIDS
- Acute Respiratory Infections and Other Invasive Bacterial Diseases
- Diarrhoeal Diseases
- Other Research Projects
- Incorporation of New Research Teams
- Research Support Services

The term "principal investigator" used in this report refers to the person acting as such within CRESIB, regardless of whether they are the principal investigator of the overall project.

Research

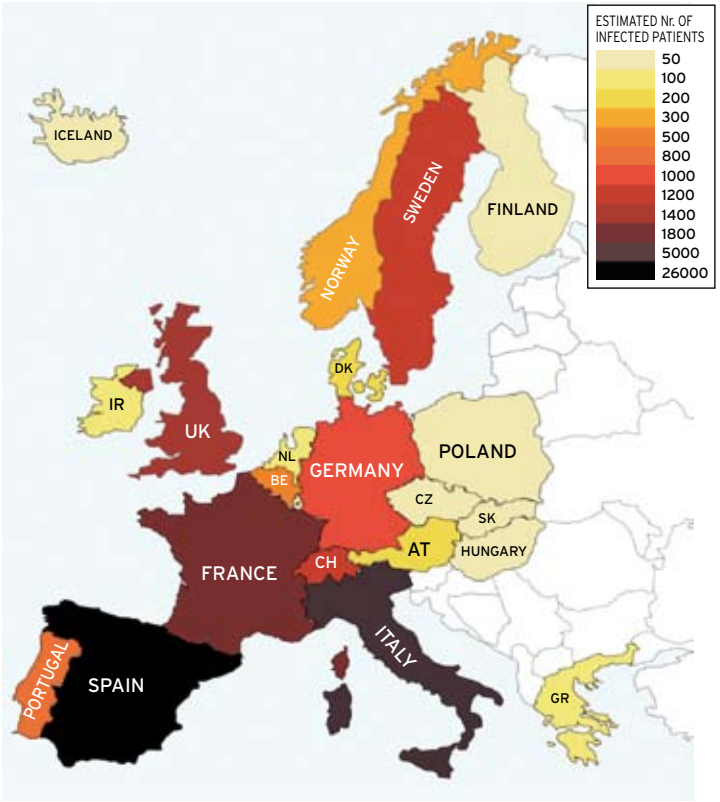
Imported Diseases

Imported Diseases

Emerging infectious diseases that threaten our public health originate from both domestic and international sources, but in recent years imported diseases from abroad have increased significantly in an era in which commerce, travel, ecological change and population movements have acquired a global dimension.

One such disease is American trypanosomiasis or Chagas disease, caused by the parasite *Trypanosoma cruzi*. Chagas disease is an important public health problem in Latin American countries, currently affecting an estimated 10-20 million people. The transmission of *T. cruzi* in areas where it is endemic occurs through a triatomine that releases excreta infected with the parasite into lacerated skin or mucosa. Other main routes of infection are blood transfusion and congenital transmission via infected mothers.
Chagas disease occurs in different phases. The acute form, which lasts from four to eight weeks, is either asymptomatic or causes a mild illness. The disease progresses then to the indeterminate phase, which is asymptomatic and can last for years to decades. Twenty to forty percent of the infected people eventually progress to the chronic phase, developing symptoms which mostly affect the digestive, cardiac and/or nervous system. Up to now only two antiparasitic drugs are efficacious during the acute phase but they are only around 20% efficacious during the chronic phase.

Migration flows from Latin America have substantially changed the epidemiology of the disease. Though it has traditionally been considered a disease of poverty associated with rural poor housing in



Eur Heart J. 2008 Nov; 29(21): 2587-91. Epub 2008 Oct 7. PMID: 18840880

Latin America because the insects are often harboured in thatched roofs and adobe walls, it is now becoming globalized through international migration. Currently there are more than two million Latin American immigrants in Spain, 750,000 of them women of fertile age.

Several epidemiological and clinical studies have been carried out in Barcelona by CRESIB researchers since 2003. In a prospective study performed from 2005 to 2007 it was shown that the prevalence of Chagas disease among Latin American pregnant women was 3.4%, and it rose to 27.5% among Bolivian ones. The mother-to-child transmission rate was 7.3%, very similar to that found in endemic regions. It was also shown that in blood banks in Barcelona, 0.66% of the blood donated by Latin American immigrants was positive for Chagas disease. These studies combined with cost-effectiveness studies led to the taking of public health measures in Spain: since 2005 all blood bank donors who come from endemic countries or are born from mothers who come from endemic countries are screened for Chagas disease. In addition, it is expected that from January 2010 CRESIB will be involved in a Chagas disease screening programme for Latin American pregnant women in Catalonia promoted by the Catalan Government.

Chagas disease research within CRESIB focuses on epidemiological and clinical research, and on looking for markers of progression and/or cure of the disease. CRESIB also collaborates and participates in several imported disease networks:

- TropNetEurop (www.tropnet.net)
- EUNID (www.eunid.eu)
- WHO Global Network for Chagas Elimination

Platform for the comprehensive care of patients with Chagas disease in Cochabamba (Bolivia) and Barcelona (Spain)

Chagas Disease is the third most common parasitic infection worldwide and the first one in Latin American countries. Due to the migratory flows, it is also an important public health challenge in non-endemic countries. Spain is the most affected country in Europe.

As in other related poverty diseases, there is a lack of knowledge on crucial aspects of Chagas disease. The challenges range from public health policies and transmission control programmes to clinical, physiopathological and molecular aspects of the disease. In clinical practice, there is a scarcity of tools to improve the monitoring and medical surveillance of patients suffering from this disease.

The objective of this project was to contribute to the reinforcement and consolidation of Chagas disease programmes in both Bolivia and Catalonia, to improve comprehensive care for the patients and to increase knowledge on epidemiological, clinical, immunological, diagnostic and therapeutic aspects of Chagas disease. The platform develops a global intervention strategy centred on the Chagas programme which combines

measures of direct medical assistance to the patients, specialist training of health professionals from both systems and development of research protocols.

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

Working team for the Platform for the Comprehensive Care of Adult Patients with Chagas Disease

Principal investigator:
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- National Chagas Programme, Ministerio de Salud y Deportes, Cochabamba (Bolivia)

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

Duration of the project:
2008-2010.

Characterization and evaluation of the prothrombotic state in Chagas disease as a predictive marker of recovery after treatment with benznidazole

The association of thromboembolic disease with Chagas disease was described in the first publications on this 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 favours 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, has been postulated.

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. So far, there are no progression markers of the disease after drug therapy, nor other factors that help predict which patients will develop Chagas disease and which will remain unaffected. There is therefore a need to study new markers of disease evolution and to assess the therapeutic response to conventional therapy in our environment.

In view of the above, a research project has been launched on the pathophysiology of thromboembolic events associated with infection by *Trypanosoma cruzi*, consisting of two phases. The first phase is a comparative and cross-sectional case-controlled study designed to measure the association between *T. cruzi* infection and/or Chagas disease and 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 provide a maximum reduction in the genetic variability of the values of the prothrombotic markers. The first results of this phase are under revision in an international journal.

Patient during a visit to the clinic for Chagas disease at the Hospital Clínic de Barcelona

The second phase consists of a cohort study that 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. In 2009 the enrolment of all patients and controls necessary for the study has been completed, and the follow-up of the recruited patients started.

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Funder:
Fundación Mundo Sano, Madrid (Spain).

Duration of the project:
2007-2011

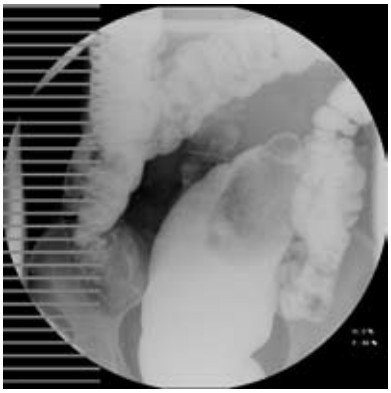
DIGESCHA : Characterization of patients with Chagas disease and gastrointestinal involvement in an endemic area (Cochabamba, Bolivia) and a non-endemic area (Barcelona, Spain)

Gastrointestinal involvement in patients infected with *Trypanosoma cruzi* is up to 20% and causes significant morbidity that requires specific management. However, gastrointestinal symptoms may be due to diseases other than *T. cruzi* infection.

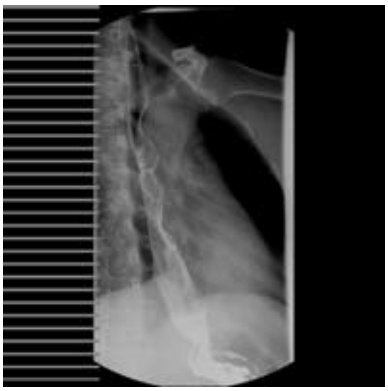
The main objective of this study was to define the prevalence of Chagas gastrointestinal disease in an endemic and non-endemic area by determining the prevalence of colonic and oesophageal involvement in *T. cruzi* infected patients and controls. A secondary objective was to develop new diagnosis strategies, such as oesophageal manometry, for dealing with early stages of the disease.

Helicobacter pylori infection and intestinal pathogens (helminths and other parasites) were investigated in order to find other pathogens that might affect these patients. The final aim of this study was to establish useful guidelines for early diagnosis, management and treatment of gastrointestinal disease in patients with *T. cruzi* infection.

The enrolment of patients and controls in Spain started in June 2009 and will be completed during the first quarter of 2010 (sample size in patients=50, and controls=50). In Cochabamba the enrolment started in December 2009.



Digestive affectation (megacolon) in a patient with Chagas disease



Chagasic esophageal disease

Principal investigator:
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Co-investigators:
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In collaboration with:

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- Jimy Pinto. Plataforma de atención integral al paciente adulto con Enfermedad de Chagas, Cochabamba (Bolivia).
- Jaime Sarabia. Instituto Gastroenterológico Boliviano-Japonés, Cochabamba (Bolivia).
- José Ignacio Elizalde, Fausto Gimeno, Gloria Lacima. Hospital Clínic de Barcelona, Barcelona (Spain).

Funders:
Agencia Española de Cooperación Internacional para el Desarrollo (AECID), Madrid (Spain).
Fundación Mundo Sano, Madrid (Spain).

Duration of the project:
2009-2010

Publications:

- Pinazo MJ, Cañas E, Elizalde JI, García M, Gascón J, Gimeno F, Gomez J, Guhl F, Ortiz V, Posada E D, Puente S, Rezende J, Salas J, Saravia J, Torrico F, Torrus D, Treviño B. Diagnosis, management and treatment of chronic Chagas' gastrointestinal disease in areas where *Trypanosoma cruzi* infection is not endemic. **Gastroenterol Hepatol**. 2010 Mar;33(3):191-200. [*Ahead of print 2009*].

Research

- **Malaria**
- Imported Diseases
- HIV/AIDS
- Acute Respiratory Infections and Other Invasive Bacterial Diseases
- Diarrhoeal Diseases
- Other Research Projects
- Incorporation of New Research Teams
- Research Support Services

The term "principal investigator" used in this report refers to the person acting as such within CRESIB, regardless of whether they are the principal investigator of the overall project.

Research

HIV/AIDS

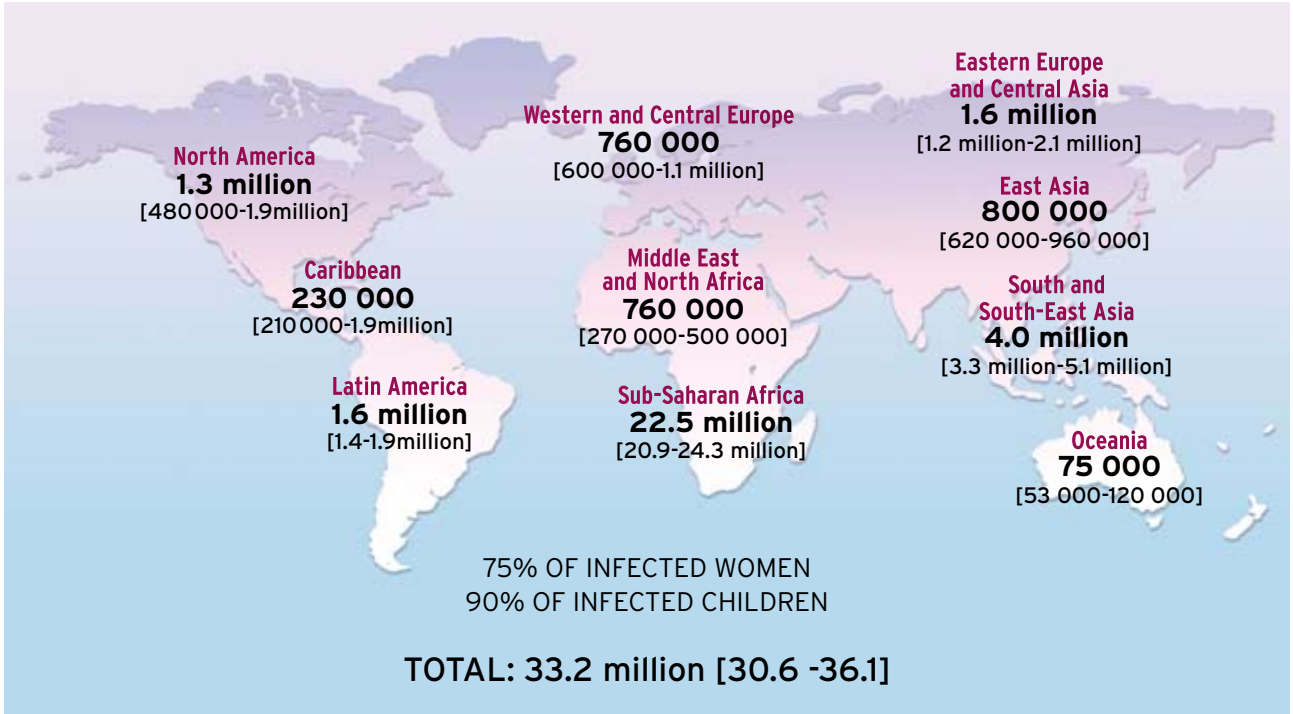
HIV/AIDS

According to the 2009 AIDS Epidemic Update (UNAIDS/WHO), the number of people living with HIV worldwide continued to grow in 2008, reaching an estimated 33.4 million, although important progress has been achieved in preventing new HIV infections and in lowering the annual number of AIDS-related deaths. Sub-Saharan Africa remains the region most heavily affected, accounting for 67% of HIV infections worldwide, 68% of new infections among adults and 91% of new infections among children in 2008. The region also accounted for 72% of the world's AIDS-related deaths in 2008.

CRESIB's research on HIV/AIDS is mostly done in close collaboration with the Centro de Investigação em Saúde de Manhiça (CISM) in Mozambique. According to UNAIDS, national prevalence in Mozambique was estimated at about 12.5%, making it one of the countries of the world that is most affected by HIV/AIDS. Data from the Vertical Transmission Prevention Programme of 2007 in Manhiça showed that more than 25% of pregnant women observed in antenatal visits were seropositive for HIV.

The research activities on HIV/AIDS carried out by CRESIB in 2009 aimed to study mother-to-child transmission of HIV, epidemiology, characterization of recent infections and response to antiretroviral treatment in adults. CRESIB also participates in a Spanish seroconverters multi-centre study addressing questions related to the incubation period and survival from HIV seroconversion. HIV/AIDS research also focuses on socio-cultural aspects of the disease and the acceptability of interventions.

Adults and children estimate to be living with HIV in 2007



From: A global view of HIV infection (UNAIDS) - Estimated adult HIV prevalence for countries in 2007 in: 2008 Report on the global AIDS epidemic (UNAIDS, WHO), ISBN 978 92 9 173711 6.

ENIC: Assessment of immunological parameters and health indicators during the first year of life in HIV-negative children born to HIV-positive mothers in Manhiça (Mozambique)

The prevalence of HIV in pregnant women exceeds 40% in certain regions of sub-Saharan Africa and rates of mother-to-child transmission of HIV vary from 10 to 50%. With such a high prevalence of HIV in pregnant women, HIV-negative children born to HIV-positive mothers will represent an increasing proportion of the African population. HIV exposure has been suggested to induce haematological and immune abnormalities as well as to elicit HIV-specific cell immunity in HIV-negative children born of HIV-infected women. It is thus essential to understand the impact of exposure to HIV on the HIV-negative child born to an HIV-infected mother in the African context. The African context refers to the dominance of HIV-1 clade C virus, a high burden of co-infections and a predominantly breastfeeding population.

This proposal will assess immunological parameters over the first year of life in HIV-negative children born to HIV-positive mothers in Manhiça, a region of southern Mozambique. Parameters assessed will include lymphocyte phenotyping, cell populations and response to routine vaccination. Furthermore, in infants who do not become infected over the first year, HIV-specific CD8 T cell responses will be assessed to investigate whether there is a correlation between the presence of HIV-specific CD8 cells and lack of infection through the breastfeeding period.

Enrolment of mother-infant pairs finished in 2009 and scheduled visits, sample processing and database cleaning will continue through 2010. The patient follow-up will finalize in July 2010. The prevalence of HIV in women delivering in the Manhiça Health Centre was found to be 49%, significantly higher than previous data from the Manhiça antenatal clinic in 2004 showing a prevalence of 23%.

Principal investigator:
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- Nilsa de Deus. Centro de Investigação em Saúde de Manhiça (CISM), Manhiça (Mozambique).

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

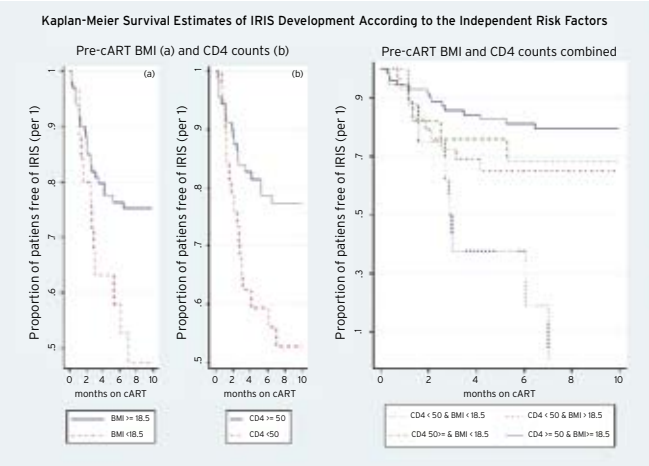
Duration of the project:
2007–2010

RITA: Evaluation of immune reconstitution following initiation of highly active antiretroviral treatment in Manhiça (Mozambique)

The HIV pandemic affects over 30 million people worldwide, with over 67% of the burden being in sub-Saharan Africa. Mozambique is one of the countries included in the programme of roll-out of antiretroviral treatment led by the World Health Organization. Although there is ample experience in implementation of highly active anti-retroviral therapy (HAART) from Western countries, regimens and approaches to therapy cannot simply be transposed to African countries. In particular, there are different subtypes of HIV-1 and most basic knowledge on HIV biology and immune responses comes from European patients infected with HIV-1 clade B, whereas the main circulating viruses in sub-Saharan Africa are HIV-1 clades C, A and D. Furthermore, the burden of co-infections is much greater in sub-Saharan Africa and could lead to different responses to HAART. Thus, baseline information on kinetics of immune restoration after HAART and dynamics of opportunistic
diseases is needed in order to design innovatives strategies for antiretroviral therapy specific to the African context.

This protocol evaluated immune parameters in patients enrolled in the HAART programme in Manhiça, Mozambique over a 16 month period. The evaluation included assessment of the dynamics of restoration of a functional immune system and the incidence and morbidity associated with immune reconstitution inflammatory syndrome (IRIS).

IRIS is observed in patients who demonstrate a good virological and immunological response to HAART but experience a paradoxical clinical worsening. In IRIS patients, the rapid restoration of functionally active antigen-specific cells following HAART is hypothesized to



This figure shows the survival estimates of IRIS development according to the independent predictors identified. Forty-five percent of patients with a CD4 count lower than 50 cells/µl at ART initiation and 55% of patients with a body mass index (BMI) lower than 18.5 had developed IRIS by 10 months on ART. When combining both predictors all patients with a CD4 count lower than 50 cells/µl at ART initiation and a BMI lower than 18.5 developed IRIS by 7 months on ART.

initially lead to an immunopathological rather than a protective effect, resulting in worsening of a known condition or an atypical presentation of unrecognized opportunistic infections.

Initial results of this study have shown a prevalence of 11.6% of IRIS associated with Kaposi sarcoma (KS). Multivariate analysis identified four independent IRIS-KS predictors: pre-treatment KS, detectable plasma Kaposi sarcoma herpesvirus DNA, anaemia and plasma HIV-1 RNA viral load.

Preliminary results have also shown a better prognosis overall in terms of immunological and health indicators for those patients who control viral load at four months as opposed to those who achieve undetectable viral load at later time points.

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In collaboration with:
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- Carla Carrilho. Department of Pathology, Hospital Central de Maputo, Maputo (Mozambique).
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Funders:
Fundació "la Caixa", Barcelona (Spain).
Agència Catalana de Cooperació al Desenvolupament (ACCD), Barcelona (Spain).

Duration of the project:
2005-2009

Publications:
- Letang E, Almeida JM, Miró JM, Ayala E, White IE, Carrilho C, Bastos R, Nhampossa T, Menéndez C, Campbell TB, Alonso P L, Naniche D. Predictors of immune reconstitution inflammatory syndrome-associated with kaposi sarcoma in Mozambique: a prospective study. **J Acquir Immune Defic Syndr.** 2010 Apr;53(5): 589-97. [*Ahead of print* 2009].

Afrevacc: The African-European HIV vaccine development network

The African-European HIV Vaccine Development Network (Afrevacc) is a partnership of European and African institutions created to build capacity for and conduct HIV vaccine trials in African countries. CRESIB is working specifically in collaboration with the Centro de Investigação em Saúde de Manhiça (CISM) in Mozambique to implement the following specific objectives:

1) To evaluate age-specific community prevalence and incidence of HIV.

2) To determine HIV viral load levels established in recent infections.

3) To assess the feasibility and community acceptability of a potential HIV vaccine which is likely not to induce sterilizing immunity from infection but will be protective of disease.

4) To identify channels of recruitment of low and moderate risk groups for potential future phase I/II trials of HIV vaccines.

Further objectives include initiating training activities and other activities designed to assure the quality of the procedures with cellular immunology samples, and to standardize 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; feasibility and attitudes towards an HIV vaccine trial; and acute and recent HIV infections. The epidemiology study seeks to assess age-specific prevalence and incidence of HIV in the community. The protocol entitled "Establishment of community prevalence of Human Immunodeficiency Virus Infection and Sexually Transmitted Infections in Manhiça district, southern Mozambique (AFEPI)" was approved by the Mozambican ethics committee in September 2009 and the first cross-sectional prevalence study will begin in the first quarter of 2010. During 2009, the feasibility study was in preparation and will be conducted in 2010.

The study of acute and recent HIV infections began in 2008 and is entitled "Characterization of acute HIV infection and viral load setpoint in adults in Manhiça, Mozambique (PRISMA)". The identification and monitoring of patients with acute HIV infection was performed in 2008 and 2009. HIV serological testing among adults presenting at the outpatient ward with reported fever revealed that 37.8% of them had previously undiagnosed established HIV infection. Among the HIV-seronegative patients, a high proportion were found to have acute HIV infection (3.3%). Recruitment for the identification of recent HIV infections for determining viral load setpoint has been underway since April 2009 and follow-up will continue until August 2010.

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- Jonathan Weber. Imperial College, London (UK).
- 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 Programme, 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 (UK).
- Jim Tartaglia. Sanofi Pasteur SA, Lyon (France).
- Hans Wolf. Institute for Medical Microbiology and Hygiene, Universität Regensburg, Regensburg (Germany).

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

Duration of the project:
2007–2011

Publications:
- Serna-Bolea C, Muñoz J, Almeida JM, Nhacolo A, Letang E, Nhampossa T, Ferreira E, Alonso P, Naniche D. High prevalence of symptomatic acute HIV infection in an outpatient ward in southern Mozambique: identification and follow-up. **AIDS**. 2010 Feb 20;24(4):603-8. [*Ahead of print* 2009].

GEMES: Spanish seroconverters multi-centre study group. Study of AIDS incubation period and survival from HIV seroconversion in seroconverter cohorts

GEMES is a multi-centre project involving epidemiologists, statisticians and virologists who collect data from more than 2500 HIV seroconverters from 10 cohorts in Spain. The majority of GEMES cohorts were established in the late 1980s but they were not articulated until 1998, achieving full development since 1999. GEMES is the only cohort of HIV seroconverters in Spain and one of the largest in Europe. In addition, GEMES is an active member of the European seroconverter cohort project CASCADE (Concerted Action on Seroconversion to AIDS and Death in Europe), of COHERE (Collaboration of Observational HIV Epidemiological Research in Europe) and of EUROCOORD (European Coordinating Committee for the Integration of Ongoing Coordination Actions Related to Clinical and Epidemiological HIV Research). GEMES also collaborates with an HIV-Causal project funded by the National Institutes of Health and coordinated by the Harvard Public Health School.

Since the beginning the main objective of GEMES has been to monitor the AIDS incubation period, survival from HIV seroconversion and the evolution of progression biomarkers in different time periods, as well as to analyse the influence of variables (treatment, prevention, etc.) on the duration of these periods.

We have been working on the evaluation of the effectiveness of highly active antiretroviral therapy, the effects of changes in treatments, gender differences, competing risk mortality and the application of new methodologies.

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Co-investigator:
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In collaboration with:
- Julia del Amo. Instituto de Salud Carlos III (ISCIII), Madrid (Spain).
- Robert Muga. Hospital Universitari Germans Trias i Pujol, Badalona (Spain).
- Patricia García de Olalla. Agència de Salut Pública de Barcelona (Spain).
- Kholoud Porter. CASCADE Collaboration, Oxford (UK).
- Miguel Hernan. HIV-Causal Collaboration. Harvard Public Health School, Boston (USA).
- Genevieve Chêne. COHERE Collaboration. Université Victor Segalen, Bordeaux (France).

Funders:
Fundación para la Investigación y Prevención del SIDA en España (FIPSE), Madrid (Spain).
Fondo de Investigación Sanitaria (FIS), Instituto de Salud Carlos III (ISCIII), Madrid (Spain).
Proyectos de investigación en evaluación de tecnologías sanitarias y servicios de salud, Instituto de Salud Carlos III (ISCIII), Madrid, Spain.

Duration of the project:
1998–2011

Publications:
- Hurtado I, Alastrue I, García de Olalla P, Albiach D, Martín M, Pérez-Hoyos S. [Preventive intervention in venues for interaction used by men who have sex with men]. **Gac Sanit.** 2010 Feb;24(1):78-80. [*Ahead of print* 2009].
- Jarrín I, Geskus R, Pérez-Hoyos S, del Amo J. [Analytical methods in cohort studies of patients with HIV infection]. **Enferm Infecc Microbiol Clin.** 2010 May;28(5):298-303. [*Ahead of print* 2009].

MDP: Social science component of the microbicides development programme

A multi-centre phase III clinical trial of a vaginal microbicide for the prevention of HIV infection was conducted through six participating research centres in four countries (Uganda, South Africa, Zambia and Tanzania). The trial enrolled 9,385 women, was completed in October 2009 and the results were announced in November 2009. The trial demonstrated conclusively that the product "PRO2000" was not effective in preventing HIV infection.

The trial included a large integrated social component focusing on studying adherence, acceptability of the product, the understanding of informed consent and the accuracy of key trial data. The social science team developed and used a comprehensive mixed-method and triangulation model—involving structured questionnaires, coital diaries and in-depth interviews– to collect more accurate and more detailed data on adherence and sexual behaviour. This was the most extensive use of mixed methods and triangulation ever used in the context of a clinical trial.

The evidence from an evaluation of this process (2 papers have been submitted) revealed significant inaccuracies in the behavioural and adherence data collected using the conventional structured interview and case record form in a clinic setting –the main source of such data in most phase III HIV prevention trials–. However, the data also showed that these inaccuracies are largely unintentional, and that it is possible to identify them relatively easily through the use of mixed methods and triangulation and to correct most of them during the study through dialogue with the participants.

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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).
- Mitzy Gafos. Africa Centre for Health and Population Studies, KwaZulu-Natal (South Africa).
- Jonathan Weber. Imperial College, London (UK).
- Richard Hayes. London School of Hygiene and Tropical Medicine, London (UK).

- Khatia Munguambe. Centro de Investigação em Saúde de Manhiça, Manhiça (Mozambique).
- Sibone Mocumbi. Maputo Central Hospital, Maputo (Mozambique).
- Sheena MacCormack. Medical Research Council, London (UK).
- Andrew Nunn. St George's Hospital Medical School, London (UK).
- Richard Mutemwa. University of Southampton, Southampton (UK).
- Charles Lacey. University of York, York (UK).

Funder:
UK Department for International Development (DFID), Medical Research Council (MRC), London (UK).

Duration of the project:
2006-2009

Publications:

- Nunn A, McCormack S, Crook AM, Pool R, Rutterford C, Hayes R. Microbicides Development Programme: design of a phase III trial to measure the efficacy of the vaginal microbicide PRO 2000/5 for HIV prevention. **Trials**. 2009;10:99.

Research

- **Malaria**
- Imported Diseases
- HIV/AIDS
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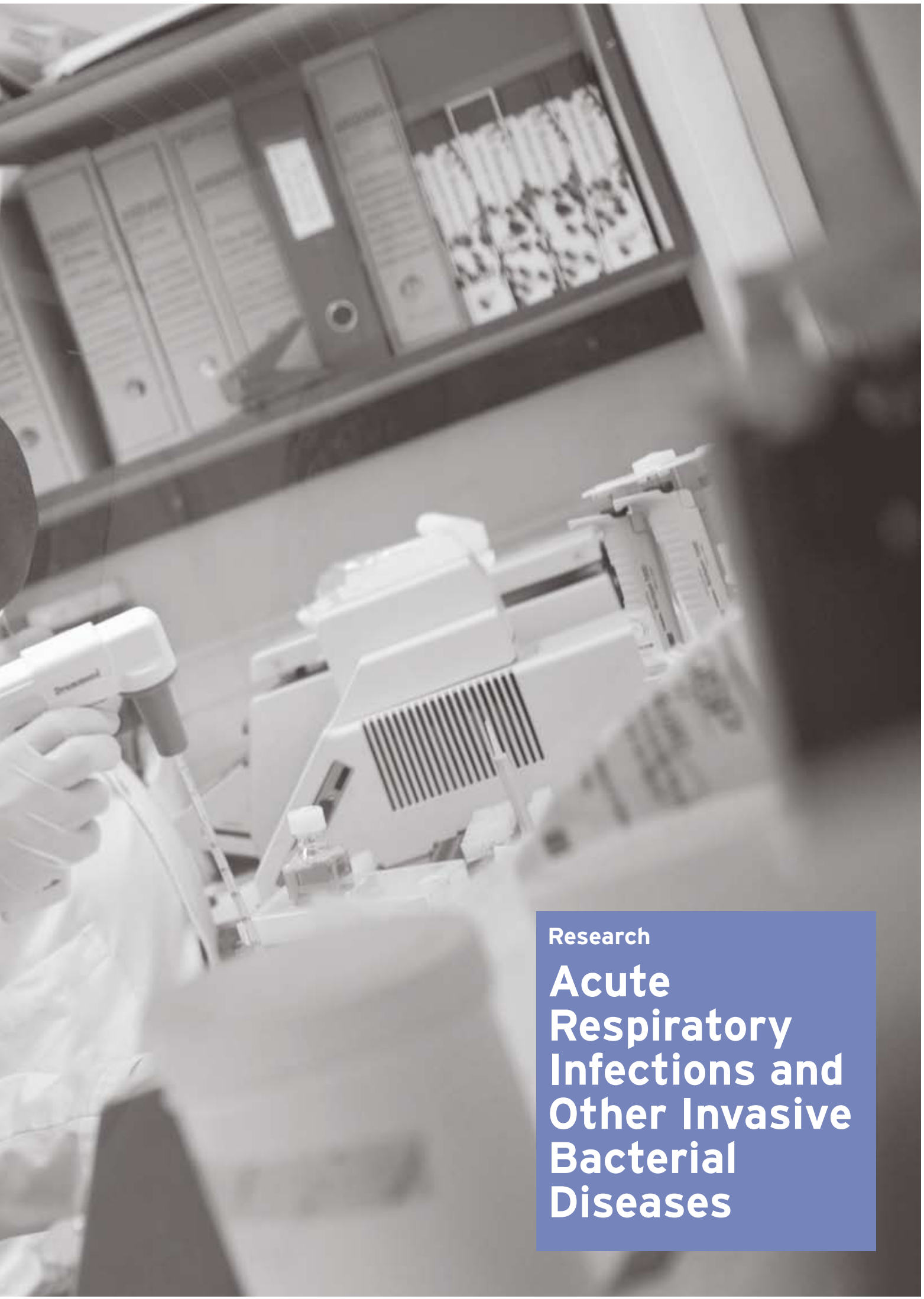


Research

Acute Respiratory Infections and Other Invasive Bacterial Diseases

Acute Respiratory Infections and Other Invasive Bacterial Diseases

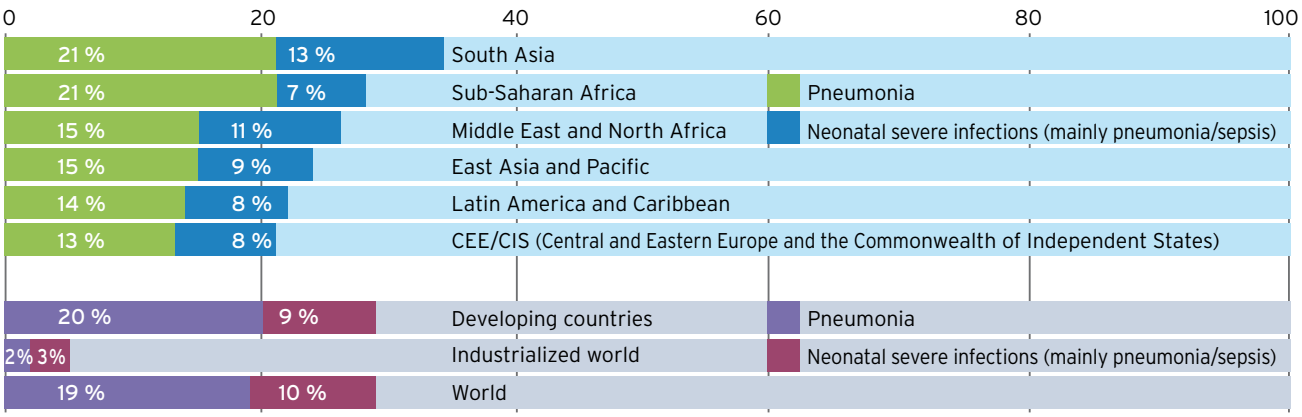
Invasive Bacterial Diseases are caused by *Haemophilus influenza type b* (Hib), *Streptococcus pneumoniae* and *Neisseria meningitidis*, among others. These organisms cause diseases with a variety of clinical presentations, including those of the brain (meningitis), lung (pneumonia) and blood stream (sepsis).

According to the World Health Organization, about 20% of all deaths in children under 5 years of age are due to acute lower respiratory infections (pneumonia, bronchiolitis and bronchitis), of which 90% are caused by pneumonia. Pneumonia kills more children under 5 years of age than any other illness in every region of the world.

Pneumonia can be caused by bacteria (most commonly *Streptococcus pneumoniae* and *Haemophilus influenza type b)*, viruses (respiratory syncytial virus, influenza and others) or fungi (especially *Pneumocystis jiroveci*, responsible for at least a quarter of all pneumonia deaths in HIV-infected infants). Key prevention measures include promoting adequate nutrition (including exclusive breastfeeding and zinc intake), reducing indoor air pollution and vaccination. Three vaccines have the potential to save millions of children's lives by reducing the incidence of pneumonia caused by the bacterial pathogens *Streptococcus pneumoniae* (pneumococcal conjugate vaccine) and *Haemophilus influenzae type b* (Hib vaccine), and by serious complications from measles (measles vaccine).

CRESIB research on acute respiratory infections and other invasive bacterial diseases focuses on improvement of diagnosis, epidemiological surveillance and evaluation of control strategies. Research is mainly done in collaboration with the Centro de Investigação em Saúde de Manhiça in Mozambique and with l´Hôpital d'Enfants de Rabat in Morocco.

Percent Total of Children Under Five Deaths (Source: UNICEF)



From: Pneumonia: The forgotten killer of children, 2006. Ed. UNICEF, WHO. ISBN-13: 978-92-806-4048-9. ISBN-10: 92-806-4048-8.

IBI: Surveillance of invasive bacterial infections among paediatric admissions in Manhiça, rural Mozambique

Invasive bacterial infection (IBI) is a leading cause of under-5 mortality worldwide, and many of the deaths are vaccine-preventable. However, coverage of the life-saving vaccines in Africa is poor, among other reasons, because of lack of local data on the disease burden, which is responsible for poor recognition of the disease. Aetiological diagnosis of bacterial infections in Africa is usually not investigated, due to the lack of facilities to do microbiological diagnosis.

Surveillance on IBI has been carried out among children under the age of 15 years admitted to the Manhiça District Hospital in collaboration with the Centro de Investigaçâo em Saúde de Manhiça (CISM) since 1998. Surveillance data indicated that non-typhoidal *Salmonella*, *Streptococcus pneumoniae* (pneumococcus), *Haemophilus influenzae* and *Neisseria meningitidis* (meningococcus) might play an important role in child mortality as a cause of bacteremia or septicaemia, acute bacterial meningitis and invasive bacterial pneumonia.

Benchtop automated hemoculture incubator in the bacteriology laboratory at CISM.

Principal investigators:
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Co-investigators:
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In collaboration with:
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Funders:
University of Maryland, Baltimore (USA).
World Health Organization (WHO), Geneva (Switzerland).
PATH, Seattle (USA).
Pneumococcal Vaccines Accelerated Development and Introduction Plan (PneumoADIP), John Hopkins University, Baltimore (USA).

Duration of the project:
1998-2009

Publications:

- Mandomando I, Macete E, Sigaúque B, Morais L, Quintó L, Sacarlal J, Espasa M, Vallès X, Bassat Q, Aide P, Nhampossa T, Machevo S, Ruiz J, Nhacolo A, Menéndez C, Kotloff KL, Roca A, Levine MM, Alonso PL. Invasive non-typhoidal *Salmonella* in Mozambican children. **Trop Med Int Health**. 2009 Dec;14(12):1467-174.
- Roca A, Bassat Q, Morais L, Machevo S, Sigaúque B, O'Callaghan C, Nhamposa T, Letang E, Mandomando I, Nhalungo D, Quintó L, Alonso P. Surveillance of acute bacterial meningitis among children admitted to a district hospital in rural Mozambique. **Clin Infect Dis**. 2009 Mar 1;48 Suppl 2:S172-80.
- Sigaúque B, Roca A, Bassat Q, Morais L, Quintó L, Berenguera A, Machevo S, Bardaji A, Corachan M, Ribó J, Menéndez C, Schuchat A, Flannery B, Soriano-Gabarró M, Alonso PL. Severe pneumonia in Mozambican young children: clinical and radiological characteristics and risk factors. **J Trop Pediatr**. 2009 Dec; 55(6):379-87.
- Sigaúque B, Roca A, Mandomando I, Morais L, Quintó L, Sacarlal J, Macete E, Nhamposa T, Machevo S, Aide P, Bassat Q, Bardají A, Nhalungo D, Soriano-Gabarró M, Flannery B, Menendez C, Levine MM, Alonso PL. Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. **Pediatr Infect Dis J**. 2009 Feb;28(2):108-13.
- Vallès X, Sarrias M, Casals F, Farnós M, Piñer R, Suárez B, Morais L, Mandomando I, Sigaúque B, Roca A, Alonso PL, Torres A, Thielens NM, Lozano F. Genetic and structural analysis of *MBL2* and *MASP2* polymorphisms in south-eastern African children. **Tissue Antigens**. 2009 Oct; 74(4):298-307.
- Bassat Q, Guinovart C, Sigaúque B, Mandomando I, Aide P, Sacarlal J, Nhampossa T, Bardají A, Morais L, Machevo S, Letang E, Macete E, Aponte JJ, Roca A, Menéndez C, Alonso PL. Severe malaria and concomitant bacteraemia in children admitted to a rural Mozambican hospital. **Trop Med Int Health**. 2009 Sep; 14(9):1011-19.

Molecular epidemiology of meningococcal disease over an 11-year period in Manhiça

Data on the aetiology of acute bacterial meningitis (ABM) in children under the age of 15 have been available from the Manhiça District Hospital, in collaboration with the Centro de Investigação em Saúde de Manhiça (CISM), since 1998. These data point at *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis* as the three main causative agents of the invasive disease. However, whereas disease caused by the first two pathogens has remained stable, the number of cases of meningococcal disease caused by the third has increased substantially in recent years.

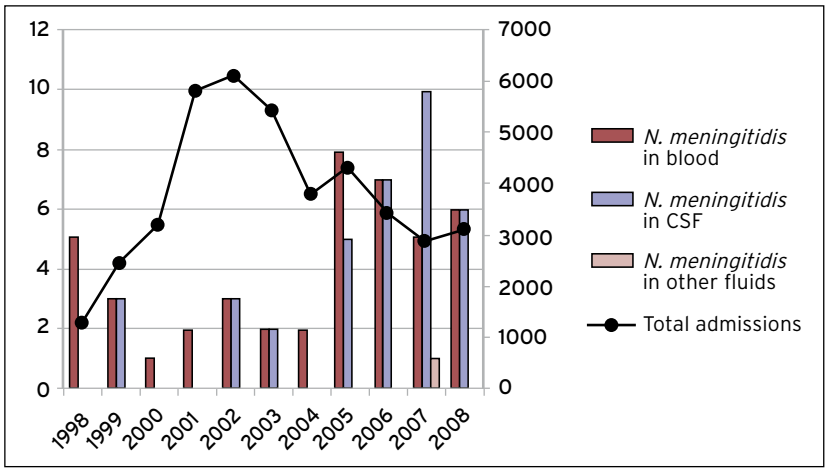
Meningococcal strains collected from 1998 to 2008 and stored at the CISM were characterized using molecular biology techniques and tested for antibiotic susceptibility to drugs commonly used in Mozambique for the treatment of ABM. The aim of the study was to genetically characterize the strains causing disease

in Manhiça over a period of 11 years, in order to ascertain whether a serogroup and strain replacement had taken place in recent years, and to detect the emergence of antibiotic-resistant strains.

Our results revealed a higher incidence of meningococcal disease than would be expected for the location of Manhiça. A substantial increase in the number of cases occurred in 2005 and was sustained throughout the two following years. The strain responsible for this increase is a serogroup W-135 meningococcus that was associated with a global outbreak related to the Hajj (pilgrimage to Mecca) in 2000, and was responsible for a similar upsurge in South Africa. Our data suggest that this strain might have been endemic in our study area before it became globally spread. Serogroup A and serogroup Y strains were also detected but represented a minor fraction of cases.

N. meningitidis isolated in Manhiça remained susceptible to the antibiotics used to treat ABM. However, we detected the presence of penicillin-resistant strains, which to date are rarely reported from anywhere else in Africa, and high levels of resistance to cotrimoxazole, which is administered as a preventive treatment for pneumonia in HIV-positive patients.

This study will be the first reliable published data on meningococcal disease in Mozambique, and advocates for the development of vaccination strategies against serogroup W-135 for African countries.



Total number of *N. meningitidis* isolated from blood, CSF (Cerebrospinal fluid) and other sterile fluids vs. total number of admissions to the MDH (Maniça District Hospital) for the 11 years of study surveillance (1998-2008).

Principal investigator:
Anna Roca

Co-investigators:
Ana Belén Ibarz, Luis Morais, Inácio Mandomando, Quique Bassat, Betuel Sigaúque, Ariel Nhacolo, Llorenç Quintó, Pedro L. Alonso

In collaboration with:
- Montse Soriano-Gabarró. GlaxoSmithKline Biologicals, Rixensart, Belgium.

Funder:
GlaxoSmithKline Biologicals, Rixensart, Belgium.

Duration of the project:
1998-2009

Aetiology, epidemiology and clinical presentation of acute respiratory infections in Mozambican children under 5 years of age

The role of viruses in paediatric pneumonia remains poorly studied, especially in sub-Saharan Africa, where at least 900,000 child deaths per year are attributed to pneumonia.

The aim of the study was to define the aetiology, epidemiology, molecular epidemiology and clinical features of viral respiratory infections among children under 5 years of age admitted to the Manhiça District Hospital with signs/symptoms of clinical severe pneumonia. The study has several objectives:

1) To calculate the incidence of viral infection by age group.

2) To describe the seasonality of each virus.

3) To calculate the case fatality rate associated with viral infections.

4) To describe associations with other common infections in the area (invasive bacterial infections, malaria and HIV).

5) To calculate the minimum incidence rate of each virus among HIV-infected and HIV-uninfected children.

During a 12-month period (September 2006 to September 2007), we collected nasopharyngeal aspirates from children

	Total n	RV n (%)	ADV n (%)	RSV n (%)	hMPV n (%)	Flu n (%)	PIV n (%)	EV n (%)	Co-infection* n (%)	*P* value
Total	394	394	135 (34) †	38 (10) †	29 (7) †	28 (7) †	20(5) †	10 (3) †	77 (20) †	
Age (months)										
< 3	50 (13)	50 (13)	14 (10)	11 (29)	6 (21)	4 (14)	4 (20)	3 (30)	7 (9)	
3 - 12	149 (38)	149 (38)	67 (50)	16 (42)	16 (55)	7 (25)	7 (35)	3 (30)	21 (27)	
12 - < 60	195 (49)	195 (49)	54 (40)	11 (29)	7 (24)	17 (61)	9 (45)	4 (40)	49 (64)	**< 0.001**
Sex (male)	250 (63)	250 (63)	85 (63)	22 (58)	20 (69)	18 (64)	12 (60)	6 (60)	52 (68)	0.974
Rainy Season	252 (64)	252 (64)	76 (56)	24 (63)	15 (52)	24 (86)	15 (75)	9 (90)	55 (71)	**0.015**
IBI ¶	38 (11)	38 (11)	16 (13)	1 (3)	3 (11)	1 (4)	1 (5)	0	10 (16)	0.408
Parasitemia ‡	57 (15)	57 (15)	19 (14)	2 (5)	3 (11)	2 (7)	4 (20)	0	16 (21)	0.181
HIV infected §	67 (25)	67 (25)	26 (30)	3 (10)	7 (33)	4 (25)	3 (19)	1 (14)	14 (25)	0.475
CFR ¥	33 (9)	33 (9)	10 (8)	2 (6)	3 (11)	1 (4)	1 (6)	1 (11)	11 (15)	0.627

† Percentages among the number of virus infected children (n=394).
¶ Percentages among children tested for IBI and with valid results (n=345)
‡ Percentages among children tested for parasitemia (n=389)
§ Percentages among children with available HIV results (n=270)
¥ Percentages among children with known outcome (n=359)

RV= Rhinovirus
ADV= Adenovirus
RSV= Respiratory syncytial virus
hMPV= Human metapneumovirus
PIV= Parainfluenza virus
EV= Enterovirus
IBI= Invasive bacterial infection
CFR= Case fatality rate

Epidemiological characteristics of different isolated viruses and viral coinfections detected among children <5 years of age admitted to MDH (Maniça District Hospital) with viral pneumonia.

attending the Manhiça District Hospital and meeting clinical criteria of respiratory distress. Viral diagnosis was performed using molecular techniques (polymerase chain reaction and real-time polymerase chain reaction). Viral detection included the following pathogens: respiratory syncytial virus, influenza and para-influenza viruses, metapneumovirus, rhinovirus, adenovirus and enterovirus. Study patients were also tested for invasive bacterial infections (through blood culture), HIV infection, and *Plasmodium falciparum* parasitaemia. Viral aetiologies were detected in almost half of the children participating in the study, the most prevalent viruses being rhinovirus and adenovirus. Overall HIV prevalence among children with viral infection was 25% and mortality was 9%, being highest among cases of invasive bacterial co-infection (OR=7, $P<0.001$) or HIV infection (OR=7, $P<0.001$).

Sample collection, laboratory (including viral and bacterial diagnosis) and statistical analysis has been completed on 2009. The epidemiological manuscript entitled "Aetiology and epidemiology of viral pneumonia among hospitalized children in rural Mozambique, a malaria-endemic area with high HIV prevalence" is expected to be finished on 2010.

Collecting nasopharyngeal aspirates from a child in the Manhiça District Hospital

Principal investigator:
Anna Roca

Co-investigators:
Cristina O'Callaghan, Núria Diez, Quique Bassat, Sonia Machevo, Llorenç Quintó, Luis Morais

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

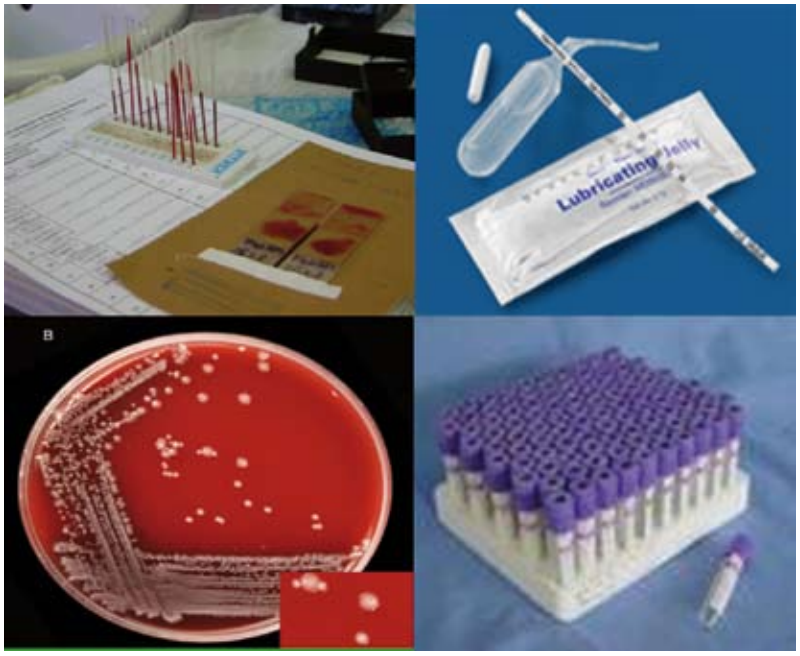
Duration of the project:
2005-2009

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

Diagnosis of common infections is still a challenge in settings of rural Africa where clinical and laboratory facilities are scarce. Health workers are daily confronted with this limitation when selecting treatment for children with infectious diseases. The lack of appropriate diagnostic tools is of special importance in cases that present with fever and other unspecific symptoms. As a consequence, diagnoses are often incorrect, leading to inappropriate treatment.

Biomarkers are used to support clinical diagnosis in developed countries and could be suitable for determining the aetiology of infections in rural Africa if used as rapid diagnostic tests (RDTs). Malaria and HIV RDTs are examples of feasibility of these tools in resource-limited settings. The project presented here explores levels of different cytokines and other proteins in blood to differentiate malaria, bacterial and viral infections in developing countries.

The study involved children under 5 years admitted to the Manhiça District Hospital with unspecific signs and symptoms of



Example of samples collected from the children recruited at the hospital. From top to bottom and from left to right: blood for determination of malaria and hematocrit, nasopharyngeal aspirate for viral determination, bacterial results from a blood culture sample and blood in EDTA to measure levels of biomarkers.

illness and fever. Child recruitment and sample processing has already finished on 2009, and healthy controls from the community have also been recruited. Data were analysed and results were reported in 2009. Attention focused on malaria and bacterial and viral pneumonia, and the biomarkers studied were PCT, CRP, EPO and G-CSF. Preliminary analyses indicate that for these markers there is a difficulty in differentiating the aetiologies mentioned above. However, PCT differentiates viral from bacterial pneumonia in the absence of malaria parasites.

Principal investigator:
Anna Roca

Co-principal Investigator:
Pedro L. Alonso

Co-investigators:
Núria Diez, Sonia Machevo, Quique Bassat, Cristina O'Callaghan, Luis Morais, Ruth Aguilar, Llorenç Quintó

In collaboration with:
- Antoni Torres. Hospital Clínic de Barcelona-Universitat de Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona (Spain).

Funder:
Bill & Melinda Gates Foundation, Seattle (USA).

Duration of the project:
2007-2009

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 clinical guidelines and political recommendations in malaria-endemic areas.

Few places in rural Africa are equipped with the necessary infrastructure to accurately diagnose malaria and/or pneumonia. The study was performed by the Centro de Investigação em Saúde de Manhiça (CISM) and CRESIB at the Hospital Distrital de Manhiça in Mozambique, where the CISM has well equipped laboratories and X-ray equipment that allow the diagnosis of both diseases.

This study collected detailed epidemiological, clinical, radiographic, and microbiological data among children under 5 years of age with a clinical suspicion of pneumonia admitted to Manhiça's Hospital Distrital during a one-year period. This has permitted a detailed and very precise aetiological classification of all recruited patients. Preliminary results have shown a great overlap of malaria with viral or bacterial infections, and risk factors for either malaria or bacterial pneumonia have been identified. The relevance of

HIV infection has also been assessed and HIV infection has been found to be more prevalent among bacterial pneumonia cases. Finally, on the basis of the risk factors found, clinical signs and symptoms in combination with laboratory determinations have been proposed as part of clinical and laboratorial algorithms for a more specific and sensitive aetiological classification in areas where chest X-ray or microscopy procedures are seldom available.

Principal investigator:
Anna Roca

Co-investigators:
Quique Bassat, Cristina O'Callaghan, Sonia Machevo, Luis Morais, Betuel Sigaúque, Llorenç Quintó, Sergi Sanz

In collaboration with:
- Martin W. Weber. World Health Organization (WHO), Geneva, Switzerland.

Funder:
World Health Organization (WHO), Geneva, Switzerland.

Duration of the project:
2006-2009

Measuring the effectiveness of introducing Hib conjugate vaccine into the routine immunization schedule in Mozambique

Disease caused by *Haemophilus influenza* type b (Hib) was a leading cause of pneumonia and meningitis among young children in industrialized countries prior to the introduction of the conjugate vaccine against the bacterium. Resource-poor countries, however, need data on the true incidence of the disease to justify the high costs associated with the vaccine.

Data on the incidence of Hib disease generated at the Centro de Investigaçâo em Saúde de Manhiça (CISM) in collaboration with CRESIB were decisive for the GAVI alliance to grant Mozambique financial support for the introduction of the Hib conjugate vaccine in the infant vaccination schedule. The vaccine was gradually introduced in the country in June 2009, and reached Manhiça District in August.

The study aimed to evaluate the impact of Hib vaccine introduction (effectiveness of the vaccine) by:

- Measuring incidence of Hib disease before and after the introduction of the conjugate vaccine.
- Quantifying the reduction in the risk of Hib disease (invasive or pneumonia) among vaccinated individuals through a case-control approach which started after vaccine introduction.

The study will also evaluate the effect of vaccine introduction on the overall mortality rate among infants aged 2-24 months.

Additionally, the study allowed the

introduction of the real-time polymerase chain reaction technique for detecting the three main causative pathogens of acute invasive meningitis—*Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis*—directly from cerebrospinal fluid samples. The use of this technique will allow us to detect an additional number of cases of invasive Hib disease that would remain undetected using standard microbiology techniques.

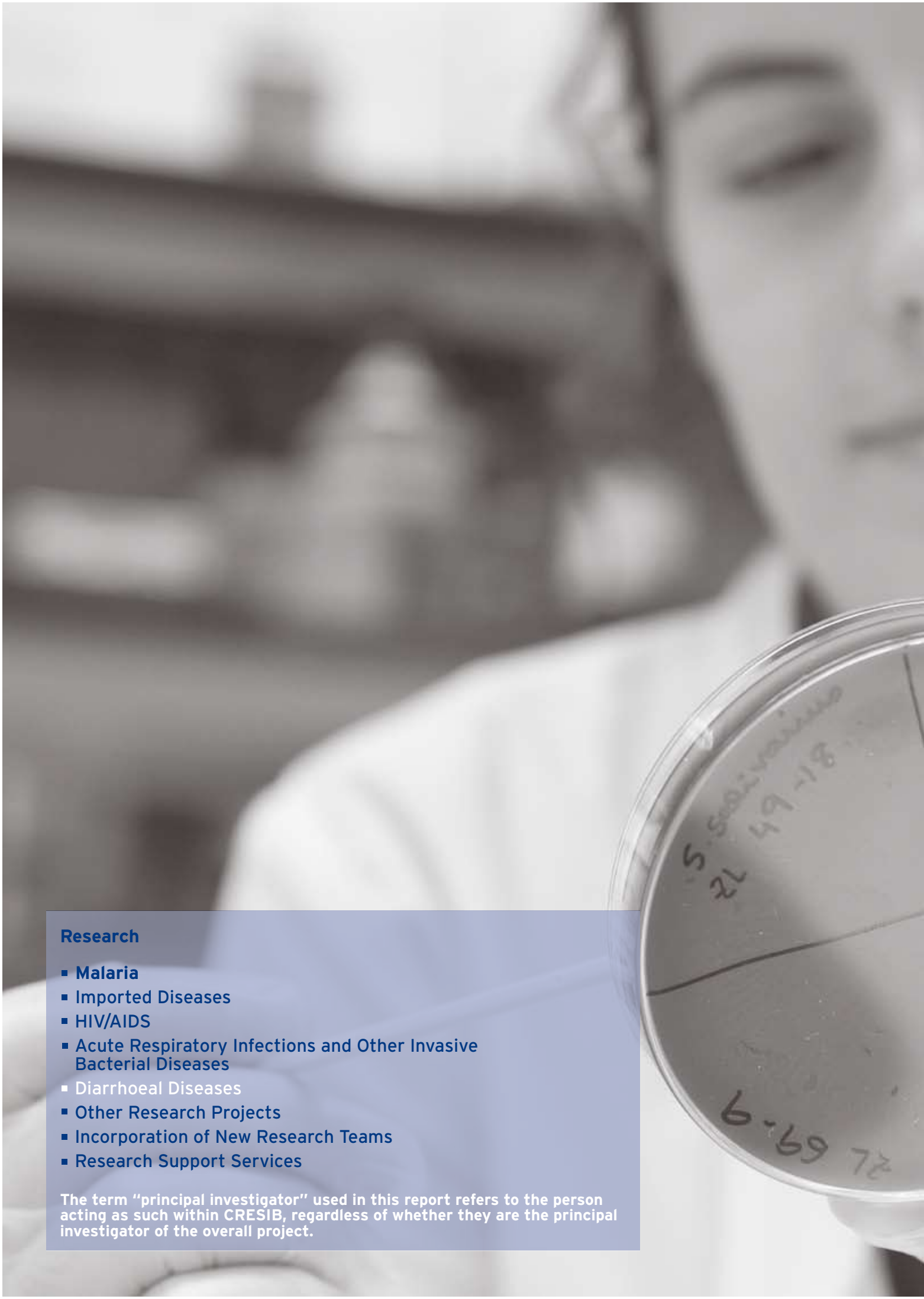
Principal investigator:
Anna Roca (until September 2009), Betuel Sigaúque (from September 2009)

Co-investigators:
Ana Belén Ibarz, Luis Morais, Sozinho Acacio, Delfino Vuvil, Inácio Mandomando, Charfudin Sacoor, Hélder Martins, João Fumane, Pedro L. Alonso

In collaboration with:
- Gloria Carvalho and Jennifer Verani. Centers for Disease Control and Prevention (CDC), Atlanta (USA).

Funder:
The Hib Initiative, John Hopkins University, Baltimore (USA).

Duration of the project:
2008-2011



Research

- **Malaria**
- Imported Diseases
- HIV/AIDS
- Acute Respiratory Infections and Other Invasive Bacterial Diseases
- Diarrhoeal Diseases
- Other Research Projects
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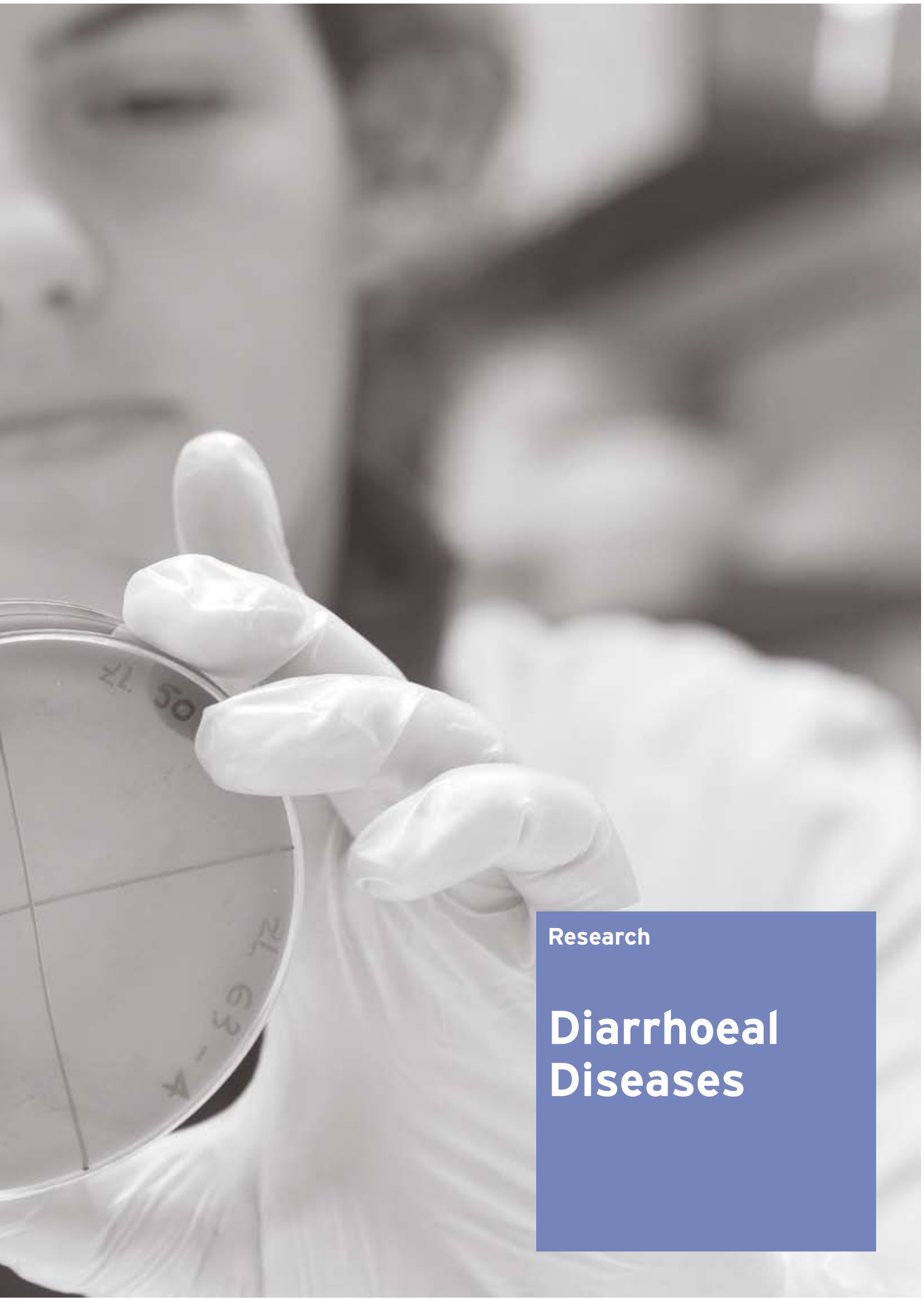
The term "principal investigator" used in this report refers to the person acting as such within CRESIB, regardless of whether they are the principal investigator of the overall project.



Research

Diarrhoeal Diseases

Diarrhoeal Diseases

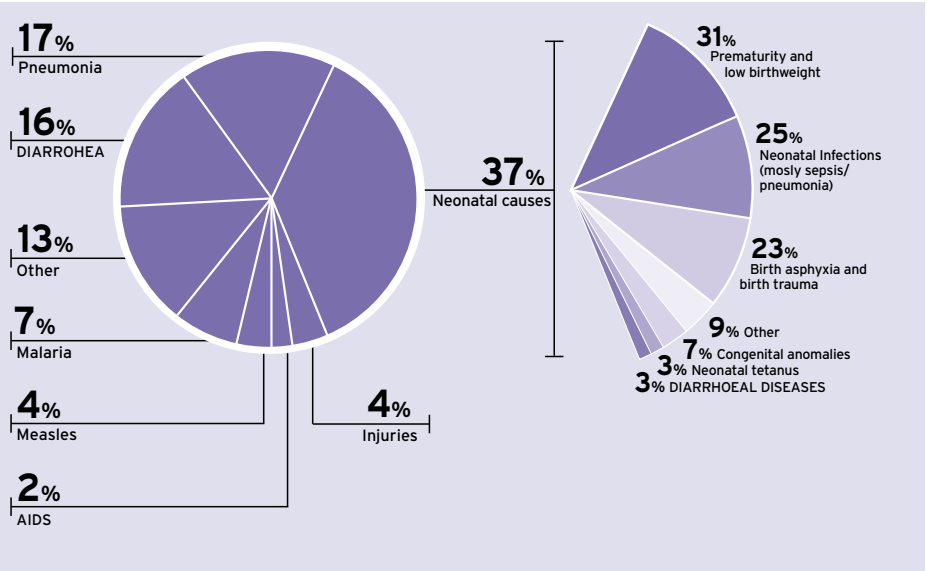
According to the World Health Organization (WHO), diarrhoea is still the second leading cause of death among children under 5 worldwide and kills more children than AIDS, malaria and measles combined (about 1.5 million children each year). Annually, an estimated 2,500 million cases of diarrhoea occur among children under 5 years of age, and estimates suggest that overall incidence has remained relatively stable over the past 20 years. More than half of these cases are in Africa and South Asia, where bouts of diarrhoea are more likely to result in death or other severe outcomes.

Diarrhoea is commonly a symptom of gastrointestinal infection and can be caused by a wide variety of pathogens, including bacteria, viruses and protozoa. However, only a few organisms are responsible for most acute cases of childhood diarrhoea.

Rotavirus is estimated to cause about 40% of all hospital admissions due to diarrhoea among children under 5 years of age worldwide, leading to some 100 million episodes of acute diarrhoea each year that result in 350,000 to 600,000 child deaths. Global rotavirus vaccine introduction has recently been recommended by the WHO.

Other major bacterial pathogens include *Escherichia coli, Shigella, Campylobacter* and *Salmonella*, along with *Vibrio cholerae* during epidemics. A major threat in treating bacterial infections is the wide spreading of antibiotic resistance. Antimicrobial resistance raises very important challenges because of the magnitude of the interconnecting global bacterial populations it involves and because of the diversity of resistance genes and genetic vectors responding to differing usage of antimicrobial agents on different populations in different parts of the world.

CRESIB's research in diarrhoeal diseases focuses on determining the aetiology and burden of diarrhoea in different geographical areas, on studying the mechanisms of antimicrobial resistance and on determining the burden of rotavirus gastroenteritis acquired in hospitals in Catalonia.



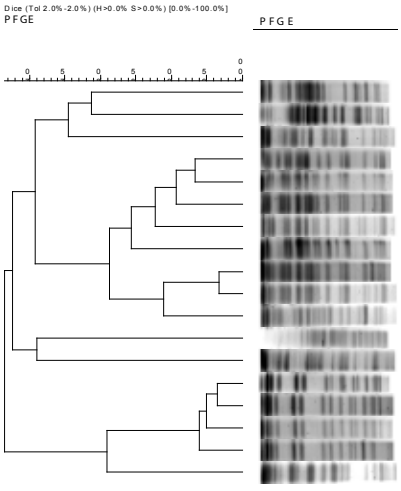
From: WHO, Global Burden of Disease estimates, 2004 update. *In*: Diarrhoea: why children are still dying and what can be done.

Epidemiological-molecular relevance and characterization of enteroaggregative *Escherichia coli* as a cause of diarrhoea in children younger than 5 years of age

Enteroaggregative *Escherichia coli* (EAEC) is one of the most important pathogens causing diarrhoea in children in developing areas. Studies have shown that EAEC isolates possess a high heterogeneity in virulence factors (VFs).

The aim of the present study is to characterize the virulence factors present in EAEC isolates causing diarrhoea in children under 5 years of age in the area of Manhiça, Mozambique.

In the course of several studies a series of diarrhoeagenic *E. coli* were collected. However, due to the identification method used (a mix of five colonies recovered from a faeces sample), the exact *E. coli* isolate remained non-identified. Thus, in 2009 the studies were designed to establish the exact EAEC isolate and as a consequence 211 EAEC strains were identified. The study will be completed on 2010 by searching for the presence of 18 different VFs (analysis in process). In the course of this analysis we have been able to show the presence of specific variants of some previously undescribed VFs that remain to be investigated in depth in further studies. In addition, the presence of these 18 VFs has been searched for in a non-diarrhoeagenic *E. coli* (control group), in order to establish their real relevance in this geographical area.

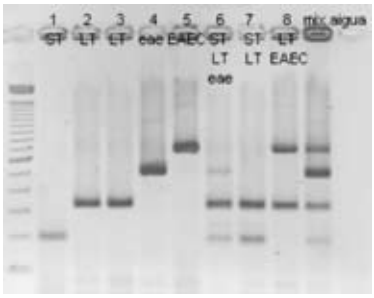


	Strain	Isolation date	Diarrhea/Control	*chu*A	*exh*A	GF	FH	ST	Grupo clonal
I	VES 256-2	28-Oct-04	Diarrhea	0	0	A	0	171	STEC 12
II	VES 256-3	28-Oct-04	Diarrhea	1	0	B2	1	NEW 1	NUEVO
III	D-7042	4-May-07	Diarrhea	1	1	D	0	NEW 5	EPEC 2
IV	D-0135	8-Aug-07	Control	0	1	B1	0	NEW 7	EHEC 2
V	D-3394	24-Sep-07	Control	1	1	D	0	106	EHEC 2
VI	D-5022	22-Oct-07	Control	0	0	B1	0	106	EHEC 2
V	D-0020	14-Nov-06	Diarrhea	1	1	D o B1	0	NEW 8	EHEC 2
VII	VES 273-2	3-Nov-04	Diarrhea	1	1	B2	0	106	EHEC 2
VIII	D-0157	22-Oct-07	Control	0	1	B1	1	106	EHEC 2
VIII	LF1237	1-Jul-09	ND	1	1	D o B1	0	106	EHEC 2
IX	LF1004	21-Apr-08	ND	0	1	B1	0	NEW 8	EHEC 2
X	F-002-1	27-Jan-87	Control	NA	0	NA	1	NA	NA
XI	D-3451	31-May-07	Diarrhea	0	0	B1	0	NEW 9	NT 5
XII	VES 230-5	2-Jun-04	Diarrhea	1	1	D	1	NEW 2	STEC 12
XIII	D-3157	4-Apr-07	Diarrhea	1	1	D	0	78	STEC 12
XIII	D-3089	10-Apr-07	Control	1	1	D	0	78	STEC 12
XIII	D-3251	09-May-08	Control	1	0	D	0	NA	NA
XIV	LF1045	25-Aug-08	ND	0	1	B1	0	NEW 6	EPEC 2

ND, non determined; NA, non aplicable; GF, phylogenetic group, FH,f hemolytic phenotype ; ST, Sequence type

Clonal and molecular characterization of verotoxigenic *Escherichia coli* recovered in periurbans areas of Lima (Peru).

Studies on antimicrobial resistance levels have shown the presence of high levels of resistance to cotrimoxazole and ampicillin in the analysed isolates, as well as the presence of extended spectrum β-lactamases among the isolates.



The detection of different diarrheogenic *Escherichia coli* by PCR.

Principal investigator:
Joaquim Ruiz

Co-investigators:
Maria J. Pons, Inácio Mandomando, Dinis Jaintilal

Additionally, the levels and mechanisms of antimicrobial resistance in a series of *Shigella* and *Salmonella* were also studied.

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

Duration of the project:
2006-2009

Publications:

- Mandomando I, Macete E, Sigaúque B, Morais L, Quintó L, Sacarlal J, Espasa M, Vallès X, Bassat Q, Aide P, Nhampossa T, Machevo S, Ruiz J, Nhacolo A, Menéndez C, Kotloff KL, Roca A, Levine MM, Alonso PL. Invasive non-typhoidal *Salmonella* in Mozambican children. **Trop. Med. Int. Health.** 2009 Dec;14(12):1467-1474.
- Mandomando I, Jaintilal D, Pons MJ, Vallès X, Espasa M, Mensa L, Sigaúque B, Sanz S, Sacarlal J, Macete E, Abacassamo F, Alonso PL, Ruiz J. Antimicrobial susceptibility and mechanisms of resistance in *Shigella* and *Salmonella* isolates from children under five years of age with diarrhea in rural Mozambique. **Antimicrob. Agents Chemother.** 2009 Jun;53(6):2450-2454.

GEMS: Global enteric multi-centre study. Diarrhoeal diseases in infants and young children in developing countries

Diarrhoea is one of the major causes of death among children under 5 years of age, particularly in developing countries. The World Health Organization ranks diarrhoeal disease as the second most common cause of mortality among children under 5 years (60 months) of age in developing countries, accounting for 18% of the 10.6 million children in this age group who die each year.

The objective of this multi-centre case-controlled study is to establish the different aetiological causes of this disease (bacterial, viral and parasitic) in children younger than 5 years of age, stratifying the monitored population into three age subgroups. The goal is to provide the information needed to guide the development and implementation of enteric vaccines and other public health interventions that can diminish morbidity and mortality from diarrheal diseases.

The results obtained to date in the population of Manhiça have shown a high

number of rotaviruses. *Cryptosporidium, Shigella*, diarrhoeagenic *E. coli* strains and *Entamoeba* were prevalent among cases, while *Giardia* was more prevalent among controls.

Principal investigator:
Pedro L. Alonso

Co-principal investigator:
Joaquim Ruiz

Co-investigators:
Inácio Mandomando, Sozinho Acácio, Tacilta Nhampossa, Delfino Vubil

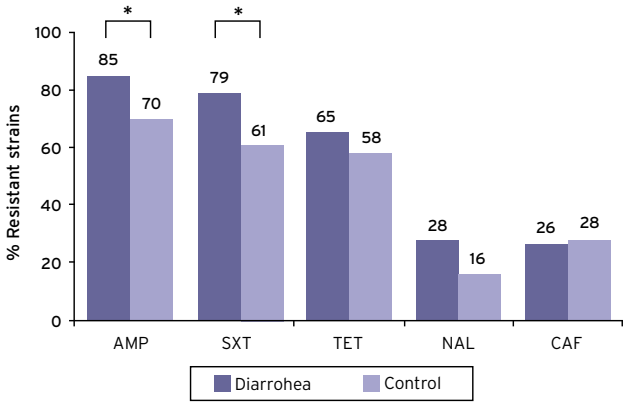
Funder:
Bill & Melinda Gates Foundation, Seattle (USA).

Duration of the project:
2007-2011

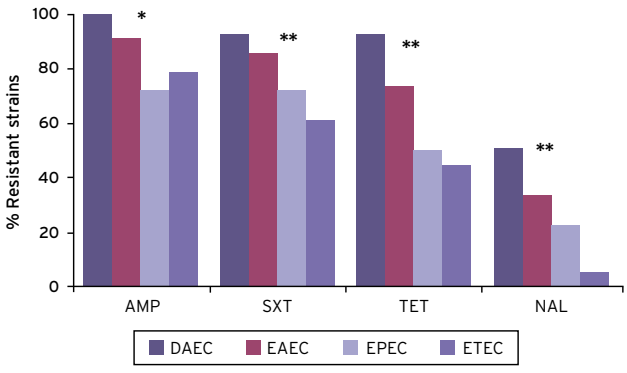
Infrastructure improvement at the Universidad Peruana Cayetano Heredia to undertake comprehensive studies on diarrhoeal diseases

Diarrhoea is an important cause of morbidity among children of peri-urban areas of Lima. However, the exact aetiological causes of the disease in this geographical area remain unknown, due to some extent to the lack of laboratory facilities. This project aims to support the acquisition of inventoried resources and to collaborate in the training of young Peruvian scientists by developing collaborative research studies and by developing or participating in courses or seminars at the Universidad Peruana Cayetano Heredia.

Research focused on the characterization



Antimicrobial resistance levels to ampicillin (AMP), cotrimoxazole (SXT), tetracycline (TET), nalidixic acid (NAL) and chloramphenicol (CAF) in *Escherichia coli* recovered from children with and without diarrhea in Chorrillos (Lima).



Antimicrobial resistance levels of different diarrheogenic patotypes of *Escherichia coli* (DAEC -diffusely adherent-; EAEC-enteroaggregative-; EPEC-enteropathogenic-; ETEC-enterotoxigenic-) to ampicillin (AMP), cotrimoxazole (SXT), tetracycline (TET) and nalidixic acid (NAL). All isolates recovered from children with diarrhoea in Chorrillos (Lima).

of the molecular mechanisms of antimicrobial resistance in a series of isolates of diarrhoeagenic and non-diarrhoeagenic *E. coli*. These studies showed the presence of transferable mechanisms of quinolone-resistance in the area, as well as a low frequency of extended spectrum beta-lactamases. Additionally, a new variant of TEM-type beta-lactamases (TEM 176) was reported and introduced in GenBank (GenBank access: GU550123.1).

Furthermore, the clonal relations in a series of 200 enteropathogenic *E. coli* were established, the results showing a high degree of clonal diversity. The enterohemorrhagic *E. coli* (20 isolates) were classified within multi-locus sequencing typing (MLST) patterns by means of the Michigan criteria. The results showed the presence of eight new described MLST patterns.

Finally, a surveillance of mothers' attitudes was performed by the Peruvian researchers, showing the frequent use of antibacterial agents (usually prescribed by physicians) by the mothers, the general opinion that antimicrobial agents were needed as a diarrhoea-therapy, and erroneous perceptions of the antibacterial nature of different substances.

Principal investigator:
Joaquim Ruiz

Co-investigator:
Maria J. Pons

In collaboration with:
- Theresa J. Ochoa. Universidad Peruana Cayetano Heredia, Lima, Perú.

Funder:
Agencia Española de Cooperación Internacional para el Desarrollo (AECID), Madrid (Spain).

Duration of the project:
2009-2013

Community and hospital-acquired rotavirus gastroenteritis in Catalonia, Spain. Analysis of disease burden (period 2003-2008)

Previous studies regarding the burden of rotavirus disease in Europe and in different autonomous regions within Spain have yielded varying estimates of the number of hospitalizations attributable to rotavirus. Few studies in Spain have assessed the burden of nosocomial rotavirus infection, though it is considered to be one of the main hospital-acquired infections in young children.

We describe here the characteristics of hospitalizations associated with rotavirus infection in the autonomous region of Catalonia during the period 2003-2008. We analysed data from the Minimum Basic Data Set for Acute-Care Hospitals of Catalonia, comprising data from all public acute-care hospitals and almost 90% of the private acute-care hospitals. Data analysis will be completed on 2010.

Principal investigator:
Edward Hayes

In collaboration with:
- Alberto López García-Basteiro, José María Bayas. Hospital Clínic de Barcelona, Barcelona (Spain).

Funder:
CRESIB (Hospital Clínic-Universitat de Barcelona), Barcelona (Spain).

Duration of the project:
2009-2010

Research

- **Malaria**
- Imported Diseases
- HIV/AIDS
- Acute Respiratory Infections and Other Invasive Bacterial Diseases
- Diarrhoeal Diseases
- Other Research Projects
- Incorporation of New Research Teams
- Research Support Services

The term "principal investigator" used in this report refers to the person acting as such within CRESIB, regardless of whether they are the principal investigator of the overall project.

Research

Other Research Projects

MorMat: Maternal mortality in sub-Saharan Africa. Contribution of HIV infection and malaria

To date little or no progress has been made in sub-Saharan Africa to reach the 5th Millennium Development Goal on maternal mortality reduction (MM). A major handicap in achieving this goal is that efforts to reduce MM in the region are not evidence-driven. Clinical records and verbal autopsies are the only source of information in most African countries. However, a high number of major clinical errors which have a significant impact on MM and question the validity of reports based on clinical data and verbal autopsies has been observed. Moreover, potentially preventable and/or treatable infectious diseases may account for over half of all maternal deaths in Africa, although these are frequently under-diagnosed.

To adress these issues, the objectives of this project were:

1) To describe the causes of maternal mortality in Mozambique.

2) To evaluate the discrepancies between clinical and pathological diagnoses.

3) To evaluate the impact of HIV infection and malaria on maternal mortality.

4) To study the cytoadherence phenomena and the inflammatory infiltrates in the parasitized organs.

We detected an extremely high maternal mortality rate (847 per 100,000 live infants). Potentially preventable or treatable infectious diseases such as HIV-related opportunistic infections, puerperal septicaemia, bronchopneumonia, meningitis and malaria accounted for over half of all maternal deaths. Major diagnostic errors were detected in a high number (40.3%) of maternal deaths. A high rate of false negative diagnoses was observed for infectious diseases which showed sensitivities under 50%: HIV/AIDS-related conditions, pyogenic bronchopneumonia, pyogenic meningitis and puerperal septicaemia. Eclampsia was the main source of false positive diagnoses.

Thus, we concluded that clinico-pathological discrepancies may have a significant impact on maternal mortality in sub-Saharan Africa. Increasing clinical awareness of the impact of obstetric and non-obstetric infections through their inclusion in the differential diagnosis, together with a thorough evaluation of cases clinically thought to be eclampsia, could significantly reduce maternal mortality.

Principal investigators:
Jaume Ordi, Clara Menéndez

Co-investigators:
Alfredo Mayor, Carlota Dobaño, Denise Naniche

In collaboration with:
- M.R. Ismail, C. Carrilho. Department of Pathology, Maputo Central Hospital, Universidade Eduardo Mondlane, Maputo (Mozambique).
- Fernanda Machungo, Nafisa Osman, Department of Obstetrics and Gynecology, Maputo Central Hospital, Universidade Eduardo Mondlane, Maputo (Mozambique).

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

Duration of the project:
2006-2009

Publications:

- Ordi J, Ismail MR, Carrilho C, Romagosa C, Osman N, Machungo F, Bombí JA, Balasch J, Alonso PL, Menéndez C. Clinico-pathological discrepancies in the diagnosis of causes of maternal death in sub-Saharan Africa: retrospective analysis. **PLoS Med**. 2009 Feb 24; 6(2):e1000036.

Aetiology of anaemia in children in a malaria-endemic rural area of Mozambique

Anaemia is one of the main causes of morbidity and mortality in children of sub-Saharan Africa. Its aetiology is complex and multi-factorial. However, there is a lack of knowledge on the anaemia risk factors and their relative contribution.

The first objective of this study was to describe the aetiology of anaemia in children from 1 month to 5 years old in a rural area in Mozambique in order to guide the development and implementation of preventive interventions. The secondary objectives were:

1) To explore the validity of actual biochemical markers to differentiate between iron deficiency and inflammation anaemia.

2) To identify pathophysiological mechanisms associated with haemolysis, spleen clearance and erythropoiesis suppression in malarial anaemia.

During 2009 we have recruited 333 cases, 39 hospital controls and 201 community controls, and we have some preliminary data showing that 72% of anaemia cases are moderate, 14% severe and 14% mild.

Data on the infections showed that:

- 46% of cases have malaria against 6% of controls.
- 74% of the severe anaemia cases have malaria.

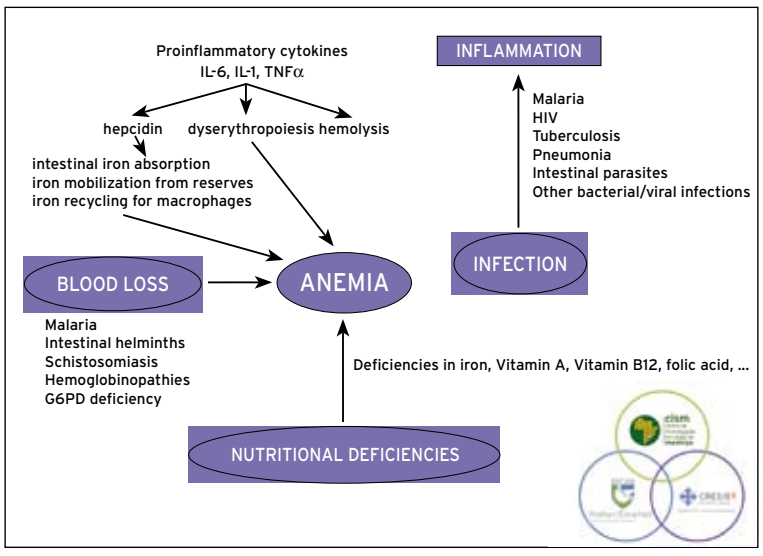


Diagram showing the potential risk factors of anaemia that are being studied

- HIV prevalence is 13% in cases, 14% in hospital controls and 1.6% in community controls.
- Epstein Barr virus prevalence in the study population is 34%, showing no differences between cases and controls.
- Parvovirus B19 prevalence in the study population is 1%.
- Presence of blood in urine and faeces is not significant in either cases or controls.

The nutritional status data showed that albumin, prealbumin and vitamin A levels are significantly lower in cases than in controls and Vitamin B12 levels are not significantly different. Iron serum levels were significantly lower in cases than in controls, while ferritin levels were significantly higher, suggesting an infection/inflammation status causing a functional-iron deficiency.

We have detected only one case of sickle cell trait in the first 200 studied children, while prevalence of G6PD deficiency was 8% in the study population.

Furthermore, the 13.2% of malarial anaemia cases with Hb ≤ 7g/dL showed suppression of erythropoiesis. This is the first time that the prevalence of this condition has been estimated. The molecular mechanisms of this suppression are being studied.

Principal investigator:
Clara Menéndez

Co-investigators:
Ruth Aguilar, Cinta Moraleda, Augusto Nhabomba, Mauricio H. Rodríguez

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

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

Duration of the project:
2008-2011

EPSIMA: Quantification of pre- and post-natal exposure to insecticides and effects on the health of the child in rural southern Mozambique

One of the ways to combat the vector that transmits malaria is by using bednets impregnated with pesticides and by fumigating the houses with pesticides with a regular periodicity (indoor residual spraying, IRS). It is well known that some of the pesticides used (DDT and pyrethroids) cross the placenta, are transferred via breast milk and have adverse effects on the child's immune system. In the district of Manhiça, Mozambique, malaria is endemic and IRS with pyrethroids is performed. These fumigations started between 2005 and 2006.

The aims of this project were:

1) To measure the exposure to pyrethroids and other persistent and semi persistent pollutants in women

and children according to whether IRS and bednets impregnated with pyrethroids (Deltaprim) are used.

2) To study the effect of exposure to pesticides from fumigations and bednets on children's health.

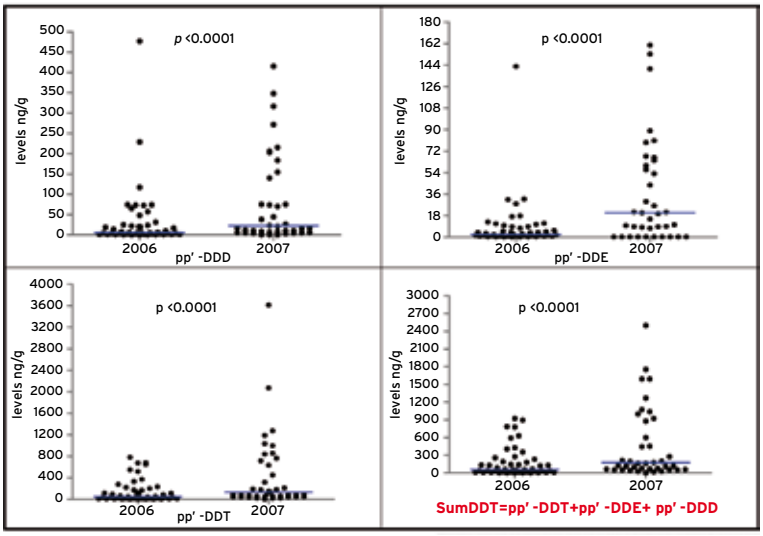
Existing information (questionnaires and biological samples) from other studies conducted by CRESIB and the Centro de Investigação em Saúde de Manhiça (CISM) will be used. A preliminary study will be performed to determine the background exposure to pesticides prior to the fumigations in straw samples from houses, puerperal women (breast milk samples) and newborns (cord blood plasma samples). A subsequent study will assess the same chemical patterns in women who have given birth during and immediately after the fumigations and will compare the results with those encountered before the fumigations.

Children's health is studied by evaluating the following parameters:

- Morbidity (by hospital admissions) according to the place of residence and IRS status.

- Immune function (cytokines and immunoglobulins) during the first months of life. The results will be related to the concentrations of pesticides at birth. The hypothesis is that exposure of pregnant women to insecticides will have an immunomodulatory or immunosuppressive effect in their neonates and children.

In 2009 we measured the levels of insecticides in straw samples and in breast milk. No differences were found between houses with and without IRS, but there was a significant increase in DDT levels between the preliminary and subsequent studies. Future activities involve the evaluation of immune responses in blood from umbilical cord and from children.



Levels of insecticides (DDT and derivatives) in straw samples from Mozambican houses in 2006 and 2007, before and after indoor residual spraying with insecticides (P value by Wilcoxon test).

Principal investigators:
Pedro L. Alonso, Clara Menéndez, Carlota Dobaño, Jahit Sacarlal

Co-investigator:
Maria Nélia Manaca

In collaboration with:
- Jordi Sunyer. Centre de Recerca en Epidemiologia Ambiental (CREAL), Barcelona (Spain).
- Joan Grimalt. Institut de Diagnosi Ambiental i Estudis de l'Aigua, Consell Superior d'Investigacions Científiques (IDAEA-CSIC), Barcelona (Spain).

Funders:
Fundació Marfà, Barcelona (Spain).
CRESIB (Hospital Clínic-Universitat de Barcelona), Barcelona (Spain).
Centre de Recerca en Epidemiología Ambiental, Barcelona (Spain).

Duration of the project:
2007-2011

Evaluation of persistence of yellow fever vaccine virus in urine

It is not known whether yellow fever vaccine virus RNA persists in urine after vaccination. The recent evidence of persistence of a related flavivirus, West Nile virus, in urine up to six years after onset of West Nile encephalitis raises the possibility that yellow fever vaccine virus might also persist in some people. Evidence to the contrary would provide reassurance that yellow fever vaccine and related chimeric vaccines constructed with a yellow fever vaccine virus "backbone" are rapidly cleared by a normal immune response. Evidence of persistence would raise possibilities that persistent vaccine virus could be transmitted from mother to infant or through blood transfusion or organ transplantation, or that the vaccine could induce long-term adverse effects. On the other hand, there may be no adverse effects of persistence of this attenuated vaccine virus, but evidence of persistence might help elucidate mechanisms of long-lasting immunity conferred by the vaccine.

This study seeks to determine whether yellow fever vaccine RNA is detected in urine following vaccination. Consenting people who have received yellow fever vaccination in the past at the Hospital Clínic's Travel Clinic and who are attending the clinic for other reasons will be asked to provide a urine sample. The urine sample will be tested for presence of yellow fever virus vaccine RNA by polymerase chain reaction.

Principal investigator:
Edward Hayes

Co-principal investigator:
Mikel Martínez

Co-investigators:
Anna Vilella, Tomas Pumarola, Joaquim Gascon

Funder:
CRESIB (Hospital Clínic-Universitat de Barcelona), Barcelona (Spain).

Duration of the project:
2009-2012

Safety and immunogenicity of yellow fever vaccine in travellers with asymptomatic human immunodeficiency virus infection

This study seeks to amplify the extremely scarce data regarding safety and immunogenicity of yellow fever vaccine in HIV-infected people. Yellow fever vaccine is recommended for people 9 months of age or older living in or travelling to areas where yellow fever is endemically transmitted. An estimated two million people infected with HIV reside in West African countries where universal yellow fever vaccination is indicated, but published data on yellow fever vaccination

of people with HIV infection is limited to a handful of mostly retrospective studies with small numbers of participants.

We proposed to prospectively evaluate the safety and immunogenicity of commercially available 17D yellow fever vaccine in asymptomatic HIV-infected prospective travellers with CD4 counts ≥ 200/mm^3 for whom the vaccine is recommended based on their proposed travel itinerary. Data regarding the frequency of solicited and non-solicited adverse events and the development of neutralizing antibody against yellow fever virus after vaccination will be collected, summarized and reported.

Principal investigator:
Edward Hayes

Co-principal investigator:
Anna Vilella

Co-investigator:
Joaquim Gascon

In collaboration with:
- Raisa Morales. Unitat d'Atenció al Viatger – Drassanes, Barcelona (Spain).
- Cristina Domingo, Matthias Niedrig. Robert Koch Institute, Berlin (Germany).
- Erin Staples. Centers for Disease Control and Prevention (CDC), Division of Vector-Borne Infectious Diseases, Fort Collins, Colorado (USA).
- Maria del Mar Lago Nuñez. Hospital Carlos III, Madrid (Spain).
- Cristina Soler Ferrer. Hospital Santa Caterina, Girona (Spain).
- Bernat Font. Hospital Parc Taulí, Sabadell (Spain).
- Lluis Valeiro. Unitat de Salut Internacional del BniM, Barcelona (Spain).
- Xavier Martínez. Hospital Vall d'Hebron, Barcelona (Spain).
- Jose M. Ramón. Hospital de Bellvitge, Barcelona (Spain).
- Carme Aramburu. Centro de Vacunación Internacional – Bergara, Barcelona (Spain).
- Eng Ong Ooi. Signature Research Program – Emerging Infectious Diseases. Duke – NUS Graduate Medical School, North Carolina (USA).

Funder:
CRESIB (Hospital Clínic-Universitat de Barcelona), Barcelona (Spain).

Duration of the project:
2009–2012

Uses of services and economic evaluation of the influenza A (H1N1) 2009 study

Influenza A (N1H1) 2009 has achieved the status of a global pandemic and has forced the health systems to prepare themselves to meet the population's health needs and the high consumption of health resources resulting from prevention and treatment of the disease and its complications. Estimating the use of health services and the incremental cost of hospital outpatients and hospitalized patients cases of pandemic influenza A, and estimating the cost-effectiveness and cost-utility of the different interventions in prevention and treatment of it, will provide highly relevant information for better decision making. The realization of a study contemporary to the pandemic and the early analysis of the results will improve the responsiveness of the Spanish health system.

The study has the following objectives:

1) To quantify the use of health services by outpatients and hospitalized patients with acute respiratory failure with influenza A (H1N1) 2009.

2) To quantify socio-economical and work-related impact in outpatients and hospitalized patients with acute respiratory failure with influenza A (H1N1) 2009.

3) To estimate the incremental cost in patients with influenza A (H1N1) 2009, both ambulatory and hospitalized for acute respiratory failure, versus controls from the community, by risk group (immunodeficient and immunocompetent) and age group.

4) To estimate the cost-effectiveness and cost-utility in pharmacological and non-pharmacological prevention and treatment interventions for influenza A (H1N1) 2009 in terms of decrease in cases, hospitalizations and length of hospitalization, complications and death, by risk group (immunodeficient and immunocompetent) and age group:

- Treatment with neuraminidase inhibitors according to the stage at which they are administered.
- Vaccine against pandemic virus A (H1N1) 2009.
- Seasonal influenza vaccine.
- 23-valent pneumococcal vaccine.

5) To compare the efficiency of the different pharmacological and non-pharmacological prevention and treatment interventions for influenza A (H1N1) 2009, estimating the cost-utility ratio by risk group (immunodeficient and immunocompetent) and age group.

During the last few months of 2009 the literature on costs in primary and secondary sources was reviewed, the definition of the variables which compose the cost of pharmacological and non-pharmacological measures and use of services was established, and the researchers agreed on the database design.

Principal investigator:
Elisa Sicuri

In collaboration with:
- Jordi Alonso. Health Services Research Group, IMIM-Hospital del Mar, Barcelona (Spain).

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

Duration of the project:
2009-2012

PRISMA: Reflecting the positive diversities of European priorities for research and measurement in end-of-life care

PRISMA is a programme that aims to inform best practice for cancer patient care and to harmonize research on palliative care (diseases in advanced and terminal phases) in Europe through the comparison and interchange of approaches, experiences and research priorities. In order to meet its objective of improving end-of-life care for patients and families, PRISMA incorporated a work package on the influence of culture on end-of-life care, of which Dr. Marjolein Gysels (CRESIB) is the principal investigator.

The deliverables achieved during 2009 are detailed below:

- In May, the work package on culture and end-of-life care successfully completed a session of presentations at the 11th Congress of the European Association for Palliative Care held in Vienna. CRESIB also hosted the PRISMA All-Assembly Meeting, held in Sitges during the same month.

- In December, the work package on culture and end-of-life care delivered the literature scoping of evidence on cultural issues in end-of-life care in eight European countries (the United Kingdom, Germany, Norway, the Netherlands, Belgium, Italy, Spain and Portugal).

- During the course of 2009, the project set up a network of experts in cultural issues in end-of-life care. In order to build an expert contacts database, potential participants from the following categories were contacted: published authors; persons recommended by project participants; persons who had responded to a published advertisement; persons identified by various national and regional palliative care associations in Europe; and persons identified from relevant conferences and workshops. A total of 510 experts were contacted, of whom 167 agreed to participate in the network and completed a short questionnaire.

- The PRISMA project launched its own website (www.prismafp7.eu). In addition, the culture and end-of-life care team set up a blog concerning cultural issues in end-of-life care (http://cultureeol.wordpress.com/).

Principal investigator:
Marjolein Gysels

Co-investigators:
Robert Pool, Arantza Meñaca, Natalie Evans, Erin Andrew

In collaboration with:
- Richard Harding, Irene J. Higginson, Sue Hall and Fliss Murtagh. King's College London (KCL), London (UK).
- Stein Kaasa, Dagny Faksvaag Haugen, 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 en Saúde da Universidade de Coimbra, Coimbra (Portugal).
- Luc Deliens, Bregie 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 Vlieger. 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 Van den Berghe, Johan Menten and Trudie van Iersel. Federatie Palliatieve Zorg Vlaanderen, Wemmel (Belgium).

Funder:
FP7 Programme, European Union.

Duration of the project:
2008-2011

Research

- **Malaria**
- Imported Diseases
- HIV/AIDS
- Acute Respiratory Infections and Other Invasive Bacterial Diseases
- Diarrhoeal Diseases
- Other Research Projects
- Incorporation of New Research Teams
- Research Support Services

The term "principal investigator" used in this report refers to the person acting as such within CRESIB, regardless of whether they are the principal investigator of the overall project.

Research

Incorporation of New Research Teams

During the period spanning the Strategic Plan (2010-2013), several strategic incorporations have been confirmed and the researchers have already joined CRESIB. The research lines that will be developed under the leadership of these researchers are summarized here:

- **Dr. Antoni Trilla:** assistant professor of Preventive Medicine and Public Health at the Universitat de Barcelona, director of the Evaluation, Support and Prevention Unit of the Hospital Clínic de Barcelona and since January 2010 Research Professor at CRESIB.

  The research within his group aims at improving prevention and control of communicable diseases –in particular hospital infections and healthcare emergencies– through safety and quality in clinical practice and in the use of vaccines.

  The main research focus within Dr. Trilla´s group is on:

  - **Hospital Infections**
  - **Epidemiology and health emergencies**
  - **Clinical quality and safety**

  Furthermore, Dr. Trilla's group has expertise in ethics in clinical research and in risk communication and will provide support on education and training activities on these topics.

  Main publications of 2009

  - Cardeñosa N, Domínguez A, Carratalà J, Ricarte JI, Jansà JM, Arnau J, Camps N, Chanovas M, Mas A, Trilla A. Usefulness of simulated cases for assessing pandemic Influenza preparedness plans. **Clin Microbiol Infect.** 2009 Dec 23 [*Ahead of print*]
  - Martínez JA, Piazuelo M, Almela M, Blecua P, Gallardo R, Rodríguez S, Escalante Z, Robau M, Trilla A. Evaluation of add-on devices for the prevention of phlebitis and other complications associated with the use of peripheral catheters in hospitalised adults: a randomised controlled study. **J Hosp Infect.** 2009 Oct;73(2):135-42.
  - Vilella A, Trilla A. [Influenza A (H1N1): A new e-pidemic]. **Med Clin Barc.** 2009 May 30;132(20):783-74.

- **Dr. Jaume Ordi:** Senior lecturer in Pathological Anatomy at the Universitat de Barcelona, senior specialist at the Pathological Anatomy Service of the Hospital Clínic de Barcelona and

since January 2010 Research Professor at CRESIB.

The main research focus within Dr. Ordi´s group is on:

- **Histological and immunohistochemical characterization of placental malaria:** There have been a number of joint projects and publications with CRESIB researchers on this topic. During 2009, Dr. Ordi has been leading the quality control programme of the placental histological evaluation of the MiP Consortium studies.

- **Descriptive studies on maternal mortality:** There have already been a number of joint projects and publications with CRESIB researchers on this topic, and the current focus is on analysing the causes of maternal mortality in different settings of sub-Saharan Africa in order to determine whether maternal death can be significantly reduced by implementing prevention tools.

- **Human papilloma virus and cervical cancer:** The main focus is on studying the role of human papilloma virus in the pathogenesis of human cancer and on biomarkers of progression/ regression of cervical cancer.

Main publications of 2009

- Ordi J, Ismail MR, Carrilho C, Romagosa C, Osman N, Machungo F, Bombí JA, Balasch J, Alonso PL, Menéndez C. Clinico-pathological discrepancies in the diagnosis of causes of maternal death in sub-Saharan Africa: retrospective analysis. **PLoS Med.** 2009 Feb 24;6(2):e1000036.

- Alos L, Moyano S, Nadal A, Alobid I, Blanch JL, Ayala E, Lloveras B, Quint W, Cardesa A, Ordi J. Human papillomaviruses are identified in a subgroup of sinonasal squamous cell carcinomas with favorable outcome. **Cancer.** 2009 Jun 15;115(12):2701-2709.

- Ordi J, Alejo M, Fusté V, Lloveras B, Del Pino M, Alonso I, Torné, A.. HPV-negative vulvar intraepithelial neoplasia (VIN) with basaloid histologic pattern: an unrecognized variant of simplex (differentiated) VIN. **Am J Surg Pathol.** 2009 Nov;33(11):1659-1665.

- **Dr. Jordi Vila:** Leader of the clinical molecular microbiology and parasitology group at IDIBAPS, member of the Microbiology Service at the Hospital Clínic de Barcelona, professor of the Department of Pathological Anatomy,

Pharmacology and Microbiology at the Faculty of Medicine of the University of Barcelona and since January 2010 research professor at CRESIB and leader of the Viral and Bacterial Infections Programme.

The main research focuses within Dr. Vila's group are:

- **Diagnosis of bacterial infections.**
- **Mechanisms of resistance to antibiotics.**
- **Pathogenesis:** studies on the proteins secreted by bacterial pathogens and their correlation with virulence.
- **Prevention of premature delivery:** causes and markers of early neonatal sepsis.
- **Drug discovery for multi-resistant bacteria:** tuberculosis and others.

Main publications of 2009

Fabrega, A., du Merle, L., Le Bouguenec, C., de Anta, M.T.J., Vila, J. 2009. Repression of Invasion Genes and Decreased Invasion in a High-Level Fluoroquinolone-Resistant *Salmonella Typhimurium* Mutant. **PLoS One 4** (11), e8029.

Mendez Arancibia, E., Pitart, C., Ruiz, J., Marco, F., Gascon, J., Vila, J., 2009. Evolution of antimicrobial resistance in enteroaggregative *Escherichia coli* and enterotoxigenic *Escherichia coli* causing traveller's diarrhoea. **Journal of Antimicrobial Chemotherapy** 64 (2), 343–7.

- Roca, I., Marti, S., Espinal, P., Martínez, P., Gibert, I., Vila, J. 2009. CraA, a major facilitator superfamily efflux pump associated with chloramphenicol resistance in *Acinetobacter baumannii*. **Antimicrob Agents Chemother.** 53 (9), 4013-4.

- **Dr. Tomas Pumarola:** Head of the Virology Section of the Biomedical Diagnostic Centre of the Hospital Clínic de Barcelona, professor of Microbiology at the Universitat de Barcelona and from January 2010 will be Research Professor at CRESIB.

The microbiology laboratory of the Hospital Clínic de Barcelona has been recognized as a National Influenza Centre by the Global Influenza Surveillance Network of the World Health Organization (WHO) since the 1960s. It is the reference laboratory in Catalonia for respiratory infections of viral aetiology, measles, rubella and

mumps and belongs to the Community Network for Reference Laboratories of the European Influenza Surveillance Scheme (EISS) and the European Centre for Disease Control (ECDC). Furthermore, it was qualified by the WHO in May 2009 to perform polymerase chain reaction diagnosis of the new influenza A virus (H1N1). This laboratory aims to become the Laboratory of Viral Emergencies in Catalonia.

Dr. Pumarola's main research interest is in:

- **Viral emergent and re-emergent infections**
- **Arbovirus diagnosis**
- **Arbovirus pathogenesis**

Main publications of 2009

- Garriga C, Pérez-Elías MJ, Delgado R, Ruiz L, Pérez-Alvarez L, Pumarola T, López-Lirola A, González-García J, Menéndez-Arias L, Spanish Group for the Study of Antiretroviral Drug Resistance. HIV-1 reverse transcriptase thumb subdomain polymorphisms associated with virological failure to nucleoside drug combinations. **J. Antimicrob. Chemother.** 2009 Aug;64(2):251-258.

Castro P, Plana M, González R, López A, Vilella A, Argelich R, Gallart, T.; Pumarola, T.; Bayas J M, Gatell JM, García F. Influence of a vaccination schedule on viral load rebound and immune responses in successfully treated HIV-infected patients. **AIDS Res. Hum. Retroviruses.** 2009 Dec;25(12):1249-1259.

- Bermejo-Martin JF, Ortiz de Lejarazu R, Pumarola T, Rello J, Almansa R, Ramírez P, Martin-Loeches I, Varillas D, Gallegos MC, Serón C, Micheloud D, Gomez JM, Tenorio-Abreu A, Ramos MJ, Molina ML, Huidobro S, Sanchez E, Gordón M, Fernández V, Del Castillo A, Marcos MA, Villanueva B, López CJ, Rodríguez-Domínguez M, Galan JC, Cantón R, Lietor A, Rojo S, Eiros JM, Hinojosa C, Gonzalez I, Torner N, Banner D, Leon A, Cuesta P, Rowe T, Kelvin DJ. Th1 and Th17 hypercytokinemia as early host response signature in severe pandemic influenza. **Crit Care.** 2009;13(6):R201.

Research

- **Malaria**
- Imported Diseases
- HIV/AIDS
- Acute Respiratory Infections and Other Invasive Bacterial Diseases
- Diarrhoeal Diseases
- Other Research Projects
- Incorporation of New Research Teams
- Research Support Services

The term "principal investigator" used in this report refers to the person acting as such within CRESIB, regardless of whether they are the principal investigator of the overall project.

Research

Research Support Services

CRESIB offers its researchers a Biostatistics Unit, laboratory management and administrative support through the Office of International Cooperation. In addition, CRESIB has agreements with its founder institutions that enable CRESIB researchers to use their platforms and research support services:

- Animal House (UB)
- Biobank of HCB-IDIBAPS
- Bioinformatics Unit (IDIBAPS)
- Cell Culture Unit (HCB)
- Cytomics Unit (IDIBAPS)
- DNA Unit (HCB)
- Electron Microscopy Unit (SCT-UB)
- Evaluation, Support and Prevention Unit (HCB)
- Genomics Unit (IDIBAPS)
- Medical Imaging Platform (IDIBAPS-HCB)
- Medical Library (UB)
- Microscopy Section (SCT-UB)
- Nanobiotechnology Unit (IDIBAPS)
- Neurological Tissue Bank (HCB-UB)
- Proteomics Unit (IDIBAPS-UB-PCB Platform)
- Tumor Bank (HCB)
- Unit for Optical Recording of Cell Signals (IDIBAPS-UB)

8.1. Biostatistics Unit (UBIOES)

The mission of the the Biostatistics Unit (UBIOES) is to process the data of health research projects, applying a holistic approach to study design, statistical analysis and interpretation of results, ensuring quality and excellence, and providing training and support to researchers in the field of biostatistics.

Statistical Support

The UBIOES plays an important role in carrying out design, data collection, analytical plans and analysis for the vast majority of CRESIB's research projects. Its main activity is to work with the principal investigators and/or partners in the different stages of their research projects, and to provide regular advice to researchers on statistical issues.

Services Offered:

- Review of the statistical requirements (protocols) of research grant applications.
- Advice on study design options.
- Determining suitable sample sizes.
- Advice on the design of databases.
- Support in the preparation of data cleansing plans and analytical plans.
- Determining appropriate analysis techniques to address key research questions.
- Advice for researchers wishing to conduct their own analyses.
- Scheduling data cleansing.
- Performing suitable data management and statistical analysis.
- Development of applications for automating data management, data cleansing and analysis and report generation (tables and graphs).
- Reporting on statistical analyses, including interpretation of results.
- Assistance in the preparation or review of scientific articles.

Training

The UBIOES is also involved in the statistical training of CRESIB researchers. To this end, it organizes a workshop on statistics with the Stata statistical package to train researchers to perform and interpret their own analyses. These face-to-face courses have a duration of 20 hours in weekly four-hour sessions spread over five weeks.

Subjects:

Workshop 1: Data Management

- Workshop 2: Descriptive Statistics
- Workshop 3: Bivariate Statistics
- Workshop 4: Linear Regression
- Workshop 5: Logistic Regression

The UBIOES also conducts customized training in specific areas of data management, statistical techniques and management of statistical programs for CRESIB researchers.

Staff

Head of the Unit: John J. Aponte
Biostatistics: Sergi Sanz, Edgar Ayala, Santiago Pérez-Hoyos and Llorenç Quintó

8.2. Laboratory management

CRESIB (Hospital Clínic-Universitat de Barcelona) has a laboratory management structure that provides the necessary support to researchers working in the laboratory. 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 Centro de Investigação em Saúde de Manhiça (CISM)
- Equipment and infrastructure maintenance and management
- Laboratory coordination
- Support for new staff
- Coordination with the platforms and scientific/technical services of the UB University Campus

8.3. Office of International Cooperation

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

OIC staff and organization is described in section Personnel.

Education and Training

On the basis of research experience focusing on the generation and transmission of knowledge, CRESIB's mission is to be a reference institution and facilitator in the field of international health education and training. The Centre develops training programmes on its own and in collaboration with different institutions, with four fundamental objectives:

1. To promote and spread awareness and knowledge of global health problems.

2. To train highly qualified researchers in specific areas related to international health (mainly through master's, 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 to manage imported diseases, and in low- and middle-income countries to address endemic health problems.

4. To train technicians, doctors and scientists from the poorest countries.

CRESIB, together with the Faculty of Medicine of the Universitat de Barcelona, is a member of the tropED network for education and training in the field of international health (www.tropEd.org), and of the Eurolife International Health Alliance (EIHA) (www.eurolifeuniversities.org/).

The centre is currently carrying out the following training programmes in relation to international health:

POSTGRADUATE TRAINING

1. MASTER'S PROGRAMMES

CRESIB collaborates in the following master programmes:

- Universitat de Barcelona: Master's Degree in Tropical Medicine and International Health, Master's Degree in Internationalization, Master's Degree in Advanced Microbiology.
- Universitat Autònoma de Barcelona: Master's Degree in International Health and Tropical Medicine.
- Universitat Pompeu Fabra: Master's Degree in Public Health.

2. DOCTORAL PROGRAMMES

CRESIB participates as a research centre in the Doctoral Programme of Medicine of the Faculty of Medicine at the Universitat de Barcelona (www.ub.edu/web/ub/ca/estudis/oferta_formativa/doctorat/doctorat.html).

The centre also collaborates with the FCRB in a "Training Fellowship" programme, which aims to provide scientific training to young graduates in Mozambique (in collaboration with the Centro de Investigação em Saúde de Manhiça [CISM]) and Morocco, so they can embark on research of interest for their own country. This programme provides support for postgraduate, master's and/or doctoral studies.

DOCTORAL THESES

Doctoral thesis presented in 2009 at the Faculty of Medicine of the Universitat de Barcelona:

Dr. Esperança Julia Pires Sevene

Thesis: Availability and safety of drugs in vulnerable population. The case of pregnant women in developing countries

Supervisors: Dr. Xavier Carné and Dr. Clara Menéndez.

Date: 20 January 2009

Dr. María Lahuerta Sanaú

Thesis: Molecular epidemiology and HIV-1 vertical transmission control in a malaria-

endemic area in South Mozambique.
Supervisor: Dr. Denise Naniche

Date: 26 February 2009

Dr. Inacio Munduapege Mandomando

Thesis: The epidemilogy of *Salmonella, Shigella and Escherichia coli* infections in Mozambican children.

Supervisors: Dr. Pedro L. Alonso and Dr. Quim Ruíz

Date: 22 May 2009

Dr. Betuel Lázaro Sigaúque

Thesis: The epidemiology and clinical presentation of invasive bacterial infection among children admitted to a rural hospital in Mozambique.

Supervisors: Dr. Pedro L. Alonso and Dr. Anna Roca.

Date: 22 May 2009

Dr. Caterina Guinovart Florensa

Thesis: The epidemiology of malaria in Mozambique: implications for malaria vaccine trial design and interpretation.

Supervisor: Dr. Pedro L. Alonso

Date: 29 May 2009.

Dr. Enrique Bassat Orellana

Thesis: Malaria in the paediatric wards of a rural Mozambican hospital and the clinical development of new antimalarial drugs.

Supervisor: Dr. Pedro L.Alonso

Date: 22 June 2009

Dr. Jahit Sacarlal

Thesis: Clinical Development of RTS,S as a vaccine for the prevention of malaria in Mozambican children.

Supervisors: Dr. Pedro L. Alonso and Dr. C. Ascaso

Date: 13 July 2009

OTHER TRAINING PROGRAMMES

CRESIB organizes short courses and continuous training programmes, seminars, workshops, conferences and similar activities on its own and in collaboration with other institutions. These training programmes include weekly seminars and an annual workshop on Chagas disease that are organized by the Centre and constitute a platform for the updating and sharing of research expertise.

The weekly seminars are taught by expert researchers on different disciplines of global health from around the world, and are open to the public. These were the seminars given in 2009:

- 7/01/2009. **Dr. Ana Villegas-Mendez.** HumProTher Laboratory, Université Joseph Fourier (France). **"Cellular delivery of WW-fusion proteins using Adenoviral subparticles: from concept to application".**
- 14/01/2009. **Dr. Alfredo Mayor.** CRESIB (HC-UB) (Spain). **"Pregnancy-associated humoral immunity against maternal and non-maternal *Plasmodium falciparum* antigens is parity- and gender-dependent".**
- 21/01/2009. **Dr. Joaquim Ruiz.** CRESIB (HC-UB) (Spain). **"Mecanismos mo-**

leculares de resistencia a quinolonas".

- 04/02/2009. **Dr. Jaime Saravia.** Instituto de Gastroenterología Boliviano Japonés de Cochabamba (Bolivia). **"Megacolon y vólvulo de sigmoides. Fisiopatología".**

- 18/02/2009. **Elena del Cacho.** Farmacia Hospitalaria, Hospital Clínic de Barcelona (Spain). **"Laboratorio de Producción de Medicamentos. Medicus Mundi Catalunya. Campamentos de Refugiados Saharauis. Tindouf, Argelia".**

- 25/02/2009. **Dr. Manel Juan.** Immunology Unit, Hospital Clínic de Barcelona (Spain). **"CCL4 i variants: Model de doble variabilitat genòmica en la resposta immunitària. Peculiaritats poblacionals i paper en la infecció per HIV".**

- 04/03/2009. **Dr. Imane Jroundi.** INAS and CRESIB (HC-UB) (Morrocco and Spain). **"Malaria Eradication in Morocco".**

- 11/03/2009. **Dr. Edward Hayes.** CRESIB (HC-UB) (Spain). **"Zika virus outbreak."**

- 18/03/2009. **Francesca Mateo.** Universitat de Barcelona (Spain). **"Acetylation and the regulation of the cell cycle".**

- 01/04/2009. **Enric Grau.** Office of International Cooperation, Fundació Clínic per a la Recerca Biomèdica (Spain). **"Marruecos, una plataforma para la mejora de la salud materno infantil basada en la investigación, la formación especializada y la asistencia sanitaria".**

- 15/04/2009. **Dr. Klaus Gossens.** The Biomedical Research Centre, University of British Columbia (Canada). **"The thymic "zip-code" unravelled".**

- 29/04/2009. **Dr. Elisa Sicuri.** CRESIB (Hospital Clínic-Universitat de Barcelona) (Spain). **"Economic evaluation of Chagas disease screening of pregnant Latin American women and of their infants in a non-endemic area".**

- 06/05/2009. **Dr. Kara Hanson.** Health Policy Unit, London School of Hygiene and Tropical Medicine (United Kingdom). **"Delivering the goods: a health systems perspective on the challenge of increasing use of nets and antimalarial drugs to reach global targets".**

- 11/05/2009. **Dr. Franco Pagnoni.** Business Line on Evidence for Antimalarial Policy and Access, WHO/TDR (Special Programme for Research and Training in Tropical Diseases) Geneva (Switzerland) **"Home Management of Malaria".**

- 14/05/2009. **Dr. Gita Ramjee.** HIV Prevention Research Unit, Medical Research Council, Durban (South Africa). **"HIV Prevention Research".**

- 03/06/2009. **Dr. Ivo Müller.** Vector Borne Disease Unit , PNG Institute of Medical Research (Papua New Guinea). **"The epidemiology of *P. vivax* in Papua New Guinea".**

- 09/06/2009. **Dr. Douglas Golenbock.** Division of Infectious Diseases & Immunology, University of Massachusetts Medical School (U.S.A.). **"The Malaria Toxin".**

- 10/06/2009. **Miguel Casado.** General Direction for Co-operation Policies and Evaluation. Foreign Affaires and Co-operation Ministry (Spain). **"Salud en el III Plan Director de la Cooperación al Desarrollo".**

- 17/06/2009. **Dr. Robert Pool.** CRESIB (HC-UB) Barcelona (Spain). **"Using qualitative methods to collect more accurate data in HIV prevention trials: the example of the Microbicides Development Programme".**

- 01/07/2009. **Elizabeth Posada.** CRESIB (HC-UB) (Spain). **"Enfermedad de Chagas, una mirada integral".**

- 17/07/2009. **Juan Garay.** Children Rights and Needs, Human and Social Development Unit, DG Development, European Commission (European Union). **"La Unión Europea ante los desafíos de la Salud Global. El nuevo énfasis en la cobertura universal a través de sistemas nacionales de salud".**

- 14/09/2009. **Dr. Frans van den Boom.** IAVI, Country and Regional Programmes (Netherlands) **"AIDS Vaccine Research: Work in Progress".**

- 29/09/2009. **Dr. Luiz Otavio Penalva.** Children's Cancer Research Institute, Health Science Center, University of Texas (U.S.A.) **"The Musashi tale: adventures of an RNA binding protein in cancer and stem cell biology".**

- 02/10/2009. **Prof. Peter Siba.** Institute of Medical research (Papua New Guinea). **"Lessons learnt and history of the PNG IMR".**

- 09/10/2009. **Dr. David Ross.** University of Pennsylvania (U.S.A.) **"An embarrassment of riches: Mining parasite genome databases for biological function".**

- 14/10/2009. **Dr. Arantza Meñaca.** CRESIB (HC-UB) (Spain). **"¿Qué estudian los antropólogos en Salud Internacional? Conceptos generales de Antropología Médica."**

- 21/10/2009. **Dr. Fernando Baquero.** Microbiology Unit, Hospital Ramon y Cajal, Madrid (Spain). **"Selección en múltiples niveles jerárquicos: la evolución de las beta-lactamasas de espectro extendido".**

- 02/11/2009. **Dr. Fabiana Piovesan Alves.** Drugs for Neglected Diseases initiative (DNDi), Latin America regional office (Brazil). **"Perspectives in Neglected Diseases: DNDi strategy for development of new treatments".**

- 17/11/2009. **Dr. Juan Carlos Palomino.** Mycobacteriology Unit, Institute of Tropical Medicine, Antwerp (Belgium). **"The challenge of tuberculosis control: old technologies and new technologies in the fight against the disease".**

- 25/11/2009. **Dr. Xavier Fernández-Busquets.** Biomolecular Interactions Team, Nanobioengineering Group, Institute for Bioengineering of Catalonia, Barcelona (Spain). **"Nanotechnology against malaria: Strategies for the identification of new drugs and their targeted delivery".**

- 27/11/2009. **Dr. Jordi Vila.** Microbiology Unit, Hospital Clínic de Barcelona (Spain). **"Microbiología clínica. Líneas de investigación".**

- 02/12/2009. **Dr. Maria Mota.** Malaria Unit, Instituto de Medicina Molecular, Universidade de Lisboa (Portugal). **"Approaching malaria from the host side".**

- 09/12/2009. **Dr. Carmen Contreras.** Laboratorio de Enfermedades Entéricas y Nutrición. Instituto de Medicina Tropical Alexander von Humboldt. Universidad peruana Cayetano Heredia (Peru). **"Estudio de DEC (diarrhoeagenic *Escherichia coli*) aisladas de niños peruanos".**

- 11/12/2009. **Dr. Daniel Frans Lozano.** CEADES Salud y Medioambiente (Bolivia). **"Chagas y cardiopatía del adulto, Situación Actual y Retos para el Futuro".**

- 16/12/2009. **Dr. Alfred Cortés.** IRB Barcelona (Spain). **"Variant expression in *Plasmodium falciparum*: beyond var genes".**

- 16/12/2009. **Ricardo Ataide.** Department of Medicine, University of Melbourne (Australia). **"Modelling Monocyte Interactions in the Placenta Phagocytosis Assay".**

- 21/12/2009. **Dr. Pilar Requena.** Department of Biochemistry and Molecular Biology, Universidad de Granada (Spain) **"Mechanism of action of bovine glycomacropeptide as an intestinal anti-inflammatory agent".**

- 22/12/2009. **Dr. Eva Codina.** Immunology Unit, Institut de Biotecnologia i Biomedicina, Universitat Autònoma de Barcelona (Spain). **"Spleen-expanded T lymphocytes contribute to pancreatic insulitis in onset of human type 1 diabetes".**

The annual workshops on Chagas disease organized by CRESIB (the fifth workshop on Chagas disease was given in February 2009) brought together international researchers and experts on this disease. These workshops resulted in the generation and editing of consensus documents and clinical guidelines endorsed by the *Sociedad Española de Medicina Tropical y Salud Internacional* (SEMTSI).

In collaboration with Ministry of Health of the Catalan Government *(Generalitat de Catalunya)*, CRESIB organized a course on Arbovirus *(Chikungunya and Dengue)* addressed to professionals working in the International Health Units of the Catalan Health System in July 2009, given by Drs. Edward Hayes, Roger Eritja, Carles Aranda and Joaquim Gascon.

COOPERATION PROJECTS IN THE FIELD OF INTERNATIONAL HEALTH TRAINING

PROGRAMME TO SUPPORT THE CREATION OF A SPECIALIZATION IN EPIDEMIOLOGY AND BIOSTASTICS IN THE INSTITUT NATIONAL D'ADMINISTRATION SANITAIRE (INAS) IN MOROCCO

Participating entities: Fundació Clínic per a la Recerca Biomèdica, CRESIB, Universitat de Barcelona, Universitat Pompeu Fabra, Institut National d'Administration Sanitaire of Morocco and Agencia de Salud Pública de Barcelona.

Funder: Fundació La Caixa.

Amount: €200,000

Period: 2007-2009

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 and Universidade Eduardo Mondlane and CISM.

Funder: Fundació La Caixa.

Amount: €272,000

Period: 2008-2010

UNIVERSITY SCHOLARSHIP PROGRAMME FOR MOZAMBICAN WOMEN

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

Amount: Fundació La Caixa.

Quantity: €300,000

Period: 2008-2014

International Cooperation

CRESIB's strategy for collaborative action in projects with local partners in the research platforms in Mozambique, Morocco and Bolivia include training and capacity building of health professionals and health cooperation with a view to providing institutional reinforcement of counterparts and of their health policies, programmes and facilities. Furthermore, biomedical research leads and fosters the creation and consolidation of scientific talent and the application of its results in public health decisions aimed at breaking the vicious circle of disease and poverty.

Through the right combination of the three areas of action (training and capacity building, health cooperation and biomedical research), in close collaboration with national counterparts and health systems, it is possible to influence the principles of ownership, alignment and managing for results laid down in the Paris Declaration on Aid Effectiveness. The health cooperation actions carried out so far have been channeled primarily through the ministries of health in each country, strengthening the leadership of their own policies and initiatives, developing their planning and management capacities and thus contributing to the optimization of available resources.

In the past year, development cooperation funds provided by the Agencia Española de Cooperación Internacional para el Desarrollo (AECID) have been used to further strengthen the leadership role in research on neglected diseases in Africa of the Centro de Investigaçao em Saude de Manhiça (CISM), Mozambique, to modernize the centre's facilities and scientific equipment, and to introduce new technologies to improve the organization and management of several ongoing studies. Furthermore, a research laboratory is being created at l´Hôpital d'Enfants de Rabat, Morocco, which forms part of the Centre Hospitalier Ibn Sina and aims to become a national reference for research in the major infectious diseases affecting children under 5 years of age.

For several years the cooperation funds from the Agència Catalana de Cooperació al Desenvolupament (ACCD) have been used to reinforce the national HIV/AIDS programme in Mozambique. More recently, they have fostered the creation of the joint platform for research on Chagas disease of the Universidad Mayor de San Simón (Cochabamba, Bolivia) and CRESIB in Barcelona, a pioneering and innovative two-way cooperation initiative involving researchers and specialists from both countries. Finally, in 2009 the *A*gència de Cooperació Internacional de les Illes Balears (ACIB) supported the CISM's research efforts to study the interaction between severe malaria and anaemia.

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