ACTIVITY REPORT 2009-2010

cism
centro de investigação em saúde de manhiça

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Published by Fundação Manhiça
Rua 12 Manhiça
Mozambique
www.manhica.org

Editorial Committee
Teresa Machai
Tânia Machonisse
Enric Jané

Graphic design and layout
Aguiló Gràfic SL

Cover photo and photos on pages 15, 17, 29, 36, 48 and 52 courtesy of the Bill & Melinda Gates Foundation. Photos on pages 56 and 59 courtesy of Javier Molina. Photo on page 19 courtesy of Kim Manresa.

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FOREWORD

Pascoal M. Mocumbi, MD
Honorary Founder and President
Manhiça Foundation

On behalf of the Manhiça Foundation Board of Trustees, I would like to congratulate the CISM on its achievements in 2009-10. The Manhiça Foundation was created to undertake and promote activities in the fields of health care and scientific and technological development to address the needs of Mozambique and strengthen national capacities in these areas. The CISM plays a vital role in helping to achieve the objectives of the foundation, from which it receives the necessary support to carry out its mission and prepare for future challenges.

The Manhiça Foundation is the result of the conviction on the part of its trustees that research is key to improving health and developing Mozambique and sub-Saharan Africa. The development of drugs, vaccines, and other prevention and diagnostic tools improves health and contributes to the economic and social development of the population. It is crucial to continue to build and strengthen health centers in countries such as Mozambique in order to address the key health challenges in sub-Saharan Africa.

In recent years, the CISM has become one of the most respected research centers in sub-Saharan Africa, thanks to both the quality of its scientific work and its contributions to training and health care. To maintain this level of quality, it is essential to critically and objectively analyze the center's performance and identify areas for improvement. It was with this aim that the Manhiça Foundation created an External Scientific Committee. The committee met for the first time in 2009 to learn about the CISM's activities and evaluate its strategic plan for 2010-13. The founding of the committee was a key step towards ensuring the continued quality of the work conducted by the CISM.

One of the most outstanding aspects of the CISM's work is its capacity for translating research into health policy. With respect to the 2009-10 period, I would like to highlight the fact that the CISM played a key role in an international consortium whose work led to the World Health Organization's recommendation that intermittent preventive treatment should be adopted as a malaria control tool in infants. This is just one example of how an initial concept can be built on and developed until it is ultimately adopted as a healthy policy.

The Manhiça Foundation's capacity to recruit the participation of key national actors is crucial to the ongoing development of the CISM. The appointment of the Universidade Eduardo Mondlane and the Foundation for Community Development to the Board of Trustees in 2010 was a major achievement that will strengthen the Manhiça Foundation and improve its capacity to carry out its mission.

The CISM faces an exciting period, in which one of the main challenges will be to successfully implement its ambitious strategic plan. One of the keys to meeting this challenge will be to continue to prioritize quality and training.

Finally, I would like to thank everybody who has supported the Manhiça Foundation in the development of its activities, and in particular, those who offer continued support to the CISM. In an increasingly globalized world, the success of research centers will hinge on their ability to establish strategic partnerships that will better equip them to meet the global health challenges.

FOREWORD

Pedro L. Alonso, MD, PhD
President of the Board of Governors
Manhiça Foundation

It is always a great pleasure to welcome the CISM's activity report. The 2009-10 period was very exciting and I hope that you will enjoy learning more about the CISM and the activities described in this report.

In recent years, the CISM has developed a broad research agenda and successfully contributed to better health, both in Mozambique and sub-Saharan Africa. Since its creation in 1996, the center has successfully seized many opportunities and identified strategic areas for development. However, after the creation of the Manhiça Foundation in 2008, the Board deemed it necessary to develop a strategic plan incorporating renewed and ambitious vision and mission statements to guide the CISM's future.

In late 2008 the center undertook the development of its Strategic Plan for 2010-13, which was approved by the Manhiça Foundation Board and endorsed by the foundation's External Scientific Committee. The plan redefines the center's vision, mission and values, and identifies a set of goals, objectives and strategies for the four-year period. The center's strategy for 2010-13 is built on three pillars: research, people and sustainability.

Research occupies a central place in the CISM's mission and vision. The strategic plan comprises a set of goals and objectives built around the center's research program, scientific training and research platforms. Among the center's aims is to generate clinical and epidemiological evidence that will guide public health policymakers in their decisions and support the introduction of new tools and strategies, while maintaining a healthy research agenda in molecular biology, physiopathology and immunology. This requires the center to optimize its comparative advantages by building even stronger research platforms and maintaining a translational research pipeline ranging from basic research to public health. In addition, our continued contribution to the training of future generations of Mozambican researchers is key to strengthening the national health research system and ensuring the long-term success of the CISM's mission.

The people working at the center are its most important asset. In an open, multicultural and competitive world, the success of the CISM will depend on its capacity to maintain a stimulating working atmosphere and to attract, retain and develop outstanding staff. To this end, the strategic plan incorporates strategies designed to strengthen its internal training and professional development plans.

Sustainability is of critical importance, particularly in the context of the global economic crisis. The strategic plan addresses the strengthening of financial and scientific management capacities, the improvement of budgeting processes, the development of partnerships and networks at national and international levels and the enhancement of external communications at the national level.

The CISM's Strategic Plan for 2010-13 is ambitious and aims to position the center as a global player in global health, demonstrating its commitment to tackling pressing local and national health problems. I hope you will share my excitement at seeing how this plan unfolds and allows the CISM to continue to contribute to better health in Mozambique and globally.

FOREWORD

Eusébio V. Macete, MD, PhD
Director
Manhiça Health Research Centre

The 2009-10 period covered by this report has been very exciting and productive. In the following lines I would like to highlight some of the developments that took place during this period and I hope that you will be encouraged to read more about these and other activities described in the report.

One major development was that the CISM strengthened the translational character of its research program. With the creation of the Monitoring and Evaluation Unit in 2009, the center took an important step towards bridging the gap between the clinical development of control tools and the evaluation of the effectiveness of these tools in real-life implementations. This unit is currently undertaking projects aimed at improving understanding of the impact of malaria control tools in our study area and other regions of Mozambique. There are, however, plans to expand activities to other areas with a focus on the development of methodologies and tools for effectiveness evaluation. In 2009-10, the CISM also generated clinical and epidemiological data on invasive bacterial infections that are key to guiding public health policies and treatment guidelines. Another example of how the center is generating data to inform public health policymakers is the study undertaken by the CISM to evaluate the effectiveness of the Hib vaccine deployment which was launched in mid 2009.

The CISM participated in the pooled analysis of the safety and efficacy of intermittent preventive treatment in infants (IPTi) published in The Lancet in 2009. This publication culminates the work of an international consortium that has successfully translated an initial concept into a public health recommendation adopted by the World Health Organization. Studies conducted at the CISM in previous years were key to the development of this consortium, and the center is now in a unique position to help national policymakers decide on the implementation of IPTi.

In addition, the center has expanded its research portfolio in malaria, HIV/AIDS, tuberculosis, pneumonia, invasive bacterial infections and diarrheal diseases. I would like to highlight some of our contributions to the development of prevention tools in these areas, such as the RTS,S/AS01E phase III clinical trial that started in 2009, our work in the development of microbicides for the prevention of HIV infection, our investigation of alternative drugs for IPT in pregnancy, and our project to estimate the incidence of tuberculosis infection in children to prepare the center for vaccine trials.

The strengthening of scientific management and coordination mechanisms was key to supporting the development of research activities in 2009-10. The creation of an Internal

Scientific Committee in 2009 has improved coordination and decision-making processes involving the most senior researchers at the CISM. Furthermore, the implementation of a new information platform means that research information generated by the center is now readily available to all staff.

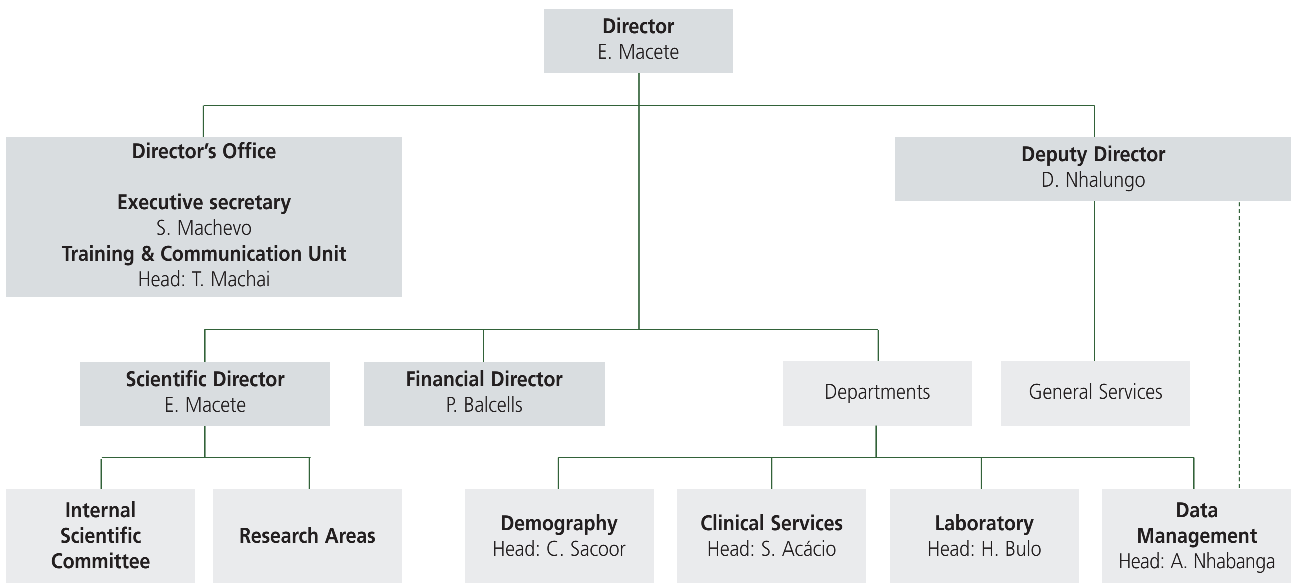
All this research activity needs to be supported by cutting-edge platforms and facilities. Significant improvements were made in this area during 2009-10, including the renovation of electrical installations, the upgrading of servers and the improvement of the telecommunications infrastructure. The laboratory has also undergone dramatic improvements thanks to the ISO 9001:2008 certification obtained in 2009, the implementation of a laboratory information system in collaboration with Siemens and the completion of a new BSL-3 laboratory that will support research in tuberculosis. Finally, the implementation of the data management program, OpenClinica, has allowed the center to significantly upgrade its standards in this area.

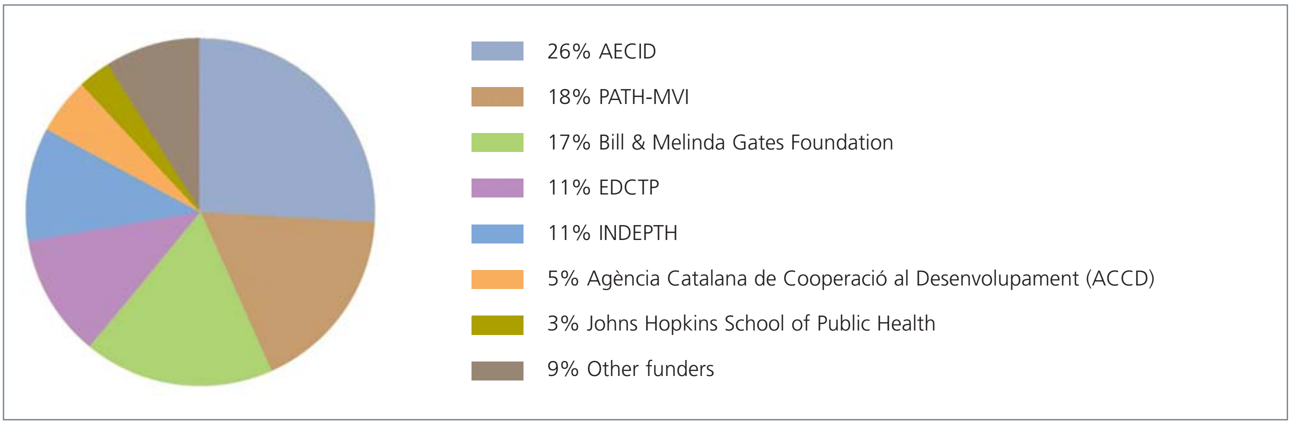
The center also expanded its training activities during 2009-10. The center's longstanding Training Fellowship Program remains the flagship of our research training activities. This program is key to the center's long-term sustainability and the development of the health research sector in Mozambique. In addition, our support to international researchers through internships at the center strengthens our international relations and creates an international environment that also benefits our staff. Finally, the center continued to offer training opportunities to its technical staff and recently developed a training policy designed to stimulate career development within the center through the provision of an improved training framework.

Finally, the CISM has strengthened its relationship with the community through the creation of a Community Consultative Committee. This board is a forum through which the center can communicate its activities and seek advice from community representatives, as well as provide a formal channel for community involvement in the center's activities.

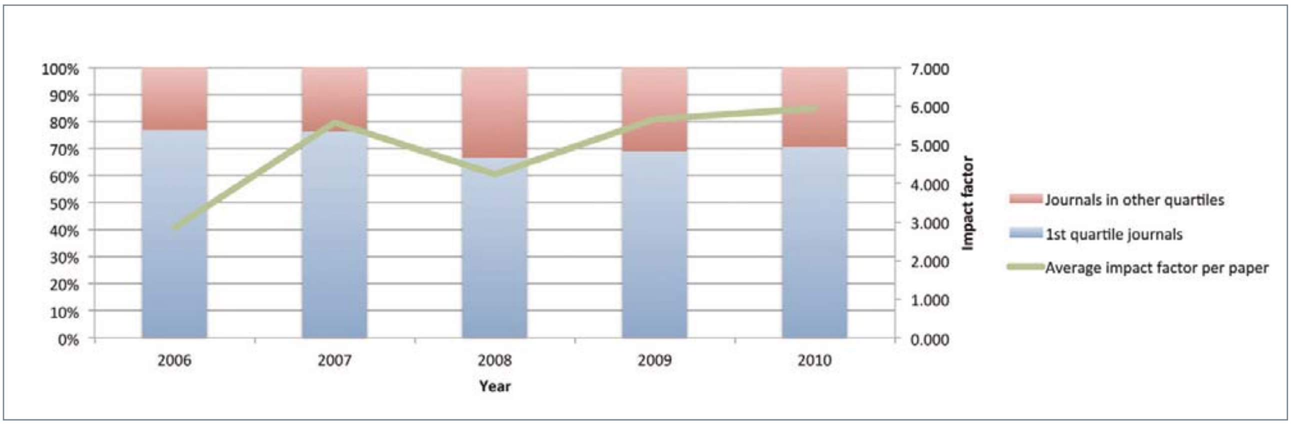
These lines highlight just a few of the activities and achievements described in this report. It goes without saying that none of this would have been possible without the contribution of our outstanding staff and our funders and partners. But above all, my last words are to thank the community which we serve, which is always willing to collaborate with the center. It is thanks to its day-to-day participation in the center's activities that the CISM is able to fulfill its mission.

Organizational Chart of the CISM

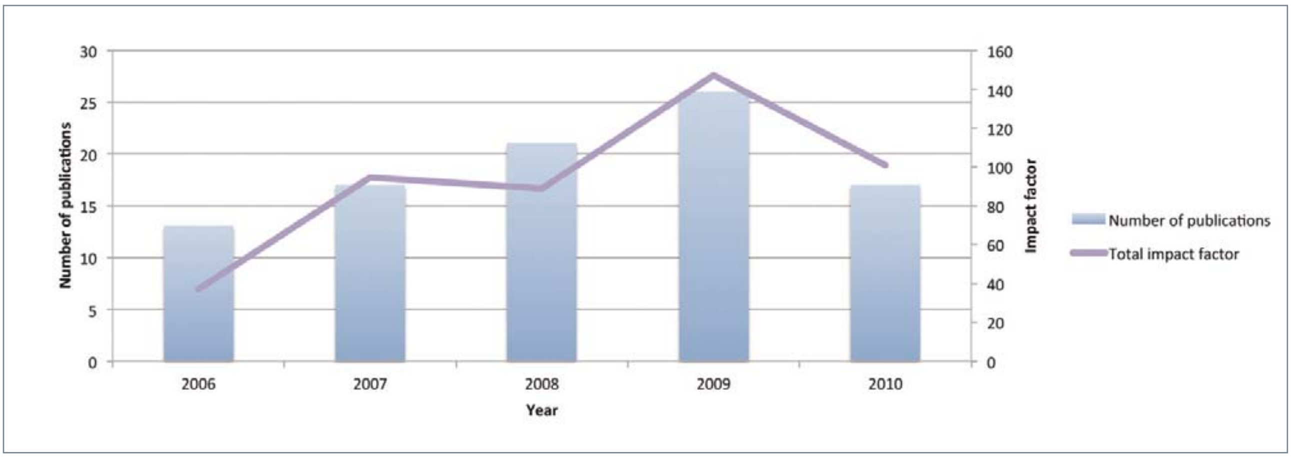




Funders (2009)



Percentage of papers published in journals in the first quartile and average impact factor per paper 2006-10



Number of publications and total impact factor 2006-10

OVERVIEW OF THE CISM

The CISM entrance.

The CISM is located in the Manhiça District, in the South of Mozambique.

The Manhiça Health Research Centre (Centro de Investigação em Saúde de Manhiça, CISM) was created in 1996 to fight disease and safeguard the health of vulnerable populations through research, healthcare assistance and training. Since then, the center has developed a comprehensive research agenda, trained researchers and technical personnel, and contributed to healthcare assistance in the Manhiça District.

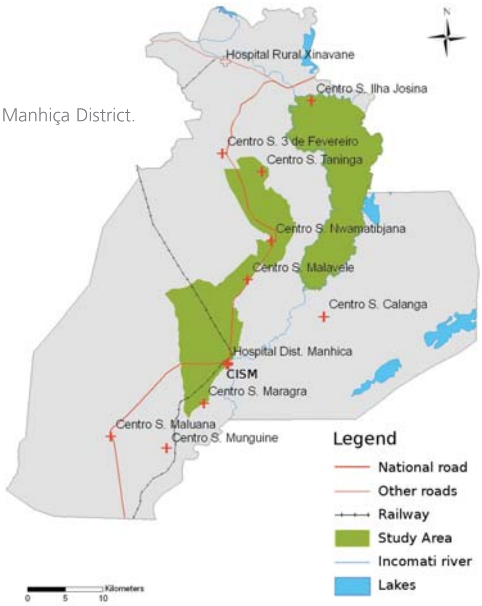
The CISM is located in Manhiça, a village located approximately 80km from Maputo city, in the northern portion of the Maputo province (in the South of Mozambique).

Vision, Mission and Values in CISM's Strategic Plan for 2010-13	
Vision	To become a center of excellence in biomedical research and in the generation of evidence to guide public health policy in Mozambique and the rest of the world
Mission	To foster and conduct biomedical research in health priority areas to promote and safeguard the health of the population
Values	Excellence, ethics and humanity

RESEARCH

The CISM's research agenda aims to tackle priority health problems in Mozambique, which are also representative of the situation in other sub-Saharan African countries.

Manhiça District.



The study area covers an area of 500 km^2 in the Manhiça District, and close to 86,000 inhabitants (approximately 55% of the total population in this district).

The CISM fosters a multidisciplinary approach to health problems to maximize the translation of results obtained in the laboratory into clinical research and the development of control and treatment tools. As a result, research projects are usually conducted by multidisciplinary teams with expertise in areas such as immunology, molecular biology, epidemiology and social sciences.

The center maintains stable research collaborations with national and international centers. In this context, the CISM has a strategic partnership with the Barcelona Center for International Health Research (CRESIB, Hospital Clínic-Universitat de Barcelona). This partnership has contributed significantly to the creation and development of the center in recent years and led to many research projects.

The CISM has three research platforms that are crucial to its activities. These platforms are the demographic, geographic and morbidity surveillance platforms and they cover a study area of 500 km^2 which has close to 86,000 inhabitants. All households in this area are geo-positioned using global positioning system (GPS) and the population is under continuous demographic surveillance. In addition, the Morbidity Surveillance System collects around-the-clock information on all pediatric outpatient visits and admissions to the Manhiça District Hospital and other health centers in the study area.

In the last few years, the CISM has contributed to the development of malaria prevention and treatment tools and gradually broadened its activities to include other priority diseases. The current research agenda of the center targets some of the main causes of death and disease in children and pregnant women.

The research projects presented in this report are grouped into the following areas:

- Malaria
- HIV/AIDS
- Tuberculosis
- Pneumonias and other invasive bacterial diseases
- Diarrheal diseases
- Maternal and reproductive health
- Social sciences
- Other diseases

Research Strategy 2010-13

<table>
<tr><th></th><th>Malaria</th><th>HIV/AIDS</th><th>Tuberculosis</th><th>Pneumonias and other invasive bacterial diseases</th><th>Diarrheal diseases</th></tr>
<tr><td>Clinical and molecular epidemiology</td><td colspan="5" rowspan="5">The CISM's research objectives are divided into strategic areas, as shown in this matrix structure. These research areas are not mutually exclusive, and are expected to create synergies and foster new and creative approaches to research questions.

These research areas encompass the core research activity for the coming years. However, we acknowledge that in a rapidly changing world, the center may need to seize opportunities as they appear, even if they have not been identified in the strategic plan.</td></tr>
<tr><td>Maternal and reproductive health</td></tr>
<tr><td>Social Sciences</td></tr>
<tr><td>Monitoring and evaluation</td></tr>
<tr><td>Clinical trials</td></tr>
</table>

TRAINING

Training is one of the CISM's primary activities. The CISM contributes to the strengthening of human resources in the country through a range of training activities targeting young researchers and technical personnel. These training activities are also crucial to guaranteeing the sustainability of the center.

HEALTHCARE ASSISTANCE

Improving healthcare provision in the Manhiça District is one of the CISM's priorities. The center works in collaboration with the Manhiça Health Centre, the Manhiça District Hospital and the national health authorities to ensure that the community benefits from the presence of the CISM and its research results.

STRUCTURE OF THE CENTER

The CISM is organized into three areas (Research, Finances and General Services), four departments (Demography, Clinical Services, Laboratory and Data Management) and two units (Training and Communications).

- The Research Area is responsible for all the center's research activities and for the coordination and follow-up of research projects (see pages 16-53).
- The Finances Area is responsible for all the financial aspects of the center and its research activities.
- The General Services Area is responsible for services such as maintenance, logistics, security, transport and human resources.
- The Demography Department manages the geographic and demographic surveillance platforms (see page 56).
- The Clinical Services Department manages the Morbidity Surveillance System and coordinates the CISM's healthcare activities in collaboration with the district health authorities (see page 57).
- The Laboratory Department is responsible for the laboratory facilities and provision of diagnostic services to the CISM's projects and the Manhiça District Hospital (see page 58).
- The Data Management Department manages research and morbidity surveillance data and ensures that paper forms are correctly transcribed into the center's databases and subsequently stored (see page 60).
- The Training Unit is responsible for the management of the CISM's training activities and programs as well as the relationships between the CISM and other academic institutions (see page 62).
- The Communications Unit manages all internal and external communications.

The center's Executive Board is formed by the Director, the Deputy Director and the Area Directors.

The CISM's Strategic Plan for 2010-13 identified the following 10 goals

1. To develop and implement a biomedical research agenda focused on health priorities and aimed at informing public policy
2. To continue to make a major contribution to the training of the next generation of health research leaders in Mozambique
3. To improve laboratory capacity and quality
4. To improve data capture and management tools
5. To improve the professional development of the center's staff
6. To strengthen scientific management
7. To improve administrative and financial management capacity
8. To secure and expand funding for core activities and projects
9. To consolidate existing partnerships and seek new national and international partners
10. To design and implement an internal and external communication strategy

THE MANHIÇA FOUNDATION

The CISM is managed by the Manhiça Foundation (Fundação Manhiça), a non-profit institution created in 2008 by the Republic of Mozambique, Spain, the Mozambican National Health Institute, the Fundació Clínic per a la Recerca Biomèdica (Hospital Clínic-Universitat de Barcelona) and Dr. Pascoal M. Mocumbi as honorary founding member. The foundation's mission is to conduct and promote activities in health, science and technology to meet the needs of the country and develop national capacities in these areas.

In 2009, the Foundation for Community Development (Fundação para o Desenvolvimento da Comunidade, FDC) and the Universidade Eduardo Mondlane joined the Manhiça Foundation as trustees.

The Manhiça Foundation is governed by a Board of Trustees and a Board of Governors, which includes the trustees of the foundation. The foundation's activities and strategies are reviewed regularly by an External Scientific Committee, which is an advisory body to the Board of Trustees and the Board of Governors. In its first meeting, held at the CISM in 2009, the External Scientific Committee undertook to gain first-hand knowledge of the overall activity of the center and to analyze the strategic plan proposed for 2010-13.

Two new committees created in 2009-10 to strengthen scientific management and community participation

Internal Scientific Committee

The CISM's Internal Scientific Committee was established in 2009 to provide scientific guidance to the center's leadership, coordinate the center's research activities, review internal research proposals and periodically analyze the center's scientific strategy. It is formed by the most senior members of the researcher staff and meets once a month. During 2009-10 the committee held 16 meetings and analyzed 28 research proposals.

The Community Consultative Committee

As a response to improve community participation in the center's life, the social sciences research group galvanized, with support from the Demography Department and the Communications Unit, the creation of the Community Consultative Committee.

This committee is crucial to ensuring a good relationship with the community and guaranteeing that key information and messages are transmitted to the members of the community in the study area covered by the CISM's activities. The committee holds two ordinary meetings every year and has met four times since its creation in early 2010.

Section 1

Research

MALARIA

According to the World Malaria Report 2008 from the WHO, malaria affects 250 million people and causes one million deaths every year. Sub-Saharan Africa is the region most affected by the disease, with 85% of all cases and 90% of all deaths. Children under five years of age and pregnant women are the most vulnerable groups and malaria continues to be the first cause of death in African children in this age group. However, according to the 2009 WHO World Malaria Report, there has been a decrease in the burden of disease in several sub-Saharan African countries. The reasons behind this fall are not well understood, but increased use of malaria control tools has probably played a key role.

Currently, malaria control is based on three basic strategies: (i) prompt and effective treatment of cases with artemisinin-based combination therapies and intermittent preventive treatment (IPT) during pregnancy; (ii) vector control with indoor residual spraying with insecticides; and (iii) a reduction in human-vector contact via insecticide-treated nets.

Malaria is one of the main research areas of the CISM, which has contributed to the development and evaluation of new control strategies in recent years, with activities including IPT strategies (for both infants and pregnant women) and studies of the RTS,S/AS malaria vaccine candidate and new artemisinin-based combination treatments. The center has also conducted work in immunological and molecular aspects of malaria such as the development of naturally acquired immunity to malaria in children and the pathogenic mechanisms involved in severe and placental malaria. The CISM recently made advances in translational research with the implementation of new monitoring and evaluation activities to monitor the burden and transmission of malaria and assess the effectiveness of control tools.

EPIDEMIOLOGICAL AND CLINICAL CHARACTERIZATION

Malaria-attributable proportion of fever and establishment of malaria case definition in children across different epidemiological settings

In many settings of stable malaria transmission, the presence of asymptomatic malaria parasite carriers is common and the definition of clinical malaria remains uncertain. On the other hand, in most rural areas diagnosis of clinical malaria is generally presumptive (based on fever or history of fever), although a positive blood film is required to confirm diagnosis in areas where laboratory facilities exist.

The CISM, in collaboration with the National Malaria Control Program and the National Institute of Health, conducted a national malaria survey in Mozambique between February 2002 and April 2003 to determine the prevalence and intensity of malaria infections and establish a case definition of malaria and explore its relation to age strata across the country. The study formed part of the routine surveys conducted by the National Malaria Control Program.

A total of 8816 children under ten years of age were selected for the study. Axillary temperature was measured in all study participants and finger-prick blood was collected to prepare thick and thin films for the identification of parasite species and the determination of parasite density. The proportion of fever cases attributable to malaria infection was estimated using logistic regression analysis of fever as a monotonic function of parasite density. Bootstrapped estimated confidence intervals, in addition to sensitivity and specificity for different parasite density cut-offs, were also calculated.

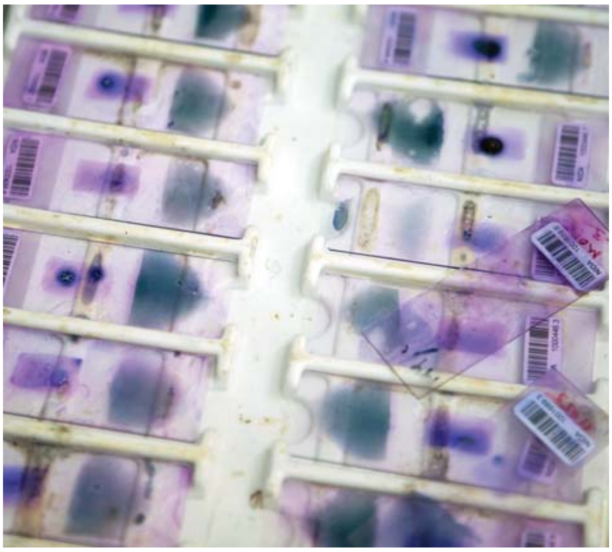
The study confirmed that malaria remains a major cause of febrile illness during childhood. It also defined the relation between parasite density and fever and showed how this varies with age and region. These findings may help to guide case definition for clinical trials of preventive tools, as well as provide definitions that may improve the accuracy of disease burden measurements.

Severe malaria and concomitant bacteremia in children

Invasive bacterial disease and malaria remain the two leading causes of pediatric mortality and morbidity in Africa. Both malaria and bacterial infections constitute an enormous burden for the under-resourced African health facilities. The extent to which the two diseases overlap may vary according to several factors, including malaria endemicity and the prevalence and etiology of bacteremia in different countries.

The CISM conducted a study to describe the relationship between severe malaria and invasive bacterial disease in children under five years admitted to the Manhiça District Hospital in Mozambique. The study was based on retrospective data systematically collected from June 2003 to May 2007 through the center's Morbidity Surveillance System.

The study concluded that the co-existence of malaria and invasive bacterial infections is a common, life-threatening condition. *Streptococcus* pneumoniae was the main pathogen identified in this interaction, possibly as a consequence of the high HIV prevalence in the area. The study suggests that measures directed at reducing the burden of both these infections are urgently needed to reduce child mortality in Africa.



Blood slides for malaria diagnosis.

CLINICAL DEVELOPMENT OF DRUGS, VACCINES AND OTHER CONTROL TOOLS

Clinical development of the RTS,S vaccine

The RTS,S vaccine, which is formulated with adjuvants from the AS0 family by GlaxoSmithKline (GSK) is currently the most promising malaria vaccine candidate. It is a pre-erythrocytic vaccine against *P. falciparum* that contains a recombinant circumsporozoite protein and the hepatitis B surface antigen. The CISM has been working on the clinical development of this vaccine since 2002 in collaboration with the PATH Malaria Vaccine Initiative (MVI) and GSK Biologicals. During this period the center has carried out various phase I, I/IIb and IIb trials.

In 2003, the center conducted the first proof-of-concept phase IIb trial in children one to four years of age that demonstrated that the RTS,S/AS02A vaccine reduced the risk of *P. falciparum* infection (by 45.0%), uncomplicated malaria (by 35.3%) and severe malaria (by 48.6%) for at least 18 months after the last vaccine dose.

The CISM subsequently conducted the first clinical trial to evaluate the safety, immunogenicity and proof-of-concept of the efficacy of RTS,S/AS02D in infants. The results of this phase I/IIb clinical trial, published in 2007, demonstrated that the RTS,S/AS02D vaccine is safe and well tolerated. The candidate vaccine also induced high anti-circumsporozoite antibody titers and the adjusted efficacy of the vaccine against infection was 65.9% (95% CI, 42.6-79.8%; $p<0.0001$).

In August 2009, the center started a phase III clinical trial designed to evaluate the safety and efficacy of the RTS,S/AS01E. Other objectives will allow the evaluation of the overall public health impact of this malaria vaccine. This trial is part of a multicenter trial involving seven African countries aimed at providing the data required to register this vaccine.

A total of 1700 participants aged 6-12 weeks and 5-17 months are going to be recruited for this trial. Primary objectives include efficacy against clinical malaria in children, while secondary objectives include efficacy against severe disease, severe anemia and hospitalization due to malaria.

Intermittent preventive treatment in infants

A pooled analysis of the safety and efficacy of intermittent preventive treatment in infants (IPTi) with sulfadoxine-pyrimethamine in Africa led by a researcher from the CISM and CRESIB was published in 2009 (Aponte et al., Lancet 2009). The analysis, which used data from six double-blind, randomized, placebo-controlled trials, showed that IPTi with sulfadoxine-pyrimethamine was safe and efficacious across a range of malaria transmission settings in Africa, suggesting that this intervention is a useful contribution to malaria control.

This result culminates the work of the IPTi Consortium, integrated by leading malaria research centers in Africa, Europe, the United States, Papua New Guinea, Australia and two United Nations agencies. The IPTi Consortium has developed evidence in areas including safety, efficacy, cost-effectiveness and acceptability to support informed decision-making on the use of IPTi as a malaria control tool.

Children under one year of age have the highest risk of severe malaria. Moreover, the implementation of the Expanded Program on Immunization (EPI) has been key in increasing vaccine coverage during the first year of life. For these reasons, the objective of the clinical development of the RTS,S is to register the vaccine for its use in infants in conjunction with other EPI vaccines.

Insights into long-lasting protection induced by the RTS,S/AS02A malaria vaccine

The pre-erythrocytic malaria vaccine RTS,S/AS02A has been shown to confer protection against clinical malaria for at least 21 months in a trial in Mozambican children. However, the underlying mechanisms that determine efficacy and its duration remain unknown.

The CISM performed a new, exploratory analysis to explore differences in the duration of protection to better understand the protection afforded by RTS,S. The analysis used data from a phase IIb double-blind, randomized controlled trial in 2022 children aged one to four years performed at the center. The trial was designed with two cohorts to assess vaccine efficacy using two different endpoints: clinical malaria (cohort 1) and infection (cohort 2).

The analysis used cohort 2 data collected for safety through the health facility-based passive case detection system. Vaccine efficacy against clinical malaria was estimated over the first six-month surveillance period (double-blind phase) and over the following 12 months (single-blind phase). Adjusted vaccine efficacy against first clinical malaria episodes in cohort 2 was 35.4% (95% CI, 4.5-56.3; p=0.029) for the double-blind phase and 9.0% (-30.6-36.6; p=0.609) for the single-blind phase.

Contrary to observations in cohort 1, where efficacy against clinical malaria did not decrease over time, in cohort 2 efficacy did decrease with time. We hypothesize that this reduced duration of protection is a result of the early diagnosis and treatment of infections in cohort 2 participants as this would have prevented sufficient exposure to asexual-stage antigens. On the other hand, the long-term protection against clinical disease observed in cohort 1 may be a consequence of prolonged exposure to low-dose blood-stage asexual parasitemia.

Clinical development of Dihydroartemisinin + Piperaquine

The CISM participated in a multicenter clinical trial on the clinical development of Dihydroartemisinin (DHA) + piperaquine (PPQ) (Eurartesim®). Five African centers (Burkina Faso, Mozambique, Kenya, Uganda and Zambia) and 1500 participants from six to 59 months of age with uncomplicated *P. falciparum* malaria were enrolled in this phase III study and followed for the first 42 days after treatment. During the trial, participants were randomly allocated to receive DHA-PQP or artemether-lumefantrine (Coartem®); the principal objective was to demonstrate the non-inferiority of DHA-PQP (with a margin of 5%). CISM was the primary recruiting center in the five African countries, contributing to approximately a third of all study participants.

The results of this trial, published in 2009 (Bassat et al., PLoS One), indicate that DHA-PQP is as efficacious as artemether-lumefantrine for uncomplicated malaria treatment in African children from different endemicity settings. They also showed a comparable safety profile. Moreover, the increased occurrence of new infections within the 42-day follow-up among children treated with artemether-lumefantrine suggest that DHA-PQP has a longer post-treatment prophylactic effect, which could be an advantage in favor of this drug in areas of high endemicity. The results of this trial, together with those of a similar trial performed in Asia, have been submitted to the regulatory authorities for the international registration of the drug, expected for early 2011.

Study to compare the efficacy of four possible artemisinin-based combination treatments

The CISM participated in a multicenter, randomized, non-blinded, phase IV multi-arm study designed to compare the efficacy of four antimalarial drug combinations containing an artemisinin derivative (amodiaquine-artesunate, dihydroartemisinin-piperaquine, artemether-lumefantrine and chlorproguanil-dapsone-artesunate) for the treatment of uncomplicated malaria. The study was funded by the European and Developing Countries Clinical Trials Partnership (EDCTP) and was conducted in 10 sites in seven African countries (Burkina Faso, Gabon, Mozambique, Nigeria, Rwanda, Uganda and Zambia).

The objective of the study was to establish the safety and efficacy of these new combinations for 28 days after treatment and to determine the rate of re-infection and need for re-treatment in the six following months. The CISM recruited 511 children aged six months to five years with uncomplicated malaria to be treated with one of the combinations. The children were then followed for 28 days using active detection and for six months after treatment using passive case detection. Study data are currently being analyzed and results are expected to be published in 2011.

Clinical development of Fosmidomycin+Clindamycin

Previous data from studies conducted in Africa (children) and Asia (mostly adults) have confirmed the safety and adequate efficacy of the novel, non-artemisinin-based combination therapy with fosmidomycin+clindamycin (F+C) for the treatment of uncomplicated malaria. However, more data

Sample collection by finger prick.

specifically targeting young African children are needed to substantiate the scarce data available on African children.

In January 2010, the CISM started a study to assess the safety and efficacy of F+C. In this open-label, non-randomized clinical trial, 52 children aged between 6 months and 3 years with uncomplicated malaria were recruited at the Manhiça District Hospital, treated with F+C and followed according to standard procedures until day 28. The primary endpoint was efficacy measured as standard day 28 polymerase chain reaction (PCR)-corrected cure rates. The study was carried out without incidents, and data are currently being analyzed.

This study was the first step in a more ambitious project funded by the European and Developing Countries Clinical Trials Partnership (EDCTP) for the clinical development of this drug combination in collaboration with four European and six African research centers (Bagamoyo, Tanzania, Cotonou, Benin, Lambaréné, and Gabon). This second phase is expected to start in 2011 and continue until 2013. The objective will be to ascertain the best dosage for pediatric treatment and subsequently, to evaluate the efficacy and safety of the selected dosage in four African regions with different malaria endemicity.

Other studies

See the *Maternal and reproductive health* section for research projects on malaria prevention in pregnant women and the safety of antimalarial drugs during pregnancy.

See the *Social sciences* section for research projects on the cost-effectiveness of malaria control tools and the availability and use of antimalarial drugs in the community.

MALARIA IMMUNOLOGY

Age of exposure to *P. falciparum* and development of immunity against malaria in infants

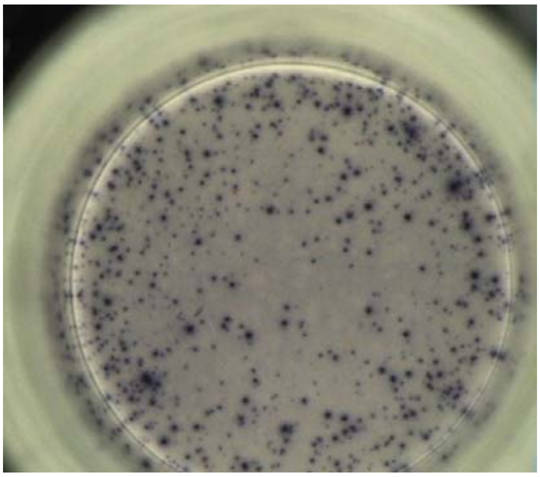
People living in regions of sub-Saharan Africa where malaria is endemic who are repeatedly exposed to infections by *P. falciparum* from birth, develop naturally acquired immunity (NAI) to the parasite. In areas with annual and stable transmission, there are almost no cases of severe malaria or deaths associated with malaria after five years of age, and the incidence of clinical malaria, along with the prevalence and the density of infection decrease with age. In high-transmission areas, NAI takes place at younger ages. The development of NAI depends on age as well as on transmission intensity, but it has been difficult to determine the contribution made by these two variables in an independent manner. The development of NAI against *P. falciparum* is still relatively unknown. Previous studies on continuous or intermittent prophylactic malaria treatment in infants suggest that the age of the first exposure to *P. falciparum* during the first year of life could be an important determinant of the development of NAI. Deepening knowledge in this area is key for future strategies to control malaria and, specifically, for malaria vaccines.

The CISM conducted a study from 2005 to 2009 to evaluate the effect of the age of first exposure to *P. falciparum* in the development of NAI in infants. The study was part of the AgeMal Consortium, which is funded by the European Union.

The study was a randomized, double-blind, placebo-controlled trial with three arms where the age of exposure to *P. falciparum* was controlled by administering chemoprophylaxis to participants during different periods of the first year of life. The risk of clinical malaria and anemia during the second year of life and the type and quality of immune responses was then compared between the different groups. The role of oxidative stress and host genetic factors in the development of NAI was also evaluated. The results of the study will be published in 2011.

Impact of intermittent preventive treatment on the development of naturally acquired immunity in infants

In areas with high malaria transmission, infants carry the burden of disease. This age group is thus one of the main targets of prevention strategies. Intermittent preventive treatment in infants (IPTi) consists of administering antimalarial drugs at specific time points in the first year of life during the Expanded Program on Immunization (EPI) visits, and has demonstrated to be effective in the prevention of malaria in children.



High in vitro cytokine response in human leukocytes after antigen stimulation by ELIspot technique.

One of the important issues regarding the implementation of malaria prevention strategies, such as IPTi, is to evaluate their impact on the development of naturally acquired immunity (NAI). Recent studies have demonstrated that the use of chemoprophylaxis in children may jeopardize the development of NAI. However, studies conducted in Tanzania and in Mozambique have indicated that IPTi not only reduces the risk of malaria without interfering in the development of NAI, but could also enhance its development. Nonetheless, there are no studies on the evaluation of immune responses to *P. falciparum* in the context of IPTi.

The CISM conducted a study to evaluate the development of immunity against *P. falciparum* in the context of a randomized, double-blind, placebo-controlled trial to assess the efficacy of IPTi with sulfadoxine-pyrimethamine (SP) administered at 3, 4 and 9 months of age through the Expanded Programme on Immunization. The study showed that IPTi is efficacious against clinical malaria, with a 22.2% decrease in the risk of clinical malaria detected during the first year of life. Antibodies against *P. falciparum*'s erythrocytic stage antigens MSP-1, AMA-1 and EBA 175 were used to measure the immune responses. These antigens play a critical role during the invasion of erythrocytes and are used in the development of malaria vaccine candidates.

The study has already shown that IPTi with SP did not modify antibody levels against *P. falciparum*'s erythrocytic-stage antigens during the first two years of life, indicating that the intervention does not negatively affect the acquisition of malaria antibodies.

Currently, analyses are being conducted to investigate the effects of IPTi with SP on other antibody responses that are thought to be involved in the acquisition of immunity, such as variant surface antigens (VSAs) and growth-inhibiting antibodies. During 2009-10, the statistical analyses of the immunological data obtained in the laboratory were completed and published. Data analyzed included antibody and cytokine responses to *P. falciparum* measured at 5, 9, 12 and 24 months of age in children from Manhiça participating in the IPTi trial. Specifically, the following markers were investigated: antibody responses to merozoite surface proteins (MSP1-19, AMA-1 and EBA-175) by enzyme-linked immunosorbent assay, antibody responses to VSAs expressed on *P. falciparum* by a flow cytometry assay (FACS), antibodies that inhibit in vitro parasite growth, and cytokine responses to *P. falciparum* by luminex and intracellular cytokine staining and flow cytometry.

The data showed that IPTi does not affect levels of VSA antibodies or cytokines or the frequency of growth inhibitory antibodies during the first two years of life. These functional immune responses were not associated with protection against malaria. However, levels of IgG1 and IgG3 antibodies to EBA-175 at 12 months of age were associated with a reduced incidence of malaria in the second year of life.

Role of maternal immunity on the clinical outcomes of malaria in pregnancy

Women are at higher risk of *P. falciparum* infection and disease when pregnant. This increased susceptibility has been explained by nutritional factors, increased attraction of mosquitoes, normal maternal immunity alterations needed for successful fetal development, and/or hormone imbalances characteristic of the gestational condition. In particular, a permissive effect on parasite replication associated with these immuno-endocrine changes has been proposed as an explanation for the massive accumulation of erythrocytes infected by *P. falciparum* in the placental intervillous spaces, a characteristic feature of malaria during pregnancy.

There is growing evidence that malaria susceptibility could largely be explained by a lack of antibodies that can block adhesion of infected erythrocytes to placental chondroitin sulfate A. However, recent results obtained in the CISM suggest that pregnancy-associated immunosuppression may explain poor pregnancy outcomes in the absence of placental infection and increased susceptibility to malaria during the early postpartum period.

The CISM started a study aimed at describing the causes of this susceptibility to malaria in pregnancy from an immunological perspective. The study will also provide a greater understanding on how pregnant women become resistant to malaria infection.

Five-hundred and thirty pregnant women will be enrolled in the study in the context of a randomized open-label superiority trial aimed at evaluating the use of mefloquine for intermittent preventive treatment in pregnancy (IPTp) (MiPPAD, Malaria in Pregnancy Preventive Alternative Drugs). Blood samples will be collected before receiving IPTp doses (the first given at at least 13 weeks of gestation and the second at least one month after the first dose), and at delivery. Antibody-mediated immunity (malaria-specific and general) and cellular immunity (effector and memory) will be determined and associated with pregnancy outcomes. Recruitment will finish at the end of 2011.

Immune correlates of protection against malaria after vaccination with RTS,S/AS01E

As the defining study for the RTS,S vaccine licensure, and probably the last trial in which there will be an unvaccinated control group, the RTS,S/AS01E Phase III trial being conducted presents the best opportunity to understand the mechanisms of vaccine action and immune correlates of vaccine-induced protection.

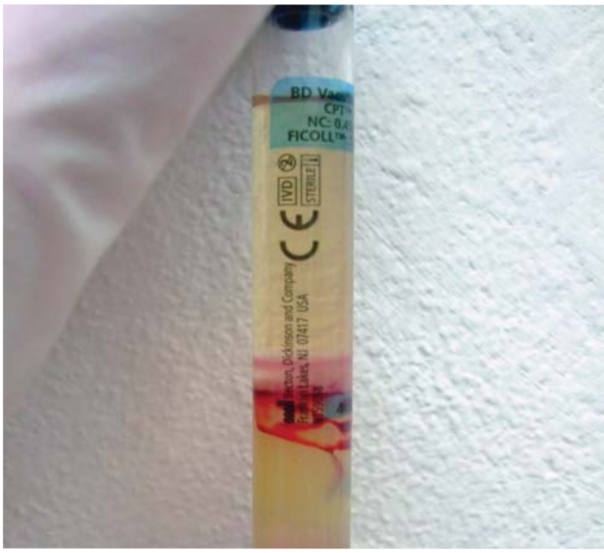
The CISM started a multicenter study ancillary to the phase III trial to investigate the immunological basis of RTS,S-induced immunity. The study will go beyond the current measurement of the vaccine-induced antibody response, that of acquired anti-circumsporozoite protein antibody titers, to include assessment of the isotype (subclasses), quality (affinity/avidity) and functionality (invasion/development inhibition/sporozoite migration) of IgG antibody responses against pre-erythrocytic antigens. In addition, the study will 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 cross-sectional visits. Finally, the induction of antibody and cellular immune responses against *P. falciparum* blood-stage antigens will be measured to further investigate potential mechanisms of RTS,S-induced long-term protection.

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

In 2009-10 blood samples were collected, processed and stored at screening and one month after the third vaccination dose from a subsample of children and infants for future immunology studies. Furthermore, there have been three meetings of the Vaccine Immunology Workgroup of the Mal055-Immunology study consortium (London, Nairobi and Barcelona) to develop the study protocol and experimental plan.

Study of asexual blood stage immunity markers associated with long-lasting protection in children vaccinated with RTS,S/AS02A

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



Separation of blood into erythrocytes, leukocytes and plasma through centrifugation.

The CISM undertook 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 lower-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 was conducted in study participants in which parasitemia was measured by microscopy and blood collected from participants to investigate blood-stage immunogenicity. No significant differences were found in the prevalence of infection between vaccine and control groups at 60 months after the first immunization, indicating that RTS,S /AS02A protection lasted for four years in the Mozambican children studied.

Antibody-based immune responses to asexual erythrocytic-stage antigens were then analyzed in a randomized subsample of RTS,S/AS02A-vaccinated and control children at study month 8.5.

After preliminary univariate crude analyses no differences were found in antibody responses between vaccinated and control children six months post-vaccination with these blood-stage immune markers, thus providing 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.

Immunity and susceptibility markers to malaria in individuals exposed to *P. falciparum* infection

With continuous exposure to *P. falciparum*, individuals acquire effective natural immunity with age, but the underlying mechanisms of protection are unknown.

To date, comprehensive analysis of the role of cellular immune responses in immunity has been impeded by the limited number of parameters that could be assayed using standard methodologies and by the large sample volumes required for such analyses. Recent advances in multiplex and high-throughput methods, however, support the simultaneous characterization of multiple mediators and cell phenotypes, allowing for the qualitative and quantitative analysis of malaria immunity.

The CISM has undertaken a comprehensive analysis of cell-mediated immunity to identify responses against *P. falciparum* which could be used as markers of immunity and/or susceptibility against malaria. In particular, this study will characterize (i) immunopathological markers of severe malaria in children, (ii) markers of clinical immunity in children and adults, and (iii) immunopathological markers of placental malaria in pregnant women.

To achieve this, a collection of cryopreserved blood samples from six studies conducted in Mozambique and Barcelona, which were designed to investigate immunity and pathogenesis against malaria from different perspectives, will be analyzed.

The characterization of correlates/surrogates of immunity will considerably facilitate the development and evaluation of new vaccine candidates and the deployment of effective interventions against malaria.

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

Microscopic analysis of blood smears is currently the most frequently used method to measure parasitemia in malaria vaccine trials. However, it is subjective and labor intensive, which complicates its utilization in large-scale evaluation programs. Flow cytometry is a powerful technique for measuring parasitemia in the peripheral blood of mice and humans and could represent an alternative method. However, due to the limited specificity achieved with currently available flow cytometry techniques, it has not been widely used in vaccine trials.

The CISM conducted an exploratory evaluation of a new flow cytometry methodology developed by the group of Jimenez-Diaz MB and Angulo-Barturen at GlaxoSmithKline in Tres Cantos, Madrid, to estimate parasite density, based on infected red blood cell count, identified by auto-fluorescence and DNA content measured after staining with YOYO-1.

Blood samples were collected from 100 individuals recruited at the Manhiça District Hospital and participants of the preparation study for Mal055. Blood drops were collected by finger prick, fixed with glutaraldehyde, incubated with RNAase, and stained with YOYO-1 in 96-well plate format. After acquisition in a 4-color Becton Dickinson FACSCalibur (with blue laser 488nm), erythrocytes gated in logarithmic side/scatter plots were analyzed in bidimensional FL-2 (585 nm)/YOYO-1 (530 nm) plots in comparison with unidimensional YOYO-1 analysis. The results are expected to be published in early 2011.

MOLECULAR EPIDEMIOLOGY AND PHYSIOPATHOLOGY

Role of *P. falciparum* adhesion and immune responses to the parasite in severe malaria

Only 1% to 2% of *P. falciparum* malaria cases lead to severe episodes, which can manifest in many forms, including deep coma (cerebral malaria), severe anemia, respiratory distress with metabolic acidosis and, less frequently, multiorgan failure. Any of these syndromes can be fatal or cause serious sequelae. However, the causal relationship between symptoms and the underlying pathogenic process is not well established and continues to be a controversial topic. Severe malaria has been attributed partly to the sequestration of *P. 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.

The CISM performed a study to determine whether *P. falciparum* isolates have intrinsic properties of cytoadherence that are correlated with clinical severity and to explore the contribution of specific human receptors in this adhesion phenotype. To this end, forty-six *P. falciparum* isolates from children under five years of age in Manhiça with severe malaria (cases) were examined and compared to 46 isolates from sex- and age-matched children with uncomplicated malaria (controls). Cytoadherence properties such as platelet-mediated clumping, rosetting and adhesion to purified receptors (CD36, ICAM1 and gC1qR) were compared between these matched pairs by non-parametric tests.

Compared to matched controls, the prevalence of platelet-mediated clumping was higher in cases (P=.019), in children presenting with prostration (P=.049) and in children with severe anemia (P=.025). The prevalence of rosetting and gC1qR adhesion was also higher in isolates from cases with severe anemia and multiple seizures, respectively (P=.045 in both cases). Our data indicate a role for platelet-mediated clumping, rosetting and adhesion to gC1qR in the pathogenesis of severe malaria. Inhibition of these cyto-adherence phenotypes may improve severe malaria outcomes.

Finally, the CISM is currently completing the comparison of immune responses in children with severe and uncomplicated malaria. The results will provide information on differential immune mechanisms involved in the severity of infection.

CISM staff performing a malaria survey.

Phenotypic, antigenic and transcriptional characterization of *P. falciparum* placental isolates

In malaria-endemic areas, the prevalence, density and severity of *P. falciparum* infection are greater in pregnant women than in the same women before they get pregnant or in women who are not pregnant.

It has been suggested that susceptibility in pregnancy could be largely explained by a lack of antibodies that are capable of blocking the adhesion of infected erythrocytes to placental chondroitin sulfate 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 (men exposed to malaria lack these antibodies), parity-dependent (antibodies increase during successive pregnancies) and has been associated with a lower risk of placental parasitemia, maternal anemia and low birth weight. In light of these experimental findings, it has been suggested that var2csa may constitute an attractive target for vaccination against malaria in pregnancy.

In this context, the CISM is investigating the molecular mechanisms involved in the adhesion of *P. falciparum* to the placenta in order to identify antigens that could be used to develop vaccines against malaria during pregnancy. Specifically, the CISM is examining the effect of parity on maternal antibody responses against *P. falciparum* isolates collected from pregnant Mozambican women and non-pregnant hosts. IgGs against the surface of *P. falciparum*-infected erythrocytes and merozoite recombinant antigens have been quantified in plasma from women at delivery and from men and children.

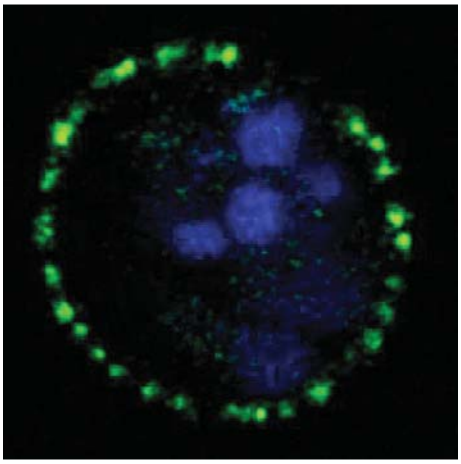
Maternal isolates, but not isolates from non-pregnant hosts, were found to transcribe var2csa. As placental infection was associated with increased levels of IgGs against the merozoite antigens and isolates tested, as well as total IgGs, we stratified comparisons by placental infection. Compared to men, infected primigravidae (PG) and uninfected multigravidae (MG) had higher levels of IgGs against isolates from pregnant women, but not against other isolates, supporting the concept of a pregnancy-specific development of immunity to these variants of the parasite. In women without placental infection, IgGs against parasites infecting pregnant and non-pregnant hosts, as well as total IgGs, were lower in PG compared to MG and men, suggesting that poor immunity to placental parasites and a general reduction of immunity to *P. falciparum* may both predispose PG to malaria.

The results of this study show that parity and placental infection can modulate antimalarial immunity during pregnancy. 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.

Molecular markers of *P. falciparum* drug resistance in the context of intermittent preventive treatment during pregnancy

Factors involved in the development of *P. falciparum* resistance to sulfadoxine-pyrimethamine (SP), particularly in the context of intermittent preventive treatment during pregnancy (IPTp), are not well known.

The CISM conducted a study aimed at determining the impact of IPTp and HIV infection on molecular markers of SP resistance and the clinical relevance of resistant infections. To this end, SP resistance alleles were determined in peripheral (n=125) and placental (n=145) *P. falciparum* isolates obtained from pregnant women enrolled in a randomized, placebo-controlled trial of IPTp in Manhiça, Mozambique. The prevalence of quintuple mutant infections was 12% (23/185) in pregnant women who received placebo and 24% (20/85) in those who received SP. When the last IPTp dose was administered in late pregnancy, mutant infections at delivery were more prevalent in placental samples (7/30, 23%) than in matched peripheral samples (2/30, 7%), in women who received IPTp-SP than in those who received placebo (odds ratio [OR] 8.13, 95% CI, 1.69-39.08) and in HIV-positive women than in HIV-negative women (OR=5.17, 95% CI, 1.23-21.66).



Surface antigens on *P. falciparum*-infected erythrocytes seen by immunofluorescence.

No association was found between mutant infections and increased parasite density or malaria-related morbidity in mothers or children. In conclusion, this study shows that IPTp with SP increases the prevalence of resistance markers in the placenta and in HIV-infected women at delivery, suggesting that host immunity is key for the clearance of drug-resistant infections. However, this effect of IPTp is limited to the period when blood levels of SP are likely to be significant and does not translate into more severe infections or adverse clinical outcomes.

Physiopathological mechanisms involved in placental malaria infections and its impact on fetal development

Malaria in pregnancy (MiP) is characterized by the accumulation of *P. 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 physiopathological mechanisms of MiP.

To assess the specificity and uniqueness of var2csa transcription in pregnancy, the CISM conducted a study in which var gene transcription patterns were assessed 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 from non-pregnant individuals, although var2csa was detected at low levels in 39/40 (98%) of isolates from non-pregnant donors. The presence of other var gene subgroups in pregnant women was detected for 15/19 (78%) of placental isolates and for all peripheral samples. The proportion of A transcripts was significantly higher in children (71% of total var genes) than in adults (38%), while B genes were more common in adults (42%) than in children (19%). Whether the presence of parasites transcribing non-var2csa var transcripts plays an important role in placental infection and the development of immunity during pregnancy needs to be further explored.

To quantify the degree of conservation of expressed var2csa variants we sequenced the CSA-binding DBL2 and DBL3 domains of var2csa from 22 placental and 21 peripheral isolates from pregnant women, and obtained 388 different sequences for DBL2 and 456 for DBL3. 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.

The center also conducted a study to investigate the cell-mediated immune mechanisms involved in placental malaria infection and their impact on fetal outcome. The aim was to evaluate the relationship between immune cell populations and immunoendocrine 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 performed.

Preliminary results showed that (i) there were higher cytokine concentrations in placental plasma compared to peripheral plasma; (ii) there were similar levels of Th1 cytokines and Th2 cytokines in the different infection groups; (iii) there were higher levels of IL-10 in the active malaria groups in both compartments and IL-8 in the periphery; and (iv) there were higher levels of the pro-inflammatory IL-1, cytokine in placental plasma in the active malaria group (but no differences were found in peripheral plasma levels among groups).

The unit for drugs trials.

MONITORING AND EVALUATION

Universal Coverage Bed Net Distribution Effectiveness Study in 4 districts in the Sofala Province of Mozambique

Long-lasting insecticide-treated nets (LLINs) are one of the main malaria control tools. Coverage of LLINs has increased in recent years and the objective of the Mozambican National Malaria Control Program (NMCP) is to achieve universal access. The NMCP has conducted several pilot universal coverage LLIN distribution campaigns, but there is a lack of evidence on which is the best distribution model and the impact on malaria burden.

The CISM, in collaboration with the NMCP, Population Service International and the President's Malaria Initiative (PMI, Centers for Disease Control and Prevention/USAID), is evaluating the effectiveness of an LLIN distribution campaign conducted in 4 districts in the province of Sofala (Gorongosa, Muanza, Nhamatanda and Cheringoma) in 2010. The study will evaluate the distribution model, the coverage and use of the nets and their impact on the prevalence of malaria and anemia in children under 5 years, the incidence of malaria at health facilities, and the intensity of *P. falciparum* transmission in the distribution area. A preliminary cross-sectional study that will be repeated annually for two more years was conducted in 2010. Finally, a passive case detection system is being implemented in four health facilities in the study area.

Cross-sectional studies to monitor the burden of malaria and the impact of different malaria control tools in Manhiça

In Mozambique, as in most of sub-Saharan Africa, the National Malaria Control Program has implemented new malaria control tools in recent years and is now increasing coverage to accelerate and intensify malaria control in the country. In this context, it is critical to evaluate time trends in the burden of malaria and intensity of malaria transmission and to estimate the impact of the introduction of malaria control tools.

As part of its monitoring and evaluation activities, the CISM is going to conduct an annual cross-sectional survey in the Manhiça area between 2010 to 2014 to monitor the prevalence of parasitemia and anemia and the coverage of malaria control tools. An age-stratified sample of 1000 individuals of all ages will be selected by random sampling from the demographic surveillance system census. The study also aims to evaluate the intensity of malaria transmission in the area through the use of a new methodology that calculates serological conversion rates from immune serological markers of exposure to *P. falciparum* erythrocytic-stage antigens. The study will also estimate the impact of the introduction of malaria control tools (bed nets, indoor residual spraying, etc.).

The first cross-sectional survey was conducted in March 2010 and data are currently under analysis.

Health facility–based cross-sectional study to evaluate the intensity of malaria transmission in Manhiça

Malaria monitoring and evaluation activities include different approaches, mainly based on routine data from health facilities and community cross-sectional surveys. These community-based studies are a useful tool to monitor the prevalence of malaria, the use of malaria control tools and the intensity of transmission in particular areas. However, they are logistically complex, especially in areas with outdated census data. New methodologies are required to conduct rapid and cheap evaluations in different areas of Mozambique. In Tanzania, a study showed that malaria transmission estimates based on serological conversion rates from a health facility–based cross-sectional survey were comparable to those obtained from a community-based cross-sectional survey.

Based on the assumption that individuals who attend health services are a good representation of the local community, health facility–based cross-sectional surveys, which include patients attending for any reason in addition to accompanying persons or visitors, are an interesting alternative for estimating local malaria transmission intensity derived from serological conversion rates as well as malaria prevalence.

In 2010, the CISM conducted a study of community members at both the Maragra and Ilha Josina health posts; the participants (n=250 at both posts) included men, women (pregnant or not) and children of all ages resident in the area who attended the health facility for any reason and voluntarily agreed to participate in the study. Malaria transmission intensity will be estimated from serological conversion rates and the prevalence of malaria will also be calculated.

The results of the study are being analyzed and will be compared with matching data collected during the community-based cross-sectional survey conducted in the same area to evaluate the level of consistency between the malaria transmission estimates derived from both studies.

Researchers

Ruth Aguilar [1, 2]
Pedro Aide [1, 3]
Pedro L. Alonso [1, 2]
John J. Aponte [1, 2]
Quique Bassat [2]
Joseph Campo [2]
Carlota Dobaño [2]
Raquel González [1, 2]
Caterina Guinovart [1, 2]
Miguel Lanaspa [1, 2]
Eusébio Macete [1, 4]
Sónia Machevo [1, 5]
Alda Mariano [5]
Maria Nélia Manaca [1]
Alfredo G. Mayor [2]
Clara Menéndez [1, 2]
Gemma Moncunill [2]
Augusto Nhambomba [1]
Diana Quelhas [1]
Montse Renom [1]
Mauricio Rodríguez [1, 2]
Eduard Rovira [2]
Jahit Sacarlal [1, 5]
Elisa Serra [2]
Esperança Sevene [1, 5]
Elisa Sicuri [2]

[1] Manhiça Health Research Centre (CISM)
[2] Barcelona Centre for International Health Research (CRESIB), Hospital Clínic – Universitat de Barcelona, Spain
[3] National Health Institute (INS), Mozambique
[4] Ministry of Health, Mozambique
[5] Universidade Eduardo Mondlane, Mozambique

PUBLICATIONS

Aide, P., J. J. Aponte, M. Renom, T. Nhampossa, J. Sacarlal, I. Mandomando, Q. Bassat, M. N. Manaca, A. Leach, M. Lievens, J. Vekemans, M. C. Dubois, C. Loucq, W. R. Ballou, J. Cohen, and P. L. Alonso. "Safety, Immunogenicity and Duration of Protection of the RTS,S/AS02(D) Malaria Vaccine: One Year Follow-Up of a Randomized Controlled Phase I/IIb Trial." PLoS One. 5.11 (2010): e13838.

Aponte, J. J., D. Schellenberg, A. Egan, A. Breckenridge, I. Carneiro, J. Critchley, I. Danquah, A. Dodoo, R. Kobbe, B. Lell, J. May, Z. Premji, S. Sanz, E. Sevene, R. Soulaymani-Becheikh, P. Winstanley, S. Anemana S. Adjei, D. Chandramohan, S. Issifou, F. Mockenhaupt, S. Owusu-Agyei, B. Greenwood, M. Grobusch, P. G. Kremsner, E. Macete, H. Mshinda, R. D. Newman, L. Slutsker, M. Tanner, P. Alonso, and C. Menendez. "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. 9 (2009): 61258–7.

Barbosa, A., D. Naniche, J. J. Aponte, M. N. Manaca, I. Mandomando, P. Aide, J. Sacarlal, M. Renom, S. Lafuente, W. R. Ballou, and P. L. Alonso. "Plasmodium falciparum-specific cellular immune responses after immunization with the RTS,S/AS02D candidate malaria vaccine in infants living in an area of high endemicity in Mozambique." Infect Immun. 77.10 (2009): 4502–4509.

Bassat, Q., C. Guinovart, B. Sigauque, I. Mandomando, P. Aide, J. Sacarlal, T. Nhampossa, A. Bardaji, L. Morais, S. Machevo, E. Letang, E. Macete, J. J. Aponte, A. Roca, C. Menendez, and P. L. Alonso. "Severe malaria and concomitant bacteraemia in children admitted to a rural Mozambican hospital." Trop Med Int Health. 14.9 (2009): 1011–1019.

Bassat, Q., M. Mulenga, H. Tinto, P. Piola, S. Borrmann, C. Menendez, M. Nambozi, I. Valea, C. Nabasumba, P. Sasi, A. Bacchieri, M. Corsi, D. Ubben, A. Talisuna, and D'Alessandro U. "Dihydroartemisinin-Piperaquine and Artemether-Lumefantrine for Treating Uncomplicated Malaria in African Children: A Randomised, Non-Inferiority Trial." PLoS One. 4.11 (2009): e7871.

Borrmann, S., W. M. Sallas, S. Machevo, R. Gonzalez, A. Bjorkman, A. Martensson, M. Hamel, E. Juma, J. Peshu, B. Ogutu, A. Djimde, D'Alessandro U, A. C. Marrast, G. Lefevre, and S. E. Kern. "The effect of food consumption on lumefantrine bioavailability in African children receiving artemether-lumefantrine crushed or dispersible tablets (Coartem) for acute uncomplicated Plasmodium falciparum malaria." Trop Med Int Health. 15.4 (2010): 434–441.

Brown, G. V., V. S. Moorthy, Z. Reed, K. Mendis, M. Arevalo-Herrera, and P. Alonso. "Priorities in research and development of vaccines against Plasmodium vivax malaria." Vaccine. 27.52 (2009): 7228–7235.

Chase, C., E. Sicuri, C. Sacoor, D. Nhalungo, A. Nhacolo, P. L. Alonso, and C. Menendez. "Determinants of household demand for bed nets in a rural area of southern Mozambique." Malar J. 8.1 (2009): 132.

Conteh, L., E. Sicuri, F. Manzi, G. Hutton, B. Obonyo, F. Tediosi, P. Biao, P. Masika, F. Matovu, P. Otieno, R. D. Gosling, M. Hamel, F. O. Odhiambo, M. P. Grobusch, P. G. Kremsner, D. Chandramohan, J. J. Aponte, A. Egan, D. Schellenberg, E. Macete, L. Slutsker, R. D. Newman, P. Alonso, C. Menendez, and M. Tanner. "The cost-effectiveness of intermittent preventive treatment for malaria in infants in Sub-Saharan Africa." PLoS One. 5.6 (2010): e10313.

Grobusch, M. P., J. J. Gabor, J. J. Aponte, N. G. Schwarz, M. Poetschke, J. Doernemann, K. Schuster, K. B. Koester, K. Profanter, L. B. Borchert, F. Kurth, P. Pongratz, S. Issifou, B. Lell, and P. G. Kremsner. "No rebound of morbidity following intermittent preventive sulfadoxine-pyrimethamine treatment of malaria in infants in Gabon." J Infect Dis. 200.11 (2009): 1658–1661.

Guinovart, C., J. J. Aponte, J. Sacarlal, P. Aide, A. Leach, Q. Bassat, E. Macete, C. Dobaño, M. Lievens, C. Loucq, W. R. Ballou, J. Cohen, and P. L. Alonso. "Insights into long-lasting protection induced by RTS,S/AS02A malaria vaccine: further results from a phase IIb trial in Mozambican children." PLoS ONE. 4.4 (2009): e5165.

Hutton, G., D. Schellenberg, F. Tediosi, E. Macete, E. Kahigwa, B. Sigauque, X. Mas, M. Trapero, M. Tanner, A. Trilla, P. Alonso, and C. Menendez. "Cost-effectiveness of malaria intermittent preventive treatment in infants (IPTi) in Mozambique and the United Republic of Tanzania." Bull World Health Organ. 87.2 (2009): 123–129.

Mabunda, S., J. J. Aponte, A. Tiago, and P. Alonso. "A country-wide malaria survey in Mozambique. II. Malaria attributable proportion of fever and establishment of malaria case definition in children across different epidemiological settings." Malar J. 8 (2009): 74.

Manco, L., P. Machado, D. Lopes, F. Nogueira, V. E. Do Rosario, P. L. Alonso, L. Varandas, M. D. J. Trovoada, A. Amorim, and A. P. Arez. "Analysis of TPI gene promoter variation in three sub-Saharan Africa population samples." Am J Hum Biol. 21.1 (2009): 118–120.

Mayor, A., E. Rovira-Vallbona, A. Srivastava, S. K. Sharma, S. S. Pati, L. Puyol, L. Quinto, Q. Bassat, S. Machevo, I. Mandomando, V. S. Chauhan, P. L. Alonso, and C. E. Chitnis. "Functional and immunological characterization of a Duffy binding-like alpha domain from Plasmodium falciparum erythrocyte membrane protein 1 that mediates rosetting." Infect Immun. 77.9 (2009): 3857–3863.

Mayor, A., E. Serra-Casas, A. Bardaji, S. Sanz, L. Puyol, P. Cistero, B. Sigauque, I. Mandomando, J. J. Aponte, P. L. Alonso, and C. Menendez. "Sub-microscopic infections and long-term recrudescence of Plasmodium falciparum in Mozambican pregnant women." Malar J. 8 (2009): 9.

Menendez, C., A. Bardaji, B. Sigauque, S. Sanz, J. J. Aponte, S. Mabunda, and P. L. Alonso. "Malaria prevention with IPTp during pregnancy reduces neonatal mortality." PLoS One. 5.2 (2010): e9438.

Montgomery, C. M., K. Munguambe, and R. Pool. "Group-based citizenship in the acceptance of indoor residual spraying (IRS) for malaria control in Mozambique." Soc Sci Med. 70.10 (2010): 1648–55.

Plowe, C. V., P. Alonso, and S. L. Hoffman. "The potential role of vaccines in the elimination of falciparum malaria and the eventual eradication of malaria." J Infect Dis. 200.11 (2009): 1646–1649.

Sacarlal, J., P. Aide, J. J. Aponte, M. Renom, A. Leach, I. Mandomando, M. Lievens, Q. Bassat, S. Lafuente, E. Macete, J. Vekemans, C. Guinovart, B. Sigauque, M. Sillman, J. Milman, M. C. Dubois, M. A. Demoitie, J. Thonnard, C. Menendez, W. R. Ballou, J. Cohen, and P. L. Alonso. "Long-Term Safety and Efficacy of the RTS,S/AS02A Malaria Vaccine in Mozambican Children." J Infect Dis. 200.3 (2009): 329–336.

Serra-Casas, E., C. Menendez, A. Bardaji, L. Quinto, C. Dobano, B. Sigauque, A. Jimenez, I. Mandomando, V. S. Chauhan, C. E. Chitnis, P. L. Alonso, and A. Mayor. "The Effect of Intermittent Preventive Treatment during Pregnancy on Malarial Antibodies Depends on HIV Status and Is Not Associated with Poor Delivery Outcomes." J Infect Dis. 201.1 (2010): 123–131.

Serra-Casas, E., C. Menendez, C. Dobano, A. Bardaji, L. Quinto, J. Ordi, B. Sigauque, P. Cistero, I. Mandomando, P. L. Alonso, and A. Mayor. "Persistence after delivery of Plasmodium falciparum parasites infecting Mozambican pregnant women." Infect Immun (2010): Ahead of print.

Sevene, E., R. Gonzalez, and C. Menendez. Current knowledge and challenges of antimalarial drugs for treatment and prevention in pregnancy. Vol. 11., 2010.

Sicuri, E., A. Bardaji, T. Nhampossa, M. Maixenchs, A. Nhacolo, D. Nhalungo, P. L. Alonso, and C. Menendez. "Cost-effectiveness of intermittent preventive treatment of malaria in pregnancy in southern mozambique." PLoS One. 5.10 (2010): e13407.

Sikora, M., A. Ferrer-Admetlla, H. Laayouni, C. Menendez, A. Mayor, A. Bardaji, B. Sigauque, I. Mandomando, P. L. Alonso, J. Bertranpetit, and F. Casals. "A variant in the gene FUT9 is associated with susceptibility to placental malaria infection." Hum Mol Genet. 18.16 (2009): 3136–3144.

Wells, T. N., P. L. Alonso, and W. E. Gutteridge. New medicines to improve control and contribute to the eradication of malaria. Vol. 8., 2009.

HIV/AIDS

In 2008, the HIV epidemic affected more than 35 million individuals in the world. Sub-Saharan Africa was the most affected region, with close to 70% of all cases registered worldwide, according to the WHO.

Mozambique is one of the countries most affected by HIV/AIDS in the world. According to data from the Ministry of Health of Mozambique, the estimated prevalence of infection by HIV in pregnant women in 2005 was 9% in the north of the country and 27% in the south. Data from the Vertical Transmission Prevention Program of 2007 in Manhiça showed that more than 25% of pregnant women observed in antenatal visits were seropositive for HIV, which reflects the severity of the situation in this area of the country.

MOTHER-TO-CHILD TRANSMISSION

Impact of maternal HIV on birth outcomes and infant survival

There have been few studies characterizing the impact of HIV infection during pregnancy on mothers and their infants and even fewer in rural African settings.

The CISM performed a study to assess the impact of HIV infection on birth outcomes and infant survival in a rural area. The study also evaluated the effect of unsupervised single-dose intrapartum and neonatal nevirapine administration for the prevention of mother-to-child transmission of HIV on HIV RNA viral load at delivery and the prevalence of neonatal nevirapine resistance mutations.

Pregnant women attending the antenatal clinic were recruited for the study. These women and their infants were followed for one year. Birth outcomes were assessed at delivery and infant HIV status was determined at 1 and 12 months of age.

The study showed that women positive for HIV were more likely to have anemia at delivery than those negative for HIV (51.3% versus 35.4%). Infants born to HIV-positive mothers had a significantly higher post-neonatal mortality rate than those born to HIV-negative mothers (7.8% versus 1.9%). The rate of transmission of HIV by breastfeeding during the first year of life was 15.1% (95% CI, 7.6-22.4).

Evaluation of immunological parameters and health indicators in the first year of life in HIV-negative infants born to HIV-positive mothers in Manhiça

The prevalence of HIV in pregnant women in certain regions of Africa is as high as 40%, with vertical transmission rates ranging between 10% and 50%. The consequence is a growing population of children exposed to HIV. There are, however, certain children who, even in the absence of intervention, do not become infected during the perinatal period, despite the fact that they are exposed to the virus for many months during breastfeeding.

Many studies have analyzed the specific immune responses in non-infected adults exposed to HIV. But it also essential to understand the role of this type of response in children in the African context, as well as to increase knowledge of the impact of this exposure on immunological and hematological parameters, response to vaccines administered during infancy, and the risk of morbidity in this population. To meet this need, the CISM is conducting a trial to assess immunological parameters and health indicators in the first year of life in infants of HIV-positive mothers in Manhiça. Data analysis is currently underway and results will be published in 2011.

CHARACTERIZATION OF HIV INFECTION AND RESPONSES TO ANTIRETROVIRAL THERAPY

Asymptomatic acute HIV infections

Acute HIV infection (AHI) is the initial phase of HIV infection in which the virus is actively replicating but seroconversion has not yet occurred. It has been suggested that AHI and the early months of HIV infection may contribute disproportionally to the transmission of HIV and constitute a major motor of the HIV pandemic.

The CISM conducted a study to determine the prevalence of AHI in HIV-seronegative adults presenting with reported fever at a district hospital in southern Mozambique and to evaluate clinical, immunological and virological parameters of AHI. A total of 346 adults presenting with reported fever at an outpatient ward at the Manhiça District Hospital were screened for AHI by HIV rapid serology testing, followed by HIV-RNA testing in HIV-seronegative individuals. Follow-up visits on day 7 and after 4 and 10

Technicians working at the immunology laboratory.

months were performed for clinical examination, HIV testing and assessment of HIV-RNA, CD4 cell counts and percentage of activated CD8+ T cells.

HIV testing revealed that 37.8% (95% CI, 32.7-43.2) of the adults had previously undiagnosed established HIV infection. Among the HIV-seronegative patients, 3.3% (95% CI, 1.3-6.7) were found to have AHI as demonstrated by positive HIV-1 RNA testing.

The study suggests that the high prevalence of AHI in southern African populations may warrant the investigation of tools and target populations for AHI screening as a novel way to address HIV prevention.

Predictors of immune reconstitution inflammatory syndrome associated with Kaposi sarcoma

The impact and relevance of immune reconstitution inflammatory syndrome associated with Kaposi sarcoma (IRIS-KS) has not been assessed in sub-Saharan African countries, where the bulk of HIV-1 and KS-associated herpesvirus (KSHV) coinfection occurs. Understanding the risk factors for developing IRIS-KS would help to identify high-risk patients and improve their clinical management.

The CISM conducted a study to investigate risk factors for developing IRIS-KS in the context of a larger prospective observational study. Sixty-nine consecutive HIV-1 and KSHV coinfected Mozambican adults initiating combined antiretroviral therapy (cART) were prospectively followed for the development of IRIS-KS over 10 months. A survival analysis was performed to assess potential risk factors for developing IRIS-KS.

During the first 10 months of cART, eight patients (8/69, 11.6%) experienced IRIS-KS at a median of 13.8 weeks after cART initiation. Multivariate analysis identified four independent IRIS-KS predictors: clinical pretreatment KS, detectable plasma KSHV DNA, hematocrit <30% and plasma HIV-1 RNA viral load. Treatment with cART either alone or combined with systemic chemotherapy led to partial or complete clinical response in 62.5% (5/8) of IRIS-KS patients.

This study identified four independent predictors of IRIS-KS which may help to develop screening tools that will aid the identification of patients at high risk of IRIS-KS for whom close clinical supervision is warranted.

Clinical and socio-demographic characteristics of HIV-infected children attending the outpatient clinic at the Manhiça District Hospital

Approximately 2.1 million children under 15 years of age were infected with HIV worldwide in 2008; 430,000 of these children (91% of whom were living in sub-Saharan Africa) were estimated to be newly infected. Most of these new infections are attributed to mother-to-child transmission in utero or during delivery or breastfeeding (WHO 2009). HIV infection has different impacts across different countries and sub-regions. Data from the latest epidemiological report of HIV and AIDS for Mozambique from the UNAIDS/WHO working group (2009) showed that an estimated 1.5 million people were living with HIV in 2007, including approximately 100,000 children under 15 years. Specific highly active antiretroviral treatment (HAART) coverage for children was not reported, but the estimated number of children receiving HAART was 6320.

Technician working at the molecular biology laboratory.

In 2003, the CISM, in collaboration with and following the recommendation of the Ministry of Health of Mozambique, established a voluntary counseling and testing center and a day hospital for the treatment and care of HIV-infected patients. The roll-out of ART at the hospital started in 2005. Since early 2009 the management of HIV-infected patients has been integrated into the management of other diseases.

Despite the high HIV burden, few data have been published on pediatric HIV infection in Mozambique. It is important to understand the characteristics of HIV infection in children, one of the most vulnerable groups, and the true development of national policies on site in order to better guide the implementation of HIV prevention and treatment programs. For this reason, in 2010 the CISM conducted a descriptive study at the HIV outpatient clinic. The aim was to describe the HIV-infected pediatric population in Manhiça in terms of clinical presentation with a focus on factors such as clinical and immunological severity at presentation and follow-up, nutritional status, growth profile and developmental skills. The data analyzed included the time interval between diagnosis and commencement of ART and the patients' socio-demographic characteristics. The study is under analysis and results are expected to be published during 2011.

Comparison of rotavirus infection among HIV-positive and HIV-negative children under five years of age with acute and severe diarrhea

Rotavirus is the primary cause of diarrhea in children throughout the world, and is associated with high mortality. In Mozambique, diarrhea is a serious problem in children under five years of age. Moreover, information about the association between diarrhea and rotavirus in Mozambique is limited.

Vaccination against rotavirus has been implemented in many countries around the world, but its effectiveness in African countries has been low. One of the factors influencing this low efficacy may be the presence of HIV infection in vaccinated children, as HIV produces suppression of the immune system. Some studies have shown that immunodeficient children infected by the rotavirus have a worse prognosis than their immunocompetent counterparts.

The CISM has begun a study to determine the impact of HIV on the clinical course of natural infection by rotavirus and the effect of extraintestinal distribution in the course of infection in children under five. At the same time, it also plans to genetically characterize the rotavirus strains detected.

Other studies

See the *Maternal and reproductive health* section for research projects on the safety of antiretrovirals in pregnancy.

See the *Social sciences* section for research projects on perceptions and acceptance of HIV prevention and treatment tools.

DEVELOPMENT OF PREVENTION TOOLS

Capacity-building for the clinical development of HIV/AIDS vaccines

As in the case of other infectious diseases, a safe, effective and accessible vaccine would be a key tool in controlling HIV/AIDS, especially in less industrialized countries. Accordingly, one of the aims of the CISM is to contribute to the development of new vaccines for HIV. The center joined the African-European HIV Vaccine Development Network (AfrEVacc) funded by the European and Developing Countries Clinical Trials Partnership (EDCTP). AfrEVacc aims to develop a joint network between European and African centers, using the data and knowledge of each center to improve the capacities to conduct HIV vaccine trials in Mozambique, South Africa and Tanzania.

This network works in parallel with another network (which includes Tanzania and Mozambique) that prepares centers to conduct HIV vaccine trials. It also collaborates with other international networks including the EUROPRISE Consortium, the Global HIV Vaccine Enterprise and the International AIDS Vaccine Initiative.

The activities in the context of the AfrEVacc Network in the CISM include the conduct of studies to characterize the incidence and prevalence of HIV in the area and evaluate the feasibility of vaccine trials (see the *Social Sciences* section). The first community-based cross-sectional study to determine age-specific HIV prevalence in adults was conducted in mid 2010.

The CISM laboratory capacities will be strengthened in the context of this network to introduce cellular immunology techniques that are necessary for the development of HIV vaccine trials.

The network aims to conduct a phase I trial to evaluate the safety and immunogenicity of an HIV vaccine candidate.

Development of microbicides

See the *Social sciences* section

Researchers

Pedro L. Alonso [1, 2]
Carlos Bavo [1]
Catarina David [1, 5]
Nilsa de Deus [1]
Kizito Gondo [1]
Raquel González [1, 2]
Nayra Gutiérrez [1]
Emili Letang [1]
José Machado Almeida [1]
Maria Maixenchs [1]
Clara Menéndez [1, 2]
Sibone Mocumbi [4]
Cinta Moraleda [1]
Khátia Munguambe [1, 3]
Denise Naniche [2]
Robert Pool [2]
Cèlia Serna [2]

[1] Manhiça Health Research Centre (CISM)
[2] Barcelona Centre for International Health Research (CRESIB), Hospital Clínic – Universitat de Barcelona, Spain
[3] Foundation for Community Development (FDC), Mozambique
[4] National Health Institute (INS), Mozambique
[5] Ministry of Health, Mozambique
[6] Universidade Eduardo Mondlane, Mozambique

PUBLICATIONS

Almeida, J., E. Letang, T. Nhampossa, E. Ayala, C. David, C. Menendez, J. Gascon, P. Alonso, and D. Naniche. "Rapid suppression of HIV-RNA is associated with improved control of immune activation in a Mozambican adults initiating antiretroviral therapy with low CD4 counts." AIDS Res Hum Retroviruses (2010): Ahead of print.

Letang, E., J. M. Almeida, J. M. Miro, E. Ayala, I. E. White, C. Carrilho, R. Bastos, T. Nhampossa, C. Menendez, T. B. Campbell, P. L. Alonso, and D. Naniche. "Predictors of immune reconstitution inflammatory syndrome-associated with kaposi sarcoma in mozambique: a prospective study." J Acquir Immune Defic Syndr. 53.5 (2010): 589–597.

Naniche, D., A. Bardaji, M. Lahuerta, A. Berenguera, I. Mandomando, S. Sanz, J. J. Aponte, B. Sigauque, P. L. Alonso, and C. Menendez. "Impact of maternal human immunodeficiency virus infection on birth outcomes and infant survival in rural mozambique." Am J Trop Med Hyg. 80.5 (2009): 870–876.

Serna-Bolea, C., J. Munoz, J. M. Almeida, A. Nhacolo, E. Letang, T. Nhampossa, E. Ferreira, P. Alonso, and D. Naniche. "High prevalence of symptomatic acute HIV infection in an outpatient ward in southern Mozambique: identification and follow-up." Aids. 24.4 (2010):

TUBERCULOSIS

Tuberculosis is one of the principal causes of death worldwide, and Mozambique occupies the 18th position in the ranking of high-burden tuberculosis countries. WHO data show that there are almost 8 million new cases of tuberculosis each year and 1.7 million related deaths. The estimated annual incidence of tuberculosis in Mozambique in 2008 was 502 per 100,000 habitants, and the annual mortality was 117 cases per 100,000 habitants. There is a critical association between tuberculosis and HIV in Africa. The annual incidence of tuberculosis in adults infected with HIV/AIDS, for instance, is as high as 50%.

Research activities in tuberculosis conducted by the CISM during 2009/10 focused on increasing clinical and laboratory capacities to conduct phase IIb clinical trials of a candidate tuberculosis vaccine in children, and to estimate the incidence of tuberculosis in children under three years of age.

INCIDENCE OF TUBERCULOSIS IN BREASTFEEDING INFANTS AND CHILDREN

Tuberculosis in children contributes to 15-20% of the overall disease burden. The high morbidity and mortality are due to underdiagnosis, misdiagnosis, late diagnosis and the lack of pediatric drug formulations. It is thus essential to improve pediatric treatment and diagnostic methods. The Mozambican Ministry of Health has observed that breastfeeding infants and older children are neglected in the diagnosis, treatment and research of tuberculosis and has called for new interventions in this vulnerable group.

The diagnosis of childhood tuberculosis is currently performed using clinical methods. Research suggests that the diagnostic method of choice for childhood tuberculosis in countries with high HIV prevalence is gastric aspirate or sputum induction. With adequate mycobacteriologic laboratory facilities and quality control that permit the isolation of mycobacteria and sensitivity tests, these methods are the only ones that can provide a definitive diagnosis.

The CISM recently became involved in the international TB Vaccine Sites Network (TBVACSIN), which aims to reinforce capacities for conducting trials of a new tuberculosis vaccine in four countries (Uganda, Kenya, South Africa and Mozambique). One of the requirements is the collection of incidence data to estimate the sample size for the efficacy trial.

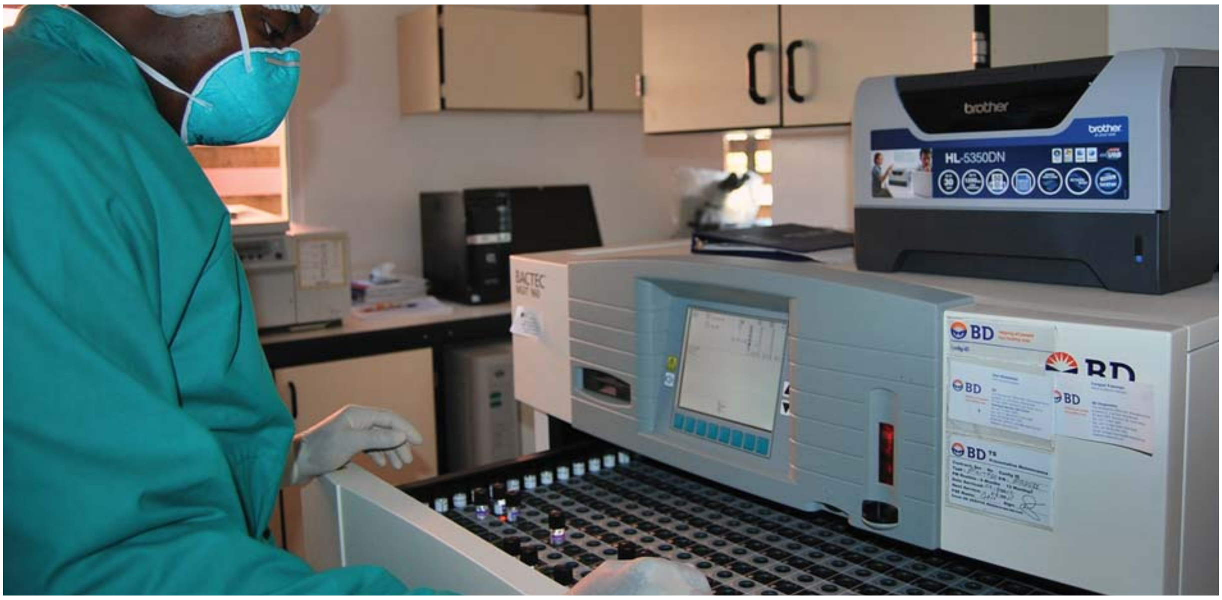
The CISM has initiated a study to assess the incidence of tuberculosis, its clinical characteristics and its consequences in children under three years of age. It also aims to compare the bacteriological performance of fluorescent microscopy with that of cultures of samples from gastric aspirates and sputum inductions, and to evaluate the rate of HIV co-infection in suspected and confirmed cases of tuberculosis. The study began in June 2010, and 200 children were recruited during the final months of the year.

EFFECTS OF ZINC SULPHATE AND VITAMIN D SUPPLEMENTS ON THE SENSITIVITY OF THE TUBERCULIN SKIN TEST (TST) IN INFANTS AND CHILDREN

The tuberculin skin test (TST) is a low-cost, simple, well-accepted technique for evaluating tuberculosis infection in children and adults. The TST has an important role in many diagnostic algorithms for childhood tuberculosis; however, it also has a number of well-known limitations. Despite global efforts to identify faster, more sensitive and more specific techniques for diagnosing tuberculosis infections, it is unlikely that any tool will replace the TST in areas with few resources in the near future. Younger children living with HIV and malnourished children run a greater risk of being underdiagnosed as immunosuppression can hinder diagnosis by the TST. Consequently, research on ways to improve the reliability of this test tool continues to be an urgent priority.

The CISM started a clinical trial to test the value of zinc and vitamin D supplements to reinforce the immune response of children identified as having a potentially weak response to skin tests. This double-blinded trial will test the efficacy of two different micronutrient interventions in increasing the sensitivity of the TST within a 72-hour period.

Tuberculosis will be identified through research in children identified in the family unit and through symptoms in screening carried out in health center and hospitalized patients. The primary objective of the study is to evaluate the concurrence between the five methods used to detect tuberculosis infection in children and infants. In this trial, 670 children aged 9 to 59 months with suspected tuberculosis will be randomly selected to receive 3% zinc sulphate or a vitamin D cream supplement (calcipotriene 0.005%) in one arm, and a placebo cream in the other arm (in the same child) to evaluate their response to the TST.



Technician working at the tuberculosis laboratory.

The results will be compared with those from Interferon Gamma Release Assays . The identification of simple ways of improving early detection of tuberculosis infections in populations with immunodeficiencies will serve as a timely call for the need for prophylaxis and treatment of the active disease, reducing morbidity and mortality in this vulnerable population.

The trial began in October of 2010 and around 100 children have been recruited and randomized to date.

MULTICENTER AERAS 402 VACCINE PHASE II TRIAL IN AFRICAN CHILDREN

The only commercially available vaccine against tuberculosis is the bacillus Calmette-Guérin (BCG) vaccine. In spite of the heterogeneous results of analyses, the BCG seems to provide partial protection against severe forms of tuberculosis during childhood; however, it appears to offer little protection against common forms of the disease such as pulmonary tuberculosis. The rapid increase in tuberculosis in African countries with a high prevalence of HIV/AIDS is a cause for concern among health institutions, despite the expansion and promotion of the use of the BCG. The development of a new vaccine against tuberculosis is therefore essential and urgent.

In the last decade, various candidate tuberculosis vaccines have been developed; some have advanced to phase I, and more recently, to phase IIa trials. The CISM has been involved, since 2007, in the international TB Vaccine Sites Network (TBVACSIN), which seeks to develop capacities for performing clinical trials for tuberculosis vaccines. The network currently includes the involvement of four African centers, namely, the University of Makerere in Uganda; KEMRI/CDC in Kisumu, Kenya; SATVI in Cape Town, South Africa; and the CISM in Manhiça, Mozambique. Since 2009, the CISM has been reinforcing clinical and laboratory capacities with support from the European and Developing Countries Clinical Trials Partnership and the Aeras Foundation.

In this context, the CISM will conduct a phase II, double-blind, randomized, placebo-controlled, multicenter, proof-of-concept trial to evaluate the safety and effectiveness of AERAS-402 in children vaccinated with BCG who are not infected with HIV.

This trial includes a phase to determine the initial dose, and a second phase on safety and efficacy, in 4096 healthy children, vaccinated with BCG and not infected with HIV. The trial is scheduled to take place in the four above-mentioned centers, and it may also be extended to other locations. Participants will be randomized to receive the AERAS 402 vaccine or placebo at between 16 and 26 weeks of age.

The primary objectives are to evaluate the safety and efficacy profiles of AERAS-402 in children, based on the evaluation criteria for tuberculosis cases, as described in the protocol.

To ensure the success of the trial, a procedures room has been built for the collection of samples, along with a safety level III laboratory (BSL3) with automatic processing of biological samples using MGIT and GeneXpert. Training sessions have also been organized for the administration of the tuberculin skin test, collection of gastric aspirate and sputum induction samples, processing of the Quantiferon test and administration of the BCG.

Lastly, in order to be able to compare the efficacy of the AERAS-402 tuberculosis vaccine for children in the four countries, the origin of the BCG vaccine must be harmonized (the centers in Uganda, Kenya and South Africa use a Copenhagen BCG strain) and the monitoring of the cold-chain system in Manhiça must also be improved. The principal objective is to introduce the 1331 Copenhagen BCG strain from a Danish factory in the district of Manhiça during the trial period to replace the BCG made in India. Staff working on the Extended Program on Immunization (EPI) will be trained in the intradermal BCG administration technique, which will be harmonized, and training will be given throughout the district of Manhiça on cold-chain management. The distribution of the new vaccine started in November of 2010 within the EPI.

EVALUATION OF A FIXED COMBINATION OF FOUR DRUGS FOR THE TREATMENT OF TUBERCULOSIS

The use of fixed-dose combination (FDC) drugs for the treatment of tuberculosis is recommended by the International Union Against Tuberculosis and Respiratory Diseases (The Union) and by the WHO. The advantages of FDC drugs include the prevention of drug resistance due to monotherapy, the reduction of the risk of incorrect dosing, simplification of the prescription process, the improvement of treatment adherence and the facilitation of direct observation of treatment. Recent studies of the bioavailability of formulas with four FDC drugs demonstrated satisfactory results, although there is still little information on the effectiveness of this strategy compared to the use of separate tablets.

The CISM participated in a multicenter study promoted and funded by The Union to evaluate a fixed combination of four drugs for the treatment of tuberculosis. The study tested the effectiveness of this compound when administered in the intensive initial phase of the treatment of new cases of pulmonary tuberculosis with positive bacilloscopy. The treatment phase was continued for four months with two FDC drugs–rifampicin and isoniazid. The conclusions of the study will be published in 2011.

OTHER STUDIES

See the *Social sciences* section for research projects on perceptions and attitudes towards tuberculosis.

Researchers

Pedro L. Alonso [1, 2]	José Muñoz [1, 2]
Kizito Gondo [1]	Jahit Sacarlal [1, 4]
Khátia Munguambe [1, 3]	

1 Manhiça Health Research Centre (CISM)

2 Barcelona Centre for International Health Research (CRESIB), Hospital Clínic – Universitat de Barcelona, Spain

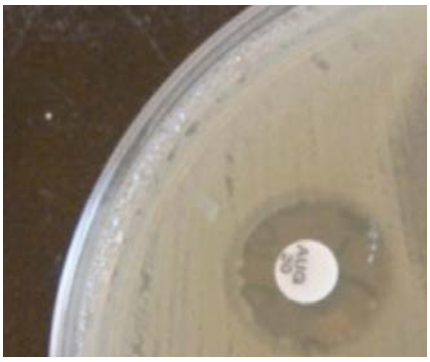
3 Foundation for Community Development (FDC), Mozambique

4 Universidade Eduardo Mondlane, Mozambique

DIARRHEAL DISEASES

According to the WHO/UNICEF, diarrhea is second only to pneumonia as the most common cause of death in children under five years worldwide. Approximately one in five child deaths (approximately 1.5 million each year) are due to diarrhea, a disease that kills more young children than AIDS, malaria and measles combined (WHO/UNICEF 2009).

Annually, an estimated 2500 million cases of diarrhea occur in children under five years of age, and estimates suggest that the overall incidence has remained relatively stable over the past two decades. Diarrhea is particularly common in developing countries, particularly in sub-Saharan Africa, largely because of a lack of safe drinking water, sanitation and hygiene. An estimated 2.5 billion people lack improved sanitation facilities, and nearly one billion people do not have access to safe drinking water.



Plaque with antibiogram.

Diarrhea is a typical symptom of gastrointestinal infection caused by a range of pathogens (bacteria, viruses and protozoa), some of which are particularly common causes of childhood diarrhea. Rotavirus, for example, is estimated to cause about 40 per cent of all hospital admissions due to diarrhea in children under five years of age worldwide, leading to some 100 million episodes of acute diarrhea each year and resulting in 350,000 to 600,000 child deaths. Global rotavirus vaccine introduction was recently recommended by the WHO.

Bacterial pathogens such as *Escherichia coli, Shigella, Campylobacter and Salmonella*, along with Vibrio cholerae during epidemics are major causes of diarrhea. The treatment of bacterial infections, however, is a cause for major concern due to the widespread emergence of multidrug resistance strains, a phenomenon that has serious implications for developing countries where newer and expensive antibiotics are often unavailable.

The diarrheal research area at the CISM is focused on the etiology and burden of diarrhea in children under five years of age and the study of antimicrobial resistance and associated mechanisms.

DIARRHEAL DISEASE IN INFANTS AND YOUNG CHILDREN - GLOBAL ENTERIC MULTI-CENTER STUDY

Since 2007, the CISM has been part of the Global Enteric Multi-Center Study (GEMS), described in the 2007-08 activity report. In the period 2009-10, the activities in this area were focused on enrolling patients with diarrhea that sought assistance in sentinel health care facilities and met the inclusion criteria of the GEMS. The children were divided into three age groups: 0-11 months, 12-23 months, and 24-59 months. Community-based controls matched by age, gender, and neighborhood were enrolled for each case. A Health Care Services Utilization and Attitudes Survey was also conducted in households with children under five in the catchment area. The enrolment for the case-control study was completed in December 2010.

The molecular characterization of isolates is currently underway as are data cleaning and analysis of clinical epidemiology and manuscript preparation. Preliminary data so far have shown elevated numbers of rotavirus (particularly in children under two), *Cryptosporidium*, *Shigella*, *diarrheagenic Escherichia coli* strains and *Entamoeba histolytica* in cases. *Giardia lamblia*, in contrast, was found to be more prevalent in controls.

ANTIMICROBIAL SUSCEPTIBILITY AND RESISTANCE MECHANISMS IN *SHIGELLA* AND *SALMONELLA* ISOLATES

Salmonella spp. and *Shigella* spp. are among the bacteria most frequently isolated in stool samples from diarrhea patients, especially in rural areas in developing countries. *Salmonella* spp. usually produce self-limited illness, whereas *Shigella* infections are likely to be more severe.

Currently, data on antimicrobial resistance in diarrheagenic bacteria in Mozambique are scarce. Few studies have inves-

Technician working at the microbiology laboratory.

tigated molecular mechanisms of antimicrobial resistance among isolates from sub-Saharan Africa, mainly due to the limited number of laboratories and research facilities with adequate infrastructure.

The CISM conducted a study to describe antimicrobial susceptibility and the molecular mechanisms of resistance in *Salmonella* and *Shigella* isolates from children with diarrhea presenting at the Manhiça District Hospital. Specifically, 109 *Shigella* and 40 *Salmonella* isolates were examined. Susceptibility to seven antimicrobial agents and mechanisms of resistance were analyzed.

The data showed that *Shigella* isolates are resistant mostly to the most available, inexpensive antibiotics by various molecular mechanisms but remain susceptible to ciprofloxacin, ceftriaxone and nalidixic acid, which is the first line of empirical treatment for shigellosis in the country. Only 3% of *Salmonella* isolates were resistant to nalidixic acid, and none were resistant to ciprofloxacin or ceftriaxone.

ANTIMICROBIAL RESISTANCE TRENDS IN BACTEREMIA ISOLATES

There is growing concern regarding the management of community-acquired infections in Africa because of the increasing prevalence of resistance to the most commonly used antibiotics in these settings and the emergence of multidrug-resistant strains. However, data for antimicrobial resistance, especially regarding trends, remain scarce throughout sub-Saharan African settings.

The CISM monitored antibiotic resistance over a five-year period (2001-2006) in bacteria isolated from the blood of children under 15 years of age admitted to the Manhiça District Hospital. Antimicrobial susceptibility patterns of bloodstream isolates and their time trends for the five most frequent causes of bacteremia in this setting were analyzed. The analysis showed a linear trend of increasing resistance throughout the study period to chloramphenicol among isolates of non-typhoidal *Salmonella*, *Escherichia coli*, *Staphylococcus aureus* and *Haemophilus influenzae*. Increasing resistance to ampicillin was also observed for *H. influenzae* isolates.

This study suggests that quinolones and third-generation cephalosporins may be needed in the short term to manage community-acquired infections.

INVASIVE NON-TYPHOIDAL SALMONELLA

In tropical Africa, non-typhoidal *Salmonella* (NTS) bacteremia is common, particularly in immunocompromised hosts. In areas where malaria and malnutrition are highly prevalent, NTS appears to be a particularly common cause of hospital admission among young children. In these areas, it is also common in HIV-infected patients and the clinical disease is associated with high mortality rates. Furthermore, the number of NTS strains resistant to the most commonly available antibiotics in these poor regions is rising.

The CISM conducted a study to describe the epidemiology and clinical presentation of NTS. The epidemiology, clinical presentation and serotype distribution of invasive NTS among children admitted to the Manhiça District Hospital between May 2001 and April 2006 were analyzed. A total of 401 NTS cases were analyzed. Fever, cough and increased respiratory rate were the most common symptoms reported, while diarrhea was present in only 29%. Young age, severe malnutrition, diarrhea and pneumonia were independent risk factors of death. *Salmonella typhimurium* (66%), and *Salmonella enteritidis* (25%) were the most frequent serotypes, with incidence rates of 240.4 and 108.6 per 100,000 child-years, respectively. Resistance to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole was high for both *S. typhimurium* and *S. enteritidis*.

The study showed that the clinical presentation of invasive NTS was non-specific and similar to that of other infections, with some factors being associated with NTS. Furthermore, antibiotic resistance was very common to currently recommended and available antibiotics for suspected sepsis.

Researchers

Sozinho Acácio [1]
Pedro L. Alonso [1, 2]
Dinís Jaintilal [1]
Tacilta Nhampossa [1, 3]
Inácio Mandomando [1, 3]
Joaquín Ruiz [2]

1 Manhiça Health Research Centre (CISM)
2 Barcelona Centre for International Health Research (CRESIB), Hospital Clínic – Universitat de Barcelona, Spain
3 Foundation for Community Development (FDC), Mozambique
4 Universidade Eduardo Mondlane, Mozambique

PUBLICATIONS

Mandomando, I., D. Jaintilal, M. J. Pons, X. Valles, M. Espasa, L. Mensa, B. Sigauque, S. Sanz, J. Sacarlal, E. Macete, F. Abacassamo, P. L. Alonso, and J. Ruiz. "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*. 53.6 (2009): 2450–2454.

Mandomando, I., E. Macete, B. Sigauque, L. Morais, L. Quinto, J. Sacarlal, M. Espasa, X. Valles, Q. Bassat, P. Aide, T. Nhampossa, S. Machevo, J. Ruiz, A. Nhacolo, C. Menendez, K. L. Kotloff, A. Roca, M. M. Levine, and P. L. Alonso. "Invasive non-typhoidal Salmonella in Mozambican children." *Trop Med Int Health.* 14.12 (2009): 1467–1474.

Mandomando, I., B. Sigauque, L. Morais, M. Espasa, X. Valles, J. Sacarlal, E. Macete, P. Aide, L. Quinto, T. Nhampossa, S. Machevo, Q. Bassat, C. Menendez, J. Ruiz, A. Roca, and P. L. Alonso. "Antimicrobial Drug Resistance Trends of Bacteremia Isolates in a Rural Hospital in Southern Mozambique." *Am J Trop Med Hyg*. 83.1 (2010): 152–157.

PNEUMONIAS AND OTHER INVASIVE BACTERIAL DISEASES

Acute respiratory infections and invasive bacterial diseases are responsible for a large number of deaths in the pediatric population. The latest report from UNICEF/WHO estimates that pneumonia is responsible for 19% of deaths worldwide in children under five years of age. A considerable proportion of pneumonia cases and deaths are caused by bacterial agents, among which *Streptococcus pneumoniae* and *Haemophilus influenzae* type b are the most prevalent. Other causes include viruses, mainly the respiratory syncytial virus.

The activities of the CISM in this area are focused on three aspects: clinical and molecular epidemiology (including studies on antibiotic resistance), improvement of diagnosis, and monitoring and evaluation of control strategies.

CLINICAL AND MOLECULAR EPIDEMIOLOGY

Acute bacterial meningitis among children

Acute bacterial meningitis (ABM) remains an important cause of mortality among African children. Epidemiologic data on ABM infection are necessary for prioritizing public health interventions.

The CISM strengthened hospital-based surveillance of ABM among children admitted to the Manhiça District Hospital starting in January 2006 in order to characterize this disease. Cerebrospinal fluid (CSF) samples were collected from children admitted to the hospital who met clinical criteria of ABM. Laboratory determinations to identify the pathogen were performed and clinical information and outcome of cases were recorded.

During the first 12 months of surveillance, CSF samples were collected from 642 children under 15 years of age with suspected meningitis (18% of all pediatric patients admitted to the hospital during that time). ABM was confirmed in 43 (7%) of the 642 cases. *Haemophilus influenzae* type b (Hib) (14 cases), pneumococcus (nine cases), and meningococcus (7 cases) accounted for approximately 70% of all confirmed cases. Four of the nine pneumococci were serotypes covered by the 7-valent pneumococcal conjugate vaccine. The case fatality rate among patients with ABM was 24% (8 of 33 with a known outcome); an additional eight patients left the hospital before discharge.

The incidence of ABM was 85 per 100,000 child-years, which peaked at 2-12 months of age at 1078 cases per 100,000 child-years. All nine pneumococci isolates were susceptible to chloramphenicol, and eight were susceptible to penicillin (the ninth had intermediate resistance). For the 10 Hib isolates tested, only one was susceptible to chloramphenicol, and five were susceptible to ampicillin.

These data highlight the importance of pneumococcal and Hib conjugate vaccines in the prevention of ABM (the Hib vaccine was not introduced in Mozambique until 2009).

Community-acquired bacteremia among children

Although community-acquired bacteremia is an important cause of childhood mortality in Africa, recognition of disease burden and potential impact of bacterial vaccines is limited.

The CISM conducted a study analyzing blood culture and clinical data from children under 15 years of age admitted to Manhiça District Hospital from 2001 to 2006. Bacteremia was identified in 8% (1550/19,896) of all pediatric hospital admissions. Non-typhoidal *Salmonella* (NTS) and *Pneumococcus* were the most prevalent pathogens isolated (26% and 25% of 1550 cases, respectively). Until 28 days of life, *Staphylococcus aureus* (39%) and group B *Streptococcus* (20%) predominated. Incidence of community-acquired bacteremia per 100,000 child-years was 1730/10 in children under 1 year old, 782/10 in 1-4 year olds, and 49/10 in children aged five and older. Case-fatality of bacteremia was 12%. Community-acquired bacteremia–associated mortality accounted for 21% (162/788) of hospital deaths. Resistance to antibiotics commonly used in Mozambique was high among invasive isolates of *Haemophilus influenzae*, *Escherichia* coli, and NTS.

This study shows that community-acquired bacteremia is an important cause of pediatric hospital admission and death in rural African hospitals. Moreover, the high burden of disease, mortality, and pattern of antibiotic resistance associated with bacteremia underscore the need for prevention in sub-Saharan Africa.

Severe pneumonia in children

The CISM conducted a study to describe the clinical presentation of severe pneumonia among hospitalized children in a malaria-endemic area with a high prevalence of HIV infection. As part of a two-year prospective study of radiologically confirmed pneumonia, chest radiographs, malaria parasite counts and bacterial blood cultures were systematically performed for children aged 0 to 23 months admitted with severe pneumonia. Radiographs were interpreted according to WHO guidelines.

Severe pneumonia accounted for 16% of 4838 hospital admissions among children aged 0-23 months; 43% of episodes had endpoint consolidation, 15% were associated with bacteremia and 11% were fatal. Fever, cough >3 days, crepitations, hypoxemia and absence of malaria parasitemia were associated with radiologically confirmed pneumonia. Nineteen per cent of children with severe pneumonia and 27% with radiologically confirmed pneumonia had clinical malaria. HIV-prevalence was 26% among children hospitalized with severe pneumonia and HIV-testing results. HIV infection, anemia, malnutrition, hypoxemia and bacteremia were associated with fatal episodes of severe pneumonia.

This study highlights the complexity of the clinical management of hospitalized children with severe pneumonia in settings with prevalent HIV and malaria. In addition to vertical programs, integrated approaches may greatly contribute to the reduction of pneumonia-related mortality.

Viral pneumonia

The role of viruses in pediatric pneumonia remains poorly studied in sub-Saharan Africa, where pneumonia-associated mortality is high. During a one-year hospital-based surveillance period, nasopharyngeal aspirate samples were collected from children aged under five years admitted to hospital in rural Mozambique with clinically severe pneumonia. The identification of 12 respiratory viruses was performed by polymerase chain reaction. Study children were also tested for invasive bacterial infection, *P. falciparum* parasitemia, and HIV.

At least one respiratory virus was detected in almost half (394/807) of the children hospitalized with clinically severe pneumonia. A total of 475 viruses were detected among these 394 children, the most prevalent of which were rhinovirus (41%), adenovirus (21%), and respiratory syncytial virus (11%). Eleven percent of virus-infected children had concomitant invasive bacterial infection, 15% had malaria parasites, and 25% had HIV coinfection. Viral infection was 5.5 to 16 times more prevalent among HIV-infected children and incidence rate ratios varied according to the virus. In-hospital mortality of virus patients was 9% and was highest in patients with IBI or HIV coinfection.

This study highlights the high prevalence of respiratory viruses among hospitalized pneumonia patients in Mozambique. HIV infection is also an important contributor to the high burden of disease and associated mortality of viral pneumonia.

Collection of nasopharyngeal aspirates.

Estimating vaccine-preventable burden of hospitalized pneumonia

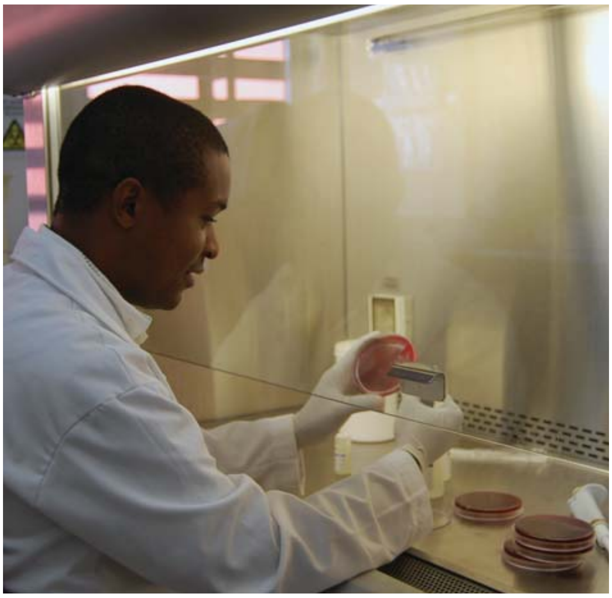
Polysaccharide-protein conjugate vaccines against *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae* have proven efficacy against radiologically confirmed pneumonia. The measurement, thus, of pneumonia incidence using radiologically confirmed cases provides a platform for estimating the vaccine-preventable burden of these diseases.

Over 24 months, the CISM conducted surveillance for radiologically confirmed severe pneumonia episodes among children under 2 years of age admitted to the Manhiça District Hospital. During this period, severe pneumonia accounted for 15% of 5132 hospital admissions and 32% of in-hospital mortality among children over 2 years of age. Almost half (43%) of the chest radiographs examined were interpreted as radiologically confirmed pneumonia. HIV infection was associated with 81% of fatal pneumonia episodes among children tested for HIV. The minimum incidence rate of radiologically confirmed pneumonia requiring hospitalization was 19 episodes/1000 child-years. Incidence rates were 9.3-19.0-fold higher in HIV-infected children than in HIV-uninfected children.

This study highlights the fact that vaccines such as Hib and pneumococcal conjugate vaccines would have a substantial impact on pneumonia hospitalizations among African children if the effects were similar to those observed in clinical trials.

Infection biology and epidemiology of staphylococci and staphylococcal diseases in sub-Saharan Africa

Staphylococcus aureus has for long been considered an opportunistic pathogen. However, due to the emergence of



Research fellow working at the microbiology laboratory.

community-acquired methicillin-resistant *S. aureus* (CA-MRSA) infections in normal hosts, *S. aureus* is a major pathogen capable of causing serious infections with considerable associated mortality. Due to the widespread use of antimicrobials, isolates with resistance against virtually all available antibiotics have been encountered in hospitals. This is a major healthcare burden since this resistance increases treatment costs and patient morbidity, and may even contribute to excess mortality.

S. aureus disease has been intensively investigated in developed countries. Corresponding data from developing countries, however, and particularly from sub-Saharan Africa, are seriously lacking due to the shortage of microbiological diagnostic facilities. Consequently, patients with suspect systemic infection are normally not diagnosed or, at best, treated empirically. This applies to patients with different bacterial, parasitic or helminthic diseases (also summarized as neglected tropical diseases).

Recently, it became evident that community-acquired infections as well as infections transmitted in hospitals caused by gram-positive cocci and gram-negative rods have a significant and clearly underestimated significance in sub-Saharan Africa. However, single reports concerning the incidence and epidemiology of *S. aureus* in Africa report a high rate of pathogen isolation. A few studies have also described the pathogenic importance of *S. aureus*. These reports support the theory that *S. aureus* is one of the most prevalent and important pathogens in systemic infection disease in sub-Saharan Africa, particular in neonates and young children with associated mortality. In addition, the coinfection problems associated with systemic *S. aureus* disease in conjunction with symptomatic HIV disease appear to be particularly serious in sub-Saharan Africa, especially in high HIV prevalence settings. Nonetheless, consistent data in these settings are not available.

To fill this gap, a German-African consortium funded by Deutsche Forschungsgemeinschaft (DFG, Germany) and coordinated by the University of Saarland in Hamburg (Germany) was created to quantify the burden and epidemiology (including the molecular epidemiology) of staphylococcal disease in Germany, Mozambique and other sub-Saharan African countries.

Data on *S. aureus* infections and associated risk factors are limited in Mozambique and the clinical relevance and associated mortality are unknown. Recently, however, *S. aureus* was identified as the third most common cause of bacteremia among children under 15 years of age admitted to the Manhiça District Hospital, and the leading cause among neonates. Overall, the minimum incidence was 178 cases per 100,000 child-years at risk and the associated hospital mortality was 6%. The isolates were highly resistant to the most commonly used antibiotics including 9% of MRSA.

Research at the CISM in this area is focused on determining the burden and epidemiology of S. aureus infections in the Manhiça District and on studying the molecular phylogeny (genetic evolution) and virulence factors of this pathogen.

In this context, in September 2010 the center launched a study called Infection Biology and Epidemiology of Staphylococci and Staphylococcal Diseases in Sub-Saharan Africa to quantify the burden of *S. aureus* disease and explore associated risk factors and clinical significance. Molecular characterization of isolates (spa typing, pulsed field gel electrophoresis, multilocus sequence typing, virulence factors), together with antimicrobial resistance and related mechanisms will be analyzed in detail.

IMPROVEMENT OF DIAGNOSIS

Procalcitonin and C-Reactive Protein for Invasive Bacterial Pneumonia Diagnosis

Procalcitonin (PCT) and C-reactive protein (CRP) are used in developed countries to differentiate between viral and bacterial causes of pneumonia. The validity of these markers needs to be further explored in Africa.

The CISM conducted a study to evaluate the use of PCT and CRP to differentiate viral from invasive bacterial pneumonia in children under 5 years hospitalized with clinically severe pneumonia in a malaria-endemic area with high HIV prevalence. The prognostic capacity of these markers was also evaluated.

Out of 835 children with clinically severe pneumonia, 87 met the definition of viral pneumonia while 89 met that of invasive bacterial pneumonia. In the absence of malaria parasites, levels of PCT and CRP were lower in the viral group than in the invasive bacterial group. However, the distribution of the markers overlapped between the clinical groups in the presence of malaria parasites. Neither of the markers could predict mortality.

This study suggests that, unlike the case in developed countries, the presence of malaria parasites should be taken into consideration for both clinical and epidemiological purposes when PCT or CRP are used to differentiate between viral and invasive bacterial pneumonia in malaria-endemic areas.

Improving the diagnosis of bacterial, viral and malarial infections

Health workers in rural facilities in Africa are confronted every day with the diagnosis and treatment of sick children with infectious diseases and lack the tools required to guide clinical management procedures including the prescription of antimalarial drugs and/or antibiotics. Misdiagnosis of malaria, bacterial infections and viral infections in children can increase morbidity and mortality, as well as increase resistance levels to antimalarial drugs and antibiotics.

As a follow-up to previous studies undertaken by the CISM on biomarkers for the diagnosis of the most frequent infections, in 2010, the center embarked on an ambitious, multicenter, collaborative project designed to discover protein biomarkers that can be used to develop rapid, inexpensive diagnostic tests to detect malaria, viral pneumonia and invasive bacterial disease. This project, funded by the Bill and Melinda Gates Foundation, will recruit patients from the Manhiça District Hospital and President Obama's pediatric hospital of Kisumu, Kenya.

Coordination tasks have been assumed by the Broad Institute and the Harvard School of Public Health (Boston, United States). The project will use genomics, proteomics and metabolomics platforms at the Broad Institute to identify candidate genes that show markedly altered levels of gene expression and assess protein level changes for the protein products of those candidate genes. Recruitment of patients and healthy community controls started in September 2010 and will continue into the last quarter of 2012. The first results are expected to become public in 2013.

MONITORING AND EVALUATION OF CONTROL STRATEGIES

Evaluation of the effectiveness of the Hib vaccine

Many resource-poor countries have not yet introduced Haemophilus influenzae b (Hib) vaccination into their national Expanded Program of Immunization (EPI). Although vaccination has been demonstrated to be highly effective in developed countries, the main constraints for its introduction in resource-poor settings, especially in Africa, are the high costs and the lack of surveillance data to prove the high burden of Hib disease.

In Africa, data on the impact of introducing Hib vaccination have been generated in Gambia and in Kenya by measuring a decrease of over 90% in the incidence rates of invasive Hib disease (before and after vaccine introduction). Since only a small proportion of Hib cases yield positive bacterial isolation in blood culture specimens, the WHO standardized a method to determine chest X-ray–confirmed pneumonia (XRP) as a more sensitive endpoint.

Demonstrating the effectiveness of the Hib conjugate vaccine against XRP could show on a larger scale the public health impact of vaccine introduction in the context of routine childhood immunization.

The CISM is conducting a study to monitor the effectiveness of immunization with the Hib vaccine within the EPI. This evaluation will use the Morbidity Surveillance System at the CISM to compare the incidence of Hib invasive disease before and after vaccine introduction and concomitantly conduct a case-control study to assess risk factors for Hib invasive disease.

OTHER STUDIES

See the *Social sciences* section for research projects on perceptions and attitudes towards acute respiratory infections.

Researchers

Pedro L. Alonso [1, 2]
Quique Bassat [2]
Ana Belén Ibarz [1, 3]
Núria Díez [2]
Miguel Lanaspa [1, 2]
Sónia Machevo [1, 5]
Inácio Mandomando [1, 4]
Luís Morais [1]
Cristina O'Callaghan [2]
Anna Roca [2]
Betuel Sigaúque [1, 4]

1 Manhiça Health Research Centre (CISM)
2 Barcelona Centre for International Health Research (CRESIB), Hospital Clínic – Universitat de Barcelona, Spain
3 CIBERESP, Spain
4 National Health Institute (INS), Mozambique
5 Universidade Eduardo Mondlane, Mozambique

PUBLICATIONS

Diez-Padrisa, N., Q. Bassat, S. Machevo, L. Quinto, L. Morais, T. Nhampossa, C. O'Callaghan-Gordo, A. Torres, P. L. Alonso, and A. Roca. "Procalcitonin and C-Reactive Protein for Invasive Bacterial Pneumonia Diagnosis among Children in Mozambique, a Malaria-Endemic Area." *PLoS One*. 5.10 (2010): e13226.

Nair, H., D. J. Nokes, B. D. Gessner, M. Dherani, S. A. Madhi, R. J. Singleton, K. L. O'Brien, A. Roca, P. F. Wright, N. Bruce, A. Chandran, E. Theodoratou, A. Sutanto, E. R. Sedyaningsih, M. Ngama, P. K. Munywoki, C. Kartasasmita, E. A. F. Simoes, I. Rudan, M. W. Weber, and H. Campbell. "Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis." *Lancet*. 375.9725 (2010): 1545–1555.

O'callaghan-Gordo C, Q. Bassat, L. Morais, N. Diez-Padrisa, S. Machevo, T. Nhampossa, D. Nhalungo, S. Sanz, L. Quinto, P. L. Alonso, and A. Roca. "Etiology and Epidemiology of Viral Pneumonia Among Hospitalized Children in Rural Mozambique: A Malaria Endemic Area With High Prevalence of Human Immunodeficiency Virus." *Pediatr Infect Dis J* (2010): Ahead of print.

Roca, A., Q. Bassat, L. Morais, S. Machevo, B. Sigauque, C. O'Callaghan, T. Nhamposa, E. Letang, I. Mandomando, D. Nhalungo, L. Quinto, and P. Alonso. "Surveillance of acute bacterial meningitis among children admitted to a district hospital in rural Mozambique." *Clin Infect Dis.* 48 Suppl 2 (2009): S172–80.

Roca, A., B. Sigauque, L. Quinto, L. Morais, A. Berenguera, M. Corachan, J. L. Ribo, D. Naniche, Q. Bassat, C. Sacoor, D. Nhalungo, E. Macete, A. Schuchat, M. Soriano-Gabarro, B. Flannery, and P. L. Alonso. "Estimating the vaccine-preventable burden of hospitalized pneumonia among young Mozambican children." *Vaccine*. 28.30 (2010): 4851–4857.

Sigauque, B., A. Roca, Q. Bassat, L. Morais, L. Quinto, A. Berenguera, S. Machevo, A. Bardaji, M. Corachan, J. Ribo, C. Menendez, A. Schuchat, B. Flannery, M. Soriano-Gabarro, and P. L. Alonso. "Severe Pneumonia in Mozambican Young Children: Clinical and Radiological Characteristics and Risk Factors." *J Trop Pediatr.* 55.6 (2009): 379–387.

Sigauque, B., A. Roca, I. Mandomando, L. Morais, L. Quinto, J. Sacarlal, E. Macete, T. Nhamposa, S. Machevo, P. Aide, Q. Bassat, A. Bardaji, D. Nhalungo, M. Soriano-Gabarro, B. Flannery, C. Menendez, M. M. Levine, and P. L. Alonso. "Community-Acquired Bacteremia Among Children Admitted to a Rural Hospital in Mozambique." *Pediatr Infect Dis J*. 28.2 (2009): 108–113.

Valles, X., A. Roca, F. Lozano, L. Morais, B. Suarez, F. Casals, I. Mandomando, B. Sigauque, D. Nhalungo, C. Esquinas, L. Quinto, P. L. Alonso, and A. Torres. "Serotype-specific pneumococcal disease may be influenced by mannose-binding lectin deficiency." *Eur Respir J*. 36.4 (2010): 856–863.

Valles, X., M. - R. Sarrias, F. Casals, M. Farnos, R. Piner, B. Suarez, L. Morais, I. Mandomando, B. Sigauque, A. Roca, P. L. Alonso, A. Torres, N. M. Thielens, and F. Lozano. "Genetic and structural analysis of MBL2 and MASP2 polymorphisms in south-eastern African children." *Tissue Antigens*. 74.4 (2009): 298–307.

MATERNAL AND REPRODUCTIVE HEALTH

The improvement of maternal and reproductive health is one of the priorities of the international community and is considered key for the social and economic development of low-income countries. A study carried out in 2005 by the WHO and other international organisms estimated that there were 536,000 maternal deaths on a global level. Of these, 99% took place in developing countries and 50% in sub-Saharan Africa.

In this section, the center's main activities in maternal and reproductive health are presented.

NEW DRUGS FOR INTERMITTENT PREVENTIVE TREATMENT DURING PREGNANCY

The emergence of resistance to sulfadoxine-pyrimethamine (SP), particularly in Eastern Africa, causes concern regarding the use of this antimalarial drug for intermittent preventive treatment during pregnancy (IPTp) in the mid and long term. Furthermore, HIV infection increases susceptibility to malaria and could reduce the effectiveness of current interventions. There is thus an urgent need to evaluate new antimalarial drugs that could be used for IPT in both HIV-negative and -positive pregnant women. Of the antimalarial drugs currently available, mefloquine (MQ) is the one that offers the most advantages.

The CISM is participating in the Malaria in Pregnancy Preventive Alternative Drugs (MiPPAD) project, a project funded by the European and Developing Countries Clinical Trials Partnership involving centers in Benin, Gabon, Kenya and Tanzania as well as in Spain, France and Germany to evaluate the use of MQ for IPTp.

The project is constituted by an initial randomized multicenter trial comparing the safety and efficacy of SP versus MQ as IPTp in the context of insecticide-treated nets. MQ tolerability is also being evaluated by comparing the administration of MQ as a single intake with its administration as a split dose in two days. In total, 4716 pregnant women will be enrolled at the antenatal clinic and followed until the infant is one year old.

Besides, in countries where HIV prevalence in pregnant women is over 10%, MQ-IPTp is being compared to placebo-IPTp in pregnant HIV-infected women receiving cotrimoxazole prophylaxis. This second trial is double-blinded and is being conducted in Kenya, Tanzania and Mozambique. It will involve 1070 pregnant women who will be followed until the infant is 2 months old.

Both trials started in 2009 and recruitment is ongoing. The CISM is responsible for data management for both of the trials and will later coordinate the data analysis.

These trials are being conducted in the context of the Malaria in Pregnancy Consortium, an international consortium that aims to improve the prevention and treatment of malaria during pregnancy.

QUANTIFICATION OF PRE- AND POST-NATAL EXPOSURE TO INSECTICIDES AND ITS EFFECTS ON CHILDREN'S HEALTH

One of the ways to combat the vector that transmits malaria is by using insecticide-treated bed nets (ITNs) and periodically fumigating households with pesticides (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 Manhiça District pyrethroid-based IRS has been periodically performed since 2005.

The CISM started a project to measure exposure to pyrethroids and other persistent and semi-persistent pollutants in women and children related to IRS and ITNs, and also to study the effect of pesticide exposure due to IRS and ITN on children's health.

Existing information (questionnaires and biological samples) from other studies conducted by the CISM are being used. A pre-study will be performed to estimate background exposure to pesticides prior to the initiation of IRS from household straw samples, breast milk samples from puerperal women and cord blood plasma samples. The post-study will assess the same chemical patterns in women who gave birth during or immediately after the fumigations and compare the results with those from the pre-study phase.

Morbidity patterns and immune evaluation will be used as indicators of children's health status.

During this reporting period, levels of insecticides in straw samples, breast milk and cord blood plasma were measured. No differences were found between houses with or without IRS, but there was a significant increase in DDT levels between the pre- and the post-study. DDT levels in cord blood plasma are under analysis. Future activities will include the evaluation of immune responses.

MICROBIOLOGICAL AND BIOCHEMICAL COMPOSITION OF BREAST MILK

Maternal breastfeeding plays a fundamental role in the growth and optimal development of infants, especially in developing countries where the prevalence of infectious and nutritional diseases is high. An important protective microbiotic component was recently described in human milk.

A community-based study was conducted in 2006 to analyze the microbiological and biochemical composition of breast milk from women living in Manhiça. The laboratory analyses were concluded recently and the first results will be published in 2011.

MONITORING THE SAFETY OF ANTIMALARIAL AND ANTIRETROVIRAL DRUGS DURING PREGNANCY

There is currently little information about the incidence of adverse reactions produced by drugs (ADRs) in Mozambique, and even less about the use of antimalarial and antiretroviral drugs in pregnant women. Mozambique is making efforts to establish a pharmaco-vigilance system, but the system has yet to be able to capture the maximum amount of information regarding ADRs. ADRs are an important cause of mortality in many countries, with levels of up to 10% reported in hospitalized patients. In the context of the introduction of new treatments against malaria and HIV/AIDS and the high comorbidity between these diseases, having information about the adverse reactions due to these drugs during pregnancy is of vital importance.

The CISM is implementing a study to evaluate the safety of different antimalarial and antiretroviral drugs during pregnancy. All ADRs that occur during pregnancy are registered in questionnaires during the antenatal visits. Immediately after birth, the health status of the babies, including the existence of congenital malformations, is also evaluated. Children are also evaluated between 2 and 12 months of age to detect any anomalies that were not diagnosed at birth. The ADRs are later evaluated to determine the existence or not of a causal relationship with the use of drugs during pregnancy.

The recruitment phase of the study ended in February of 2009 and follow-up continued until 2010. A total of 2041 women were recruited from the antenatal clinic at the Manhiça Health Centre and the Maragra Health Post. Currently data cleaning and analysis are ongoing.

This project will complement the information generated by the other surveillance systems and will inform the Ministry of Health about the safety of these drugs during pregnancy.

PREVALENCE AND RISK FACTORS OF SEXUALLY TRANSMITTED INFECTIONS AND CERVICAL NEOPLASIA IN WOMEN

There is limited information on the prevalence of sexually transmitted infections (STIs) and cervical neoplasia in rural sub-Saharan Africa.

The CISM conducted a study to describe the prevalence and the etiology of STIs and the prevalence of cervical neoplasia among women. The age-stratified cross-sectional study included 262 women aged 14 to 61 years recruited at the antenatal clinic (59%), the family-planning clinic (7%), and the community (34%).

Malaria prevention during pregnancy with intermittent preventive treatment reduces neonatal mortality

In the global context of a reduction of under-five mortality, neonatal mortality is an increasingly relevant factor. Malaria in pregnancy may affect neonatal survival, although no strong evidence exists to support this association.

In the context of a randomized, placebo-controlled trial of intermittent preventive treatment during pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) in 1030 Mozambican pregnant women, 997 newborns were followed until 12 months of age. IPTp reduced neonatal mortality by 61.3% (95% CI, 7.4%- 83.8%). Mechanisms associated with increased malaria infection at the end of pregnancy may explain the excess mortality in the less-protected malaria group. Alternatively, SP may have reduced the risk of neonatal infections.

These findings are of relevance to promote the implementation of IPTp with SP and to provide insights into the understanding of the pathophysiological mechanisms through which maternal malaria affects fetal and neonatal health.

MiPPAD team reviewing study procedures.

At least one active STI was diagnosed in 79% of women. Trichomonas vaginalis was present in 31% of all study participants. The prevalence of Neisseria gonorrhea and Chlamydia trachomatis were 14% and 8%, respectively, and syphilis was diagnosed in 12% of women. Human papillomavirus DNA was detected in 40% of women and cervical neoplasia in 12%. The risk factors associated with the presence of some of the STIs were being divorced or widowed, having more than one sexual partner and having one's partner living in another area. A higher prevalence was observed in the reproductive age group and some of the STIs were more frequently diagnosed in pregnant women.

This study confirmed the importance of STI control programs to reduce the burden of STIs, including HIV and cervical neoplasia, in rural settings.

DIAGNOSIS OF CAUSES OF MATERNAL DEATH IN SUB-SAHARAN AFRICA

Maternal mortality is a major public health problem in developing countries. Extreme differences in maternal mortality rates between developed and developing countries indicate that most of these deaths are preventable. Most information on the causes of maternal death in these areas is based on clinical records and verbal autopsies. Clinical diagnostic errors may play a significant role in this problem and might also have major implications for the evaluation of current estimations of causes of maternal death.

In the context of a maternal mortality study conducted in collaboration with the Maputo Central Hospital, a retrospective analysis of clinicopathologic correlation was carried out, using necropsy as the gold standard for diagnosis. All maternal autopsies conducted from October 2002 to December 2004 at the Maputo Central Hospital (n = 139) were included and major diagnostic discrepancies were analyzed (i.e., those involving the cause of death). Major diagnostic errors were detected in 56 (40.3%) maternal deaths. A high rate of false negative diagnoses was observed for infectious diseases, which showed sensitivities of under 50%. These diseases were HIV/AIDS-related conditions (33.3%), pyogenic bronchopneumonia (35.3%), pyogenic meningitis (40.0%), and puerperal septicemia (50.0%). Eclampsia was the main source of false positive diagnoses, with a low positive predictive value (42.9%).

This study shows that clinicopathological discrepancies may have a significant impact on maternal mortality in sub-Saharan Africa and thus questions the validity of reports based on clinical data or verbal autopsies. Increasing clinical awareness of the impact of obstetric and non-obstetric infections with their inclusion in the differential diagnosis, together with a thorough evaluation of cases clinically thought to be eclampsia, could have a significant impact on the reduction of maternal mortality.

Researchers

John J. Aponte [1, 2]	Sónia Machevo [1, 6]
Azucena Bardají [2]	Maria Maixenchs [2]
Carlos Bavo [1]	Alda Mariano [6]
Catarina David [1, 5]	Alfredo G. Mayor [2]
Nilsa de Deus [1]	Clara Menéndez [1, 2]
Carlota Dobaño [2]	Sibone Mocumbi [4]
Raquel González [1, 2]	Cinta Moraleda [1]
Nayra Gutiérrez [1]	Khátia Munguambe [1, 3]
Emili Letang [1, 2]	Denise Naniche [2]
Eusébio Macete [1, 5]	Robert Pool [2]
José Machado Almeida [1]	Esperança Sevene [1, 6]

[1] Manhiça Health Research Centre(CISM)

[2] Barcelona Centre for International Health Research (CRESIB), Hospital Clínic – Universitat de Barcelona, Spain

[3] Foundation for Community Development (FDC), Mozambique

[4] National Health Institute (INS), Mozambique

[5] Ministry of Health, Mozambique

[6] Universidade Eduardo Mondlane, Mozambique

PUBLICATIONS

de Sanjose, S., W. G. Quint, L. Alemany, D. T. Geraets, J. E. Klaustermeier, B. Lloveras, S. Tous, A. Felix, L. E. Bravo, H. R. Shin, C. S. Vallejos, P. A. de Ruiz, M. A. Lima, N. Guimera, O. Clavero, M. Alejo, A. Llombart-Bosch, C. Cheng-Yang, S. A. Tatti, E. Kasamatsu, E. Iljazovic, M. Odida, R. Prado, M. Seoud, M. Grce, A. Usubutun, A. Jain, G. A. Suarez, L. E. Lombardi, A. Banjo, C. Menendez, E. J. Domingo, J. Velasco, A. Nessa, S. C. Chichareon, Y. L. Qiao, E. Lerma, S. M. Garland, T. Sasagawa, A. Ferrera, D. Hammouda, L. Mariani, A. Pelayo, I. Steiner, E. Oliva, C. J. Meijer, W. F. Al-Jassar, E. Cruz, T. C. Wright, A. Puras, C. L. Llave, M. Tzardi, T. Agorastos, V. Garcia-Barriola, C. Clavel, J. Ordi, M. Andujar, X. Castellsague, G. I. Sanchez, A. M. Nowakowski, J. Bornstein, N. Munoz, and F. X. Bosch. "Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study." *Lancet Oncol.* 11.11 (2010): 1048–1056.

Mayor, A., E. Serra-Casas, A. Bardaji, S. Sanz, L. Puyol, P. Cistero, B. Sigauque, I. Mandomando, J. J. Aponte, P. L. Alonso, and C. Menendez. "Sub-microscopic infections and long-term recrudescence of Plasmodium falciparum in Mozambican pregnant women." *Malar J.* 8 (2009): 9.

Menendez, C., A. Bardaji, B. Sigauque, S. Sanz, J. J. Aponte, S. Mabunda, and P. L. Alonso. "Malaria prevention with IPTp during pregnancy reduces neonatal mortality." *PLoS One*. 5.2 (2010): e9438.

Menendez, C., X. Castellsague, M. Renom, J. Sacarlal, L. Quinto, B. Lloveras, J. Klaustermeier, J. R. Kornegay, B. Sigauque, F. X. Bosch, and P. L. Alonso. "Prevalence and risk factors of sexually transmitted infections and cervical neoplasia in women from a rural area of southern Mozambique." *Infect Dis Obstet Gynecol.* 2010 (2010).

Naniche, D., A. Bardaji, M. Lahuerta, A. Berenguera, I. Mandomando, S. Sanz, J. J. Aponte, B. Sigauque, P. L. Alonso, and C. Menendez. "Impact of maternal human immunodeficiency virus infection on birth outcomes and infant survival in rural mozambique." *Am J Trop Med Hyg*. 80.5 (2009): 870–876.

Ordi, J., M. R. Ismail, C. Carrilho, C. Romagosa, N. Osman, F. Machungo, J. A. Bombi, J. Balasch, P. L. Alonso, and C. Menendez. "Clinico-pathological discrepancies in the diagnosis of causes of maternal death in sub-Saharan Africa: retrospective analysis." *PLoS Med.* 6.2 (2009): e1000036.

Serra-Casas, E., C. Menendez, A. Bardaji, L. Quinto, C. Dobano, B. Sigauque, A. Jimenez, I. Mandomando, V. S. Chauhan, C. E. Chitnis, P. L. Alonso, and A. Mayor. "The Effect of Intermittent Preventive Treatment during Pregnancy on Malarial Antibodies Depends on HIV Status and Is Not Associated with Poor Delivery Outcomes." *J Infect Dis.* 201.1 (2010): 123–131.

Serra-Casas, E., C. Menendez, C. Dobano, A. Bardaji, L. Quinto, J. Ordi, B. Sigauque, P. Cistero, I. Mandomando, P. L. Alonso, and A. Mayor. "Persistence after delivery of Plasmodium falciparum parasites infecting Mozambican pregnant women." *Infect Immun* (2010): Ahead of print.

Sevene, E., R. Gonzalez, and C. Menendez. *Current knowledge and challenges of antimalarial drugs for treatment and prevention in pregnancy*. Vol. 11., 2010.

Sicuri, E., A. Bardaji, T. Nhampossa, M. Maixenchs, A. Nhacolo, D. Nhalungo, P. L. Alonso, and C. Menendez. "Cost-effectiveness of intermittent preventive treatment of malaria in pregnancy in southern mozambique." *PLoS One.* 5.10 (2010): e13407.

Sikora, M., A. Ferrer-Admetlla, H. Laayouni, C. Menendez, A. Mayor, A. Bardaji, B. Sigauque, I. Mandomando, P. L. Alonso, J. Bertranpetit, and F. Casals. "A variant in the gene FUT9 is associated with susceptibility to placental malaria infection." *Hum Mol Genet.* 18.16 (2009): 3136–3144.

SOCIAL SCIENCES

Any health intervention that involves individuals needs to and must take into account the values, beliefs, attitudes and behaviors of the target population. As such, social studies must contribute to a better understanding of these phenomena by using systematic scientific methodologies (quantitative, qualitative or mixed).

In the context of biomedical research, the social science approach adds considerable value to the information generated by other areas of knowledge, empowering even more the contribution of the CISM to the elaboration and implementation of health policies in the country.

SOCIAL AND CULTURAL PROFILE OF PRIORITY DISEASES

Perception of caretakers of children under five years of age in relation to febrile syndromes and respiratory infections

Correct and timely diagnosis is considered to be a fundamental step towards reducing infant and child mortality attributable to malaria and acute respiratory infections. The success of this depends on the recognition of febrile symptoms and respiratory problems and on the healthcare-seeking behavior of caretakers of children at risk.

The CISM undertook to document the steps taken by caretakers from the moment the first symptoms appear to the moment they contact the hospital. The study also explored how caretakers interpret symptoms and examined which symptoms trigger healthcare-seeking behavior. To do so, interviews were carried out with the caretakers of children admitted for a short stay at the Manhiça District Hospital, and follow-up was carried out after the patients were discharged. The same exercise was carried out with mothers of healthy children in the community.

The study revealed that acute respiratory infections are regarded as inherited, non-curable and chronic diseases, for which conventional medicine provides only temporary relief. The main implication of the findings of this study is that malaria is more likely to be diagnosed at early stages given that healthcare-seeking behavior is determined to a great extent by disease perceptions. Health education should thus aim to strengthen messages about acute respiratory infections to improve the identification of these infections and emphasize their infectious nature.

Perception of tuberculosis and possible relationship with the practices of prevention and healthcare-seeking behavior in Manhiça

Undocumented evidence maintains that in certain areas of Africa, tuberculosis is viewed as a disease that is acquired due to the transgression of traditional rituals. Knowing that the choice of health providers is partially determined by the cause that the patient attributes to the disease, it is probable that many tuberculosis cases never reach the health units.

In other contexts in Africa, it has been documented that 60% of patients diagnosed with tuberculosis in a health unit sought health care from alternative sources before visiting the hospital. In these cases, the control measures for tuberculosis may not have the desired impact if the interaction between local traditional practices and modern medicine is not taken into account.

A study undertaken by the CISM aims to increase understanding of the level of awareness of the community regarding tuberculosis, especially in children, and the perception of its etiology. Moreover, it aims to understand healthcare-seeking behaviors in the presence of tuberculosis in order to facilitate efforts to identify cases in the community, in the context of the National Tuberculosis Control Program.

A total of 20 interviews and seven focus group discussions were performed. Interviews were conducted with traditional healers (16) and informal drug providers (4). Preliminary results show that pediatric tuberculosis is seldom identified as such by traditional healers, informal drug providers or caretakers and is more often attributed to non-compliance with traditional rituals.

PERCEPTION AND ACCEPTANCE OF PUBLIC HEALTH INTERVENTIONS

Perception and reaction to antiretroviral treatment, the reconstitution of the immune system and adherence to treatment

Adherence to combination antiretroviral treatment (cART) and continuing this treatment for the rest of one's life in the case of HIV/AIDS patients is crucial for it to be effective. This suggests that the patient's behavior and attitude regarding treatment are important factors in determining its success. Nonetheless, the restoration of the immune responses of beneficial protection can also be accompanied by atypical opportunist infections and other associated diseases in the context of what is known as the immune reconstitution inflammatory syndrome (IRIS). These complications in the first months of treatment may influence the perception of the treatment. Moreover, when patients improve after starting cART, they may have a false sensation of being cured, and thus an increased risk of stopping treatment.

Determining the perception of cART and its effect on treatment adherence throughout the patient's life is important to guarantee that the design and implementation of cART programs are sustainable, effective, accepted and easily adhered to by the target community. The work conducted by the CISM in this area aims to determine the level of understanding regarding cART, to explore the interpretation of symptoms and its relationship with treatment adherence, as well as the reasons that lead to non-adherence.

A total of 51 patients under cART participated in a study designed to explore these aspects. Two in-depth interviews with a six month difference were conducted with each participant. The study revealed that there were no differences in perceptions and adherence levels between patients with IRIS and those without. Adherence was related more to the information given to patients, an active search of non-compliants and other obstacles such as transport costs, time available to go to the health facility and nutritional aspects.

Perceptions and behaviors related to malaria in pregnancy

Pregnant women and children aged under 5 years are the groups most vulnerable to malaria infection. To control malaria in pregnancy (MiP), Mozambique has adopted intermittent preventive treatment (IPT) with sulfadoxine-pyrimethamine (SP) combined with the distribution of insecticide-treated nets (ITNs).

The Malaria in Pregnancy Preventive Alternative Drugs (MiPPAD) project aims to evaluate the safety and efficacy of alternative antimalarial drugs to SP as IPT during pregnancy. As part of this project, a behavioral study was designed to explore perceptions and behaviors related to MiP, as well as the acceptability and use of interventions for the prevention of MiP. This study involves pregnant women (and their partners) enrolled or not in the MiPPAD study and health professionals.

The main data collection methods are in-depth Interviews, non-participant observation of clinical activities and interactions with patients, a rapid ethnographic methodology (which includes free listing of common illnesses and complaints during pregnancy) and pairwise comparisons of prevention strategies for MiP and antenatal care interventions.

Mothers waiting with their children for pediatric services at the Manhiça District Hospital.

Data collection is ongoing. This study will provide insights into factors influencing acceptability, access, and adherence to current and alternative MiP interventions in order to contribute to the success of future malaria prevention programs.

Perception and acceptability of HIV testing in pregnant women and their partners (ATHIC)

In Mozambique, and Africa in general, adherence to HIV counseling and testing remains very poor. This defeats the purpose of the efforts to control the HIV/AIDS epidemic, especially in the context of mother-to-child transmission (MTCT).

The aim of the ATHIC study is to understand the social, cultural and behavioral response to HIV testing in couples in the context of the MTCT prevention program. Specific key objectives include the determination of acceptability of HIV testing during pregnancy among pregnant women, their partners and their communities and of factors that influence caretaking options for children born to HIV-positive mothers.

The study will recruit participants (women and their partners) attending the antenatal and post-natal care services at the Manhiça District Hospital and the Manhiça, Maragra, and Nwamatibjana health centers.

The following qualitative methods will be used: direct observation at counseling and testing facilities, in-depth interviews with pregnant women and their partners, focus group discussions with community members and participant observation.

The ATHIC study will generate recommendations for community health interventions that aim to encourage couple counseling and testing to minimize the risk of seroconversion during pregnancy and breastfeeding.

Perceptions of the implementation and reception of indoor residual spraying (IRS) in Manhiça

The control of malaria continues to be a public health challenge in sub-Saharan Africa. The WHO recently reinforced its recommendation for indoor residual spraying (IRS), with the use of DDT, to reduce transmission. Since IRS was reintroduced on a national level, the programme has registered high rates of coverage in certain areas, including Manhiça, suggesting a strong level of community acceptance and collaboration. However, to ensure its sustainability, the factors that may be contributing to these high levels of acceptance must be identified.

A qualitative study was conducted using the grounded theory approach, with the aim of furthering understanding of the local development, implementation and reception of the spraying program in the district of Manhiça. Ninety-six in-depth interviews were conducted with members of family units of the neighborhoods sprayed during the 2006 and 2007 campaigns. The data were analyzed qualitatively.

Despite the good acceptance of the programme, the general perception was that the effectiveness of the insecticide against mosquitoes was limited. The following factors were identified as being related to acceptance: trust in and compliance with the health system, involvement of community leaders in raising awareness, local origin of the sprayers, perception of the effectiveness of the product in relation to insects other than mosquitoes. The few cases of non-compliance to the campaign were due to a perception of an increase in mosquitoes and other insects during the weeks after spraying, and the unavailability of family members during spraying times.

The results demonstrate that despite the fact that IRS can contribute to the control of malaria in communities, this impact is barely perceived amongst the beneficiaries, mostly because of social factors. There is a need to redefine the content of messages aimed at raising awareness and to continue to use local structures.

FEASIBILITY AND ACCEPTABILITY OF CLINICAL TRIALS

Feasibility of microbicide trials

The CISM, in collaboration with the Mozambican Foundation for Community Development (FDC) and Ministry of Health, participates in the Microbicides Development Programme network, whose objective is to develop microbicides for HIV infection prevention. A feasibility study was conducted between 2007 and 2010 to prepare the CISM for the conduct of a phase III clinical trial to test a vaginal microbicide candidate against HIV transmission.

Field worker interviewing a mother.

The study had the following objectives: (i) to evaluate the percentage of people with HIV and other sexually transmitted infections; (ii) to determine the level of awareness about HIV and AIDS in the community in general and more specifically in the study population; (iii) to identify sexual practices in the study areas; (iv) to evaluate the impact of the promotion of condoms and their use; (v) to determine the maximum attainable rate of recruitment and retention; (vi) to confirm if the materials to collect information are of good quality; (vii) to evaluate if women respond to study questions; (viii) to evaluate the willingness of women to participate in a future microbicides trial; and (ix) to evaluate if women and the community in general are prepared to take on a new fight against HIV.

The study recruited and monitored 500 HIV-negative women from the areas covered by the 1 de Junho Health Centre in Maputo and the Manhiça Health Centre. The study had 3 fundamental stages: (i) awareness-building in the community and encouragement of women to adhere to the study; (ii) recruitment and clinical follow-up of participants using the same procedures to be implemented in a microbicide trial; and (iii) socio-behavioral follow-up. This third phase involved a subsample of 100 women and their partners, who were invited to participate in in-depth interviews, focus group discussions and to experiment with alternative techniques for the collection of data on sexual practices.

Sixty participants were subsequently enrolled in a pilot study to evaluate adherence to the daily use of a placebo gel (top-up study). Data of both studies are currently under analysis.

Facilities used for the top-up study.

Feasibility of HIV vaccine trials

The AfrEVacc consortium was established to develop a network of research centers in Europe and Africa with capacity for the clinical development of HIV vaccines. The Universidade Católica de Moçambique and the CISM are the two Mozambican institutions that are part of this consortium.

Activities in this consortium include a feasibility and acceptability study of HIV vaccine trials, initiatives to improve laboratory capacities, and estimation of HIV infection prevalence and incidence in the community (see *HIV/AIDS* section).

The primary aim of the feasibility and acceptability study is to gain better understanding of how to enroll and retain adults in vaccine trials. Throughout the study the CISM will gain crucial experience of the involvement of healthy adult men and women in clinical trials. The study is currently under preparation and is expected to start in 2011.

OTHER STUDIES

Availability and use of antimalarial drugs in the community

Malaria is a great burden to the health system. A considerable portion of the population has no fast or adequate access to antimalarial treatment. Alternative sources of malaria treatment need to be identified at the community level, so that recommendations can be proposed for additional measures that may be available to widen the scope of artemisinin-based combination therapies, while recognizing the importance of protecting this critical class of medications against resistance resulting from their uncontrolled use.

The Mal-Market study is being conducted in response to this problem in collaboration with the Medicines for Malaria Venture (MMV) in order to expand the base of evidence regarding sources and factors that will facilitate antimalarial treatment and contribute to the accessibility and correct management of malaria cases in Mozambique.

The overall objective of the study is to explore issues related to demand and to further understanding of supply in the antimalarial drug market in rural areas of Mozambique.

The data collection will be carried out in three phases: (i) document analysis, (ii) qualitative evaluation, and (iii) a quantitative survey. The study began in October 2010 and will run for eight months.

Impact of parental mortality on children's health and survival

While adult mortality has progressively increased in recent decades in Mozambique, particularly due to HIV/AIDS, the consequences of these adult deaths have seldom been documented. The aim of this study is to investigate the relationship between parental mortality and child health and survival in the Manhiça district using data from the demographic and health surveillance system spanning January 1998 and December 2008.

The results of the study show that adult mortality was high and increased during the study period, particularly among males. As a consequence, the proportion of children who lost one or both parents also increased (5.8% during the study period; 64.2% of these children had lost their father). Children who lose a parent tend to have poorer health and a higher risk of death than those who do not, and the most affected are those whose mothers die. Hospital visits by children who lost a parent was also higher (79% vs. 72%) as was the frequency of hospital admissions, and the risk of death (5.6 times higher than children who have both parents). Children whose mothers are dead have the highest mortality risk in Manhiça, with an odds ratio of 8.9; the corresponding odds ratio for children whose father is dead is 3.4. Finally, children whose mother died within 42 days of deliver or during pregnancy (approximation of maternal mortality) have a seven-fold increased risk of death compared to other children.

Determinants of household demand for bed nets

Understanding what drives the purchase and use of insecticide-treated nets (ITNs) is key to finding a long-term, sustainable solution to preventing the spread of malaria. Few studies have analyzed the determinants of demand for bed nets for malaria prevention at the household level, or in particular, how demand for nets compares with demand for other mosquito-prevention methods.

The CISM conducted a study to assess the determinants of demand for bed nets in an area of endemic malaria transmission. The study looked at willingness to pay for bed nets, net ownership, usage, and past purchase behavior, alongside expenditure and frequency of use of alternative methods for malaria prevention through a household survey.

While overall net ownership in the sample was low, the evidence failed to suggest that poorer households are less likely to own bed nets when covariates were controlled for; nor did the likelihood of receiving a free net depend on socioeconomic status. Formal schooling and market knowledge seemed to indicate a higher average willingness to pay, while use of alternative methods for malaria prevention and indoor residual spraying were found to decrease demand for bed nets.

These results suggest that either full or partial subsidies may be necessary in some contexts to encourage households to purchase and use nets. It suggests that successful malaria control campaigns should invest a portion of their funds towards educating recipients of IRS and users of other preventive methods on the importance of net use even in the absence of mosquitoes.

Cost-effectiveness of intermittent preventive treatment in pregnancy

Economic evaluations of preventive strategies in pregnancy are needed to guide health policies. In particular, the cost-effectiveness of strategies such as intermittent preventive treatment in pregnancy (IPTp) are important to facilitate decision-making regarding malaria control in pregnancy.

The CISM conducted an analysis of the cost-effectiveness of IPTp in the context of a trial of IPTp with sulfadoxine-pyrimethamine (SP), where both intervention groups received an insecticide-treated net through the antenatal clinic. The cost-effectiveness of IPTp-SP with regard to maternal clinical malaria and neonatal survival was estimated. Correlation and threshold analyses were undertaken to assess the main factors affecting economic outcomes and the cut-off values beyond which the intervention is no longer cost-effective.

In 2007 US$, the incremental cost-effectiveness ratio (ICER) for maternal malaria was 41.46 US$ (95% CI, 20.5-96.7) per disability-adjusted life-year (DALY) averted. The ICER per DALY averted due to the reduction in neonatal mortality was 1.08 US$ (95% CI, 0.43-3.48). The ICER including the effect on both the mother and the newborn was 1.02 US$ (95% CI, 0.42-3.21) per DALY averted. Efficacy was the main factor affecting the economic evaluation of IPTp-SP. The intervention remained cost-effective with an increase in drug cost per dose up to 11 times in the case of maternal malaria and 183 times in the case of neonatal mortality.

This study shows that IPTp-SP is highly cost-effective for both preventing maternal malaria and reducing neonatal mortality in Mozambique. These findings are likely to hold for other settings where IPTp-SP is implemented through antenatal clinic visits. The intervention remained cost-effective even with a significant increase in drug and other intervention costs. Improvements in the protective efficacy of the intervention would increase its cost-effectiveness. Finally, provision of IPTp with a more effective although more expensive drug than SP may still remain a cost-effective public health measure to prevent malaria in pregnancy.

Researchers

Carlos Bavo [1]	Sibone Mocumbi [4]
Catarina David [1, 5]	Khátia Munguambe [1, 3]
Kizito Gondo [1]	Ariel Nhacolo [1]
Nayra Gutiérrez [1]	Robert Pool [2]
Maria Maixenchs [2]	Charfudin Sacoor [1]

[1] Manhiça Health Research Centre(CISM)

[2] Barcelona Centre for International Health Research (CRESIB), Hospital Clínic – Universitat de Barcelona, Spain

[3] Foundation for Community Development (FDC), Mozambique

[4] National Health Institute (INS), Mozambique

[5] Ministry of Health, Mozambique

PUBLICATIONS

Chase, C., E. Sicuri, C. Sacoor, D. Nhalungo, A. Nhacolo, P. L. Alonso, and C. Menendez. "Determinants of household demand for bed nets in a rural area of southern Mozambique." *Malar J.* 8.1 (2009): 132.

Conteh, L., E. Sicuri, F. Manzi, G. Hutton, B. Obonyo, F. Tediosi, P. Biao, P. Masika, F. Matovu, P. Otieno, R. D. Gosling, M. Hamel, F. O. Odhiambo, M. P. Grobusch, P. G. Kremsner, D. Chandramohan, J. J. Aponte, A. Egan, D. Schellenberg, E. Macete, L. Slutsker, R. D. Newman, P. Alonso, C. Menendez, and M. Tanner. "The cost-effectiveness of intermittent preventive treatment for malaria in infants in Sub-Saharan Africa." *PLoS One.* 5.6 (2010): e10313.

Hutton, G., D. Schellenberg, F. Tediosi, E. Macete, E. Kahigwa, B. Sigauque, X. Mas, M. Trapero, M. Tanner, A. Trilla, P. Alonso, and C. Menendez. "Cost-effectiveness of malaria intermittent preventive treatment in infants (IPTi) in Mozambique and the United Republic of Tanzania." Bull *World Health Organ.* 87.2 (2009): 123–129.

Montgomery, C. M., K. Munguambe, and R. Pool. "Group-based citizenship in the acceptance of indoor residual spraying (IRS) for malaria control in Mozambique." *Soc Sci Med*. 70.10 (2010): 1648–55.

Sicuri, E., A. Bardaji, T. Nhampossa, M. Maixenchs, A. Nhacolo, D. Nhalungo, P. L. Alonso, and C. Menendez. "Cost-effectiveness of intermittent preventive treatment of malaria in pregnancy in southern mozambique." *PLoS One.* 5.10 (2010): e13407.

OTHER DISEASES

This section presents studies focused on some of the main health problems affecting children that are particularly relevant to public health, such as anemia and the causes of death among children in rural areas such as that covered by the CISM.

Health worker showing a health card.

ETIOLOGY OF ANEMIA

Anemia continues to be one of the most difficult public health problems in the world, especially in countries where malaria is endemic. In such cases, anemia is generally a silent condition that is a major public health burden. According to estimates from the WHO, there are close to two billion anemic people in the world.

Childhood anemia is associated with a higher risk of death and deficiencies in cognitive and motor development, growth and immune functions. Children admitted to hospitals have a greater risk of death if they have anemia, and mortality ranges from 6% to 18% in those with severe anemia, even when they undergo blood transfusions. Furthermore, the majority of children at risk for severe anemia do not have easy access to facilities with the capacity to perform blood transfusions.

One of the objectives of the WHO is to decrease the prevalence of malaria and increase iron and folic acid supplementation to decrease the prevalence of anemia. However, nearly 818 million women and young children continue to have anemia. The prevalence of these two groups is higher in Africa, where up to 65% of children are anemic.

Factors that may contribute to anemia in Manhiça and those for which data are available include malaria, HIV infection (the prevalence in pregnant women is close to 25%) and malnutrition (prevalence of 56% in children seen in the outpatient clinic).

Moreover, there are no data on the prevalence of or possible contribution to childhood anemia by micronutrient deficiencies, intestinal parasites, schistosomiasis, bacteriemias and inherited hemoglobin and erythrocyte diseases. In addition, there is little knowledge about the pathophysiology of malarial anemia.

A study aimed to increase information regarding the prevalence of different risk factors and their contribution was started in 2008 in Manhiça. This information is necessary to create new control strategies and better implement those that already exist. The study also aims to improve the biochemical markers for diagnosing iron deficiencies, to determine the contribution of certain cytokines and identify new pathophysiological mechanisms in anemias associated with malaria, and to study gene expression profiles of gene expression in the bone marrow of children with malarial anaemia, in order to obtain a broader vision of the mechanisms involved in erythropoiesis suppression. The study is currently in the phase of analysis and publication of results.

CAUSES OF DEATH IN CHILDREN

Approximately 46 million of the estimated 60 million deaths that occur in the world each year occur in developing countries. Furthermore, this mortality is highest in sub-Saharan Africa, although the causes have not been well documented.

The CISM began collecting verbal autopsies through its demographic surveillance system (DSS) in 1997. Each questionnaire is reviewed independently by three physicians with experience in tropical diseases who assign the cause of death according to the International Classification of Diseases (ICD-10). Each medical doctor attributes a minimum of one and a maximum of two causes. A final diagnosis is reached when at least two physicians agree on the cause of death.

In 2009, the analysis of the first 10 years of data (1997-2006) was published. From January 1997 to December 2006, 568,499 person-years at risk (pyrs) and 10,037 deaths were recorded in the Manhiça DSS. 3,730 deaths with 246,658 pyrs were recorded for children under 15 years of age. Verbal autopsy interviews were conducted for 3002 (80.4%) of these deaths; 73.6% of deaths were attributed to communicable diseases, 9.5% to non-communicable diseases, and 3.9% to injuries.

Malaria was the single largest cause of death, accounting for 21.8% of all cases. Pneumonia, with 9.8%, was the second leading cause of death, followed by HIV/AIDS (8.3%) and diarrheal diseases (8%). The pattern of childhood mortality in the Manhiça area is typical of developing countries, where malaria, pneumonia and HIV/AIDS are major causes of death.

Researchers

Ruth Aguilar [1, 2]
Clara Menéndez [1, 2]
Cinta Moraleda [1]
Montse Renom [1]
Jahit Sacarlal [1, 3]

[1] Manhiça Health Research Centre (CISM)

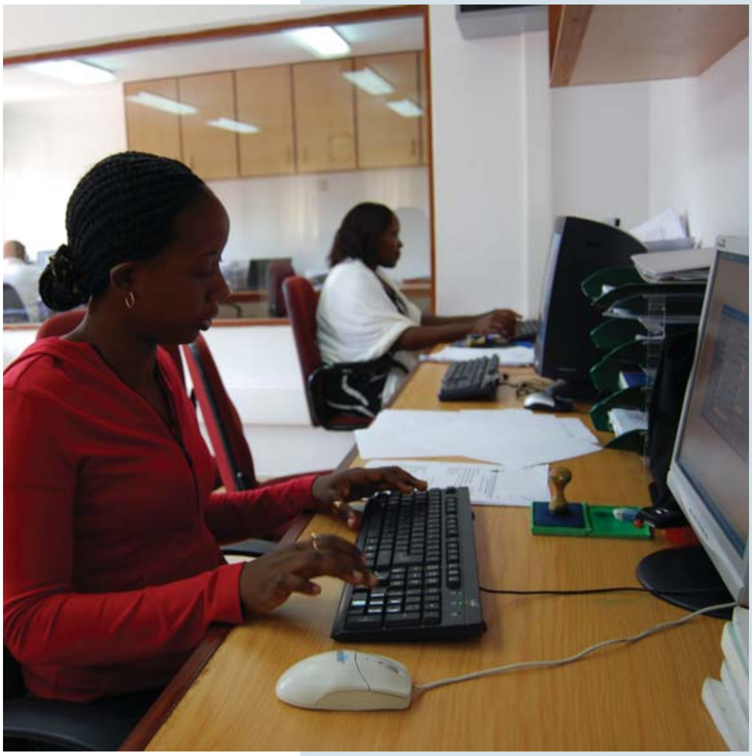
[2] Barcelona Centre for International Health Research (CRESIB), Hospital Clínic – Universitat de Barcelona, Spain

[3] Universidade Eduardo Mondlane, Mozambique

PUBLICATIONS

Sacarlal, J., A. Q. Nhacolo, B. Sigauque, D. A. Nhalungo, F. Abacassamo, C. N. Sacoor, P. Aide, S. Machevo, T. Nhampossa, E. V. Macete, Q. Bassat, C. David, A. Bardaji, E. Letang, F. Saute, J. J. Aponte, R. Thompson, and P. L. Alonso. "A 10 year study of the cause of death in children under 15 years in Manhica, Mozambique." *BMC Public Health*. 9 (2009): 67.

Section 2



Departments

DEMOGRAPHY

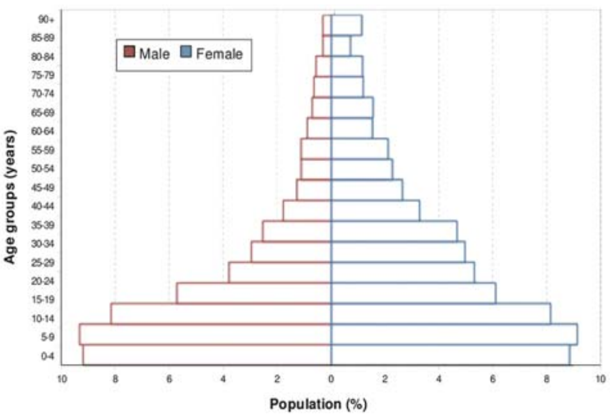
The Demography Department is responsible for the Demographic Surveillance System (DSS), which is designed to collect reliable socio-demographic data for health research in an area for which official data are scarce and unreliable. The DSS is a crucial component of the CISM's research activities and provides accurate and credible demographic indicators.

The DSS covers an area of 500 km^2 (almost one-fifth of the total district), with 18,797 households and 86,704 inhabitants (around 52% of the total district population). All the households in the study area are uniquely identified by GPS coordinates. Longitudinal demographic information is collected through three basic procedures: (i) household visits twice a year, (ii) maternity and morgue visits to record births and deaths, and (iii) contact with key community informants.

The department made significant improvements in 2009 and 2010. First, its involvement in the elaboration of the Strategic Plan for 2010-13 helped to clearly identify the department's strategic goals and objectives, the most important of which is the transition to an electronic data capture system, which is planned for 2011 in the context of the INDEPTH Effectiveness and Safety Studies of Antimalarial Drugs in Africa (INESS) project. Second, the improvement of the annual budgeting process has allowed the department to clearly plan for its annual needs and resources.

As part of the center's activities in the context of the INDEPTH Network, the department participated in the creation of the migration monograph published by the network.

The department also has a key role in the provision of support to new research centers in sub-Saharan Africa that wish to create a DSS. Specifically, the department is currently providing support to the Centro de Investigação e Treinamento em Saúde de Chokwé (CITSC) in Chokwé, Mozambique and the Centro de Investigação em Saúde de Angola (CISA) in Caxito, Angola.



Population pyramid for 2009

Field worker collecting demographic information.

Indicators	2006	2007	2008	2009	2010*
Midyear population	79,361	79,383	78,915	78,266	78,071
Infant mortality rate (per one thousand live births)	71.7	71.0	59.3	58.5	63.8
Child mortality rate (per one thousand live births) <5 years of age	133.5	126.1	108.8	122.4	119.4
Maternal mortality rate (per one thousand live births)	2.7	3.6	3.5	3.7	1.9
Life expectancy, males (years)	39.8	41.5	40.3	40.8	42.7
Life expectancy, females (years)	49.5	51.8	50.6	51.3	54.0
Life expectancy, both sexes (years)	45.2	47.1	46.0	46.8	49.1

* Information for the first six months of 2010.

CLINICAL SERVICES

The Clinical Services Department coordinates the center's activities in the health facilities in the study area. In particular, it is responsible for the implementation of the Morbidity Surveillance System and other specific research activities. The center's clinical activities are currently conducted at the Manhiça District Hospital and Health Centre, and at health posts in Maragra, Taninga, Palmeira, Ilha Josina and Malavele. All activities are conducted in close collaboration with the District Health Service and other health authorities.

The Morbidity Surveillance System collects demographic and clinical information on all outpatient and inpatient pediatric visits (<15 years of age) to the health facilities in the study area on a round-the-clock basis. This information is collected through standardized questionnaires that are subsequently entered into the CISM's databases using a double entry system. The Clinical Services Department plays a crucial role in ensuring the quality of the data collected.

Department activities include the provision of healthcare, the training of medical personnel and the preparation of case-management guidelines, principally in the maternal and pediatric areas. The center also supports the refurbishment and construction of health facility infrastructure. During 2009-10, the CISM installed a digital X-ray imaging system at the Manhiça District Hospital in the context of the RTS,S malaria vaccine trials and built a facility for sputum induction and gastric lavage for ongoing tuberculosis studies.

The CISM has also worked with national health authorities in the implementation of national health programs in recent years. The center is currently providing assistance with the implementation of the National STI/HIV/AIDS program and the National Tuberculosis Control Program with support from the Catalan Cooperation Agency for Development (Spain) and the AERAS Global TB Vaccine Foundation. The activities are aimed at guaranteeing HIV counseling and testing and vertical transmission prevention programs as a means to contribute to the control of the pandemic and to support the different studies being conducted in this area. The CISM also provides support in the diagnosis of tuberculosis and the identification of contacts.

Mother and child at the Manhiça Health Centre with health worker.

The department is also instrumental in collaboration activities with the Universidade Eduardo Mondlane through which the center and the Manhiça District Hospital receive medical students in their last year at medical school for clinical practice.

Lastly, the CISM coordinates a Pediatric Malnutrition Program at the Manhiça District Hospital funded by the non-governmental organization África Viva.

Indicators	2006	2007	2008	2009	2010
Outpatient visits	62,896	60,842	60,671	72,030	67,253
Admitted children	3,601	3,050	3,344	2,651	2,148
Intra-hospital mortality ratio	5.3%	5.1%	3.0%	4.0%	4.8%
Pediatric and adult counseling sessions	4,785	6,895	5,330	5,443	8,473
Pediatric and adult HIV tests	4,684	6,742	5,169	5,389	8,448
Children on antiretroviral treatment	59	117	142	217	299
Adults on antiretroviral treatment	661	1,399	1,746	2,155	2,739

LABORATORY

The CISM's laboratory provides infrastructure and diagnostic support to the center's research projects and healthcare activities at the Manhiça Health Centre and District Hospital.

The laboratory works in accordance with Good Clinical Laboratory Practices (GCLP) and standardized operating procedures (SOP) and obtained ISO 9001:2008 certification in 2009. The laboratory also uses a laboratory information system (Servolab®) that records all processes and sample storage in addition to special software (LabManagerPlus®) for managing laboratory stock.

The laboratory is organized into five areas: clinical analyses (hematic parasitology and hematology/biochemistry); microbiology (general bacteriology and mycobacteriology); immunology; molecular biology; and quality assurance & biosafety. In addition, it undertakes general operations such stock management, procurement & logistics, sample reception, media preparation and sample storage.

CLINICAL ANALYSES

Hematic parasitology

This area is responsible for malaria diagnosis using optical microscopy and capillary hematocrit determination. In addition to blood slides originated by research projects, all pediatric blood slides from the health facilities in the center's study area are read at the laboratory.

Malaria diagnosis is performed through quick semi-quantitative readings for clinical management and subsequent quantitative reading. In July 2009, the laboratory started to use the Lambaréné method, which is based on predefined blood volumes and provides a better estimation of parasitemia than the standard method recommended by the World Health Organization.

All blood slides are read twice and a third reading is determined using an algorithm incorporating the previous two readings. All readings are performed by different microscopists. All microscopists undergo internal and external quality control tests three times a year which they need to pass in order to be able to make quantitative readings.

Samples processed	
Parasitology	41,283
Hematology/Biochemistry	6518
General bacteriology	5151
Immunology	2909
Molecular biology	2778

Laboratory improvements during 2009-10

The laboratory significantly improved its capacities during 2009-10. Some of the major changes that took place during this period are summarized below:

- New BSL-3 for TB diagnosis: With financial and technical support from AERAS and Hospital Clínic, the center recently installed a BSL-3 laboratory with liquid and solid culture systems. This is one of the few such facilities in the country and has significantly improved the tuberculosis diagnosis capacities of the center. This laboratory is crucial to increasing the CISM's activities in this area (see the Research section).

- ISO 9001:2008 certification: Following a period in which new processes and procedures were established and existing ones improved, the laboratory obtained ISO 9001:2008 certification in 2009 from the South African Bureau of Standards (SABS). This certification offers credibility and assurance to external collaborators and a means for the laboratory to continuously improve quality. ISO 9001:2008 certification was set as an objective together with a new management and organizational structure of the laboratory.

- Laboratory Information System: In early 2010, the center installed a new laboratory information system (Servolab®) donated by Siemens. This system processes all the information generated at the laboratory and follows the samples from the moment they arrive at the laboratory to their final storage. A new barcode system was also introduced to identify all samples. This system means that the transcription of laboratory results is no longer necessary; this considerably reduces the risk of transcription errors and allows real-time access to laboratory results. Another new system installed during 2009-10 was the stock management program LabManagerPlus®.

Hematology/Biochemistry

This area processes samples for hematologic and biochemical measurements on automatic Vitros (E350 e E250) and Sysmex (XT2000-1 e KX21N) analyzers. These systems are regularly calibrated to ensure the reliability of measurements. Services are provided to both research projects and healthcare activities.

MICROBIOLOGY

General bacteriology

In this area, blood, fecal, cerebral spinal fluid and other body fluid samples are processed for bacterial isolation and identification. Most of the samples come from research projects but diagnostic services are also provided for healthcare provision.

Blood samples are cultured using an automatic system (Bactec 9050) that detects bacterial growth. Subsequently, solid culture media or parasitological diagnosis based on concentration techniques are used. Biochemistry tests, latex agglutination or specific anti-serum are used for bacterial identification. Antibiotic susceptibility tests are performed using disk diffusion tests. In addition, minimal inhibitory concentration tests for *Streptococcus pneumoniae* and *Neisseria* spp. using standard protocols (Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA) are performed.

All technicians working in this area undergo regular internal and external quality control tests.

Mycobacteriology

The laboratory significantly upgraded its facilities during 2009-10, with the construction of a BSL-3 laboratory that houses solid and liquid culture systems and other equipment for immunological and molecular techniques for mycobacterial identification. It is envisaged that the laboratory will be equipped for antibiotic susceptibility testing in the first quarter of 2011.

IMMUNOLOGY

This area evaluates cellular and humoral immunological responses to pathogens. While it mainly provides support to research projects, it has also provided CD4 counts for HIV-infected patients at the Manhiça Health Centre.

Human immunological cells are phenotyped *ex vivo* through surface coloration and identified using a cytometer (BD 4-color FACS Calibur). In vitro cultures are also used to measure immunological responses to specific antigens. Cytokine production and cytotoxicity are evaluated using intracellular cytokine coloration, flux cytometry and

Bactec system used for hemocultures

ELISpot methods. Enzyme-linked immunosorbent assays and immunofluorescence techniques are used to detect antibody responses.

This area is also responsible for Plasmodium falciparum in vitro culture, antigen preparation and other parasitological and immunohistochemical procedures.

MOLECULAR BIOLOGY

This area provides support mainly to research projects and occasionally to the Manhiça Health Centre for HIV diagnosis through polymerase chain reaction (PCR). The area has the equipment and know-how for the extraction and amplification of genetic material (DNA and RNA) and viral load determination. In particular, the laboratory has the capacity to identify, genotype and serotype microorganisms, as well as to determine clonality and antibiotic resistance markers through PCR and pulsed field gel electrophoresis.

During 2009-10, real-time PCR was introduced in the context of a project designed to evaluate the effectiveness of Haemophilus influenzae type b (Hib) vaccine introduction in the study area. Further capacity-building will extend the use of this equipment for *P. falciparum* and HIV detection.

QUALITY ASSURANCE & BIOSAFETY

This area is responsible for ensuring that all laboratory activities are conducted according to the highest quality standards and appropriate biosafety measures. All laboratory processes, SOPs, internal and external quality controls, and equipment calibration and maintenance are managed by this area. In addition, the area conducts training courses on quality assurance and quality control for laboratory personnel in addition to internal audits to ensure that all procedures are conducted following GCLP.

Since 2009 this area has also been responsible for the maintenance of ISO 9001:2008 certification.

DATA MANAGEMENT

New servers installed at the CISM.

This department is responsible for data management, including database creation, the input of questionnaire data into the system, query management and questionnaire storage. It also supports researchers in the creation of specific queries and lists for follow-up tasks, etc.

Questionnaire data are entered into the system using the double data entry system and OpenClinica®, a system which complies with international electronic data management requirements for clinical trials. This system has almost completely replaced the previous system, which is now only used for a few questionnaires designed prior to the introduction of OpenClinica®.

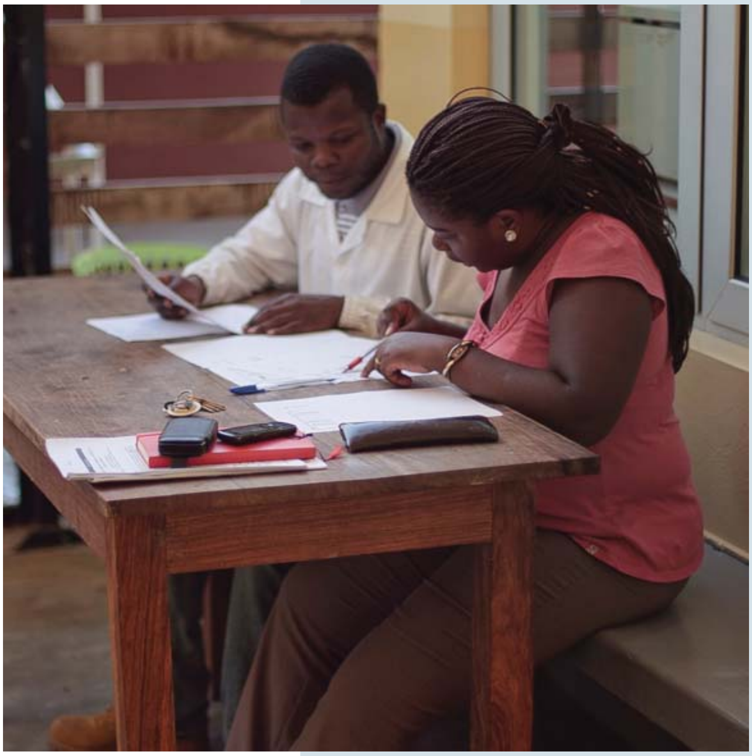
During 2009-10, approximately 166,200 questionnaires were entered and stored in the databases. Of these, approximately 64,500 corresponded to research projects and the rest to activities conducted within the Morbidity Surveillance System. The data center has also started to scan all old questionnaires so that they will be more readily available and searchable.

IT improvements during 2009-10

During 2009-10 the IT equipment and network was significantly improved to accommodate new data management applications.

- New HP servers were installed, offering improved processing and storage capacity and improved redundancy.
- The network infrastructure has been upgraded to a 1Gbps network, which allows the remote execution of applications inside the center.

Section 3



Training

TRAINING

The training of researchers and technical personnel has been a core strategic activity of the CISM since its creation. Apart from contributing to the sustainability of the center, the center's training activities support the development of health research capacities in Mozambique.

The CISM conducts its training activities in partnership and collaboration with other training institutions including research centers and universities. In particular, the Universidade Eduardo Mondlane, the Universitat de Barcelona (Spain) and CRESIB play a key role in the center's research activities.

The center's training activities are grouped into different areas:

- Research training (Training Fellowship Program)
- Post-graduate training
- Internships and short-term stays for health sciences students and professionals
- Technical personnel training
- Workshops and seminars
- Other training projects

Both internal personnel (some of whom are affiliated to Mozambican institutions such as the Universidade Eduardo Mondlane, the Ministry of Health and the National Health Institute) and external trainees benefit from the CISM's training activities.

TRAINING FELLOWSHIP PROGRAM

With a view to supporting research training in Mozambique, some years ago the CISM created the Training Fellowship Program aimed at young Mozambican graduates wishing to develop their professional career as health researchers. The program provides participants with direct research experience through involvement in research projects conducted at the center, and post-graduate training (master's or doctorates) at internationally recognized universities.

The program is implemented in collaboration with CRESIB, where training fellows do internships during their training.

While participating in this program, fellows may also do internships at other universities, research centers or international organizations. In the case of medical doctors, the program provides support for those wishing to train in a specialty.

Researchers reviewing X-ray images at the Manhiça District Hospital.

Over 20 researchers have benefited from the Training Fellowship Program since its introduction, and half of these have already completed their training. During 2009-10, Dr. Inácio Mandomando, Dr. Jahit Sacarlal, Dr. Betuel Sigaúque and Dr. Esperança Sevene completed their PhD studies.

Training Fellows during 2009-10	
Pedro Aide	Tacilta Nhampossa
Sónia Machevo	Diana Quelhas
Maria Nélia Manaca	Jahit Sacarlal
Inácio Mandomando	Charfudin Sacoor
Luís Morais	Esperança Sevene
Augusto Nhabomba	Betuel Sigaúque
Ariel Nhacolo	

POST-DOCTORAL TRAINING

The CISM fosters post-doctoral training at partner research centers of past training fellows who continue to be part of the center's research staff. For example, Dr. Inácio Mandomando, who leads some of the most important research in diarrheal and invasive bacterial diseases, currently holds a post-doctoral position at the Center for Vaccine Development at the University of Maryland (USA).

Staff attending a short course at the CISM.

INTERNSHIPS AND SHORT-TERM STAYS FOR HEALTH SCIENCES STUDENTS AND PROFESSIONALS

The CISM hosts health sciences students and professionals for short-term internships during which they have the opportunity to work alongside the center's staff on research projects or in healthcare assistance. Several types of trainees participate in this program:

- Pre-graduate medical students at the Universidade Eduardo Mondlane
- Pre-graduate international health sciences students and professionals
- National and international master's and PhD students
- Personnel from other centers with whom the CISM collaborates in the context of research projects

The CISM has an agreement with the Universidade Eduardo Mondlane School of Medicine under which it supervises medical students during their rural internship at the Manhiça District Hospital. These internships are coordinated and supervised by the Clinical Services Department. During 2009-10, the center supervised a total of 42 students under this scheme.

During 2009-10, the CISM received 27 international health sciences students and professionals from Europe and the USA, where the most prevalent diseases in Mozambique are not endemic. The internships were conducted at the Manhiça District Hospital and in some cases, at the Maputo Central Hospital.

The center also hosted a geographer from the Centro de Investigação e Treinamento em Saúde de Chokwé (CITSC) for a short-term internship at the Demography Department in order to better understand how the CISM runs its DSS.

The CISM also hosts national and international master's and PhD students who conduct research projects at the center as part of their training. In 2010, the center hosted Dr. Maura Pedrini while she performed a study on the clinical characterization of pediatric HIV/AIDS for her master's thesis at the University of Liverpool.

Finally, the center hosted personnel from the Ifakara Health Institute (Tanzania) and the Hospital Nacional Simão Mendes (Guinea-Bissau) for training in laboratory techniques and Good Clinical Practices (GCP) respectively.

TECHNICAL PERSONNEL TRAINING

The CISM contributes to the training of technical personnel through courses organized by the center and other institutions. The courses include training in GCP, GCLP, Quality Management, Biomedical Diagnosis and Analysis and Systems Programming, among others. A list of the courses conducted in 2009-10 is found in annex 5.

In 2009, six staff members were trained as trainers to enable them to provide instruction in common areas such as GCP, GCLP and biosafety, among others.

WORKSHOPS AND SEMINARS

The CISM's scientific and technical personnel participate in national and international conferences and seminars to present the results of studies conducted at the CISM, develop research networks and exchange knowledge and experience with other researchers.

Scientific sessions and journal club activities for the research and technical personnel are organized regularly at the center. These sessions are an essential training component for researchers and technical personnel and a means for sharing knowledge and research results among colleagues.

OTHER TRAINING PROGRAMS

Health Sciences Training Program in Mozambique: Development of competencies and reinforcement of academic capacities of the School of Medicine of the Universidade Eduardo Mondlane

The general objective of the Health Sciences Training Program is to develop and strengthen collaborative ties between the Universidade Eduardo Mondlane and the Universitat de Barcelona (Spain) in the health sciences area, with the support of the Fundació Clínic, the CISM, CRESIB and the "la Caixa" Foundation.

The program is particularly geared to identifying priorities and designing and implementing training activities between the two institutions that will ultimately lead to improved academic and research competencies and capacities of Mozambican health sciences professionals. It is expected that collaboration between the two universities will be strengthened. The program, which is funded by the "La Caixa" Foundation, was launched in June 2008 and completed its first phase in December 2010.

The CISM supports the management of the program in Mozambique, maintains close contact with the Fundació Clínic (the institution that manages the overall program) and participates in some of the training activities. The Community Health, Pharmacology, Pathology and Microbiology and Parasitology departments of the School of Medicine are involved in this program, which includes cross-cutting training activities in research methodologies and the training of trainers.

The following activities were conducted during 2009-10:

- Review of the School of Medicine Public Health Master's Program
- Project management training (12 administrative and research support members of staff)
- PCR training (14 researchers and technicians)
- Internship of post-graduate personnel at the Pathology Department of Hospital Clínic Barcelona by Dr. Cesaltina Lorenzoni as part of her residency in pathology
- Visit to the School of Medicine at Hospital del Mar (Barcelona, Spain) by six faculty members of the Universidade Eduardo Mondlane's School of Medicine
- Training in research methods (13 Universidade Eduardo Mondlane faculty staff, researchers and research assistants)

Training and Resources in Research Ethics Evaluation for Africa (TRREE)

TRREE is a multi-lingual web-based training program and capacity-building initiative on the ethics of research involving humans. It is headed by a consortium of interested persons from northern and southern countries.

The website provides free-of-charge and open access to:

- e-learning: distance-learning program and certification in research ethics evaluation
- e-resources: a participatory website with international, regional and national regulatory and policy resources

TRREE focuses on internationally recognized ethical principles and regulations while promoting co-learning, collaboration and capacity-building amongst partners. It integrates local issues and perspectives from low-and middle-income countries, most notably African countries, that are relevant to all those who must ensure the protection of research participants and who promote the highest ethical standards.

The CISM participates in this project by providing information related to ethical procedures in Mozambique and by translating some of the resources into Portuguese to ensure that these are utilized by lusophone countries.

Lecturing in post-graduate and undergraduate programs at the Universidade Eduardo Mondlane

Some of CISM's research staff lectures at undergraduate and post-graduate programs at the Universidade Eduardo Mondlane. During 2009-10, Dr. Eusébio Macete, Dr. Sónia Machevo, Dr. Khátia Munguambe, Dr. Jahit Sacarlal and Mr. Charfudin Sacoor gave lectures in their areas of expertise.

PhD degrees obtained during 2009-10

Inácio Mandomando, "Salmonella, Shigella and E. Coli epidemiology in Mozambican children", Universitat de Barcelona, Spain (2009)

Jahit Sacarlal, "Clinical development of RTS,S as a vaccine for the prevention of malaria in Mozambican children", Universitat de Barcelona, Spain (2009).

Esperança Sevene, "Availability and safety of drugs for pregnant women in developing countries", Universitat de Barcelona, Spain (2009)

Betuel Sigaúque, "The epidemiology and clinical presentation of invasive bacterial infection among children admitted to a rural hospital in Mozambique: the role of hospital-based surveillance systems of invasive bacterial infections as a tool to define public health priorities and interventions", Universitat de Barcelona, Spain (2009)

Master's degrees completed during 2009-10

Pedro Aide, "Epidemiology and Biostatistics", WITS University, South Africa (2009)

Tacilta Nhampossa "Public Health", Universitat Pompeu Fabra, Spain (2010)

Master's degrees in progress at the end of 2010

Luís Morais, "Biotechnology in Health and Medical Research", Fundação Oswaldo Cruz (Fiocruz), Centro de Pesquisa Gonçalo Moniz, Brasil.

Charfudin Sacoor, "Population Based Field Epidemiology", WITS University, South Africa (2009)

Internships in other institutions within the Training Fellowship program during 2009-10

Betuel Sigaúque, internship at the Hospital Sant Joan de Déu (Barcelona), Spain (2009) and Hospital Central de Maputo (Maputo), Mozambique (2009-10). Through these internships, Dr. Sigaúque specialized in Pediatrics in October 2010.

NEWS

Intermittent Preventive Treatment with sulphadoxine-pyrimethamine proves to be safe and efficient in African infants

On September 17, 2009, The Lancet published the results of a meta-analysis led by a researcher of the CISM and the CRESIB of six studies conducted in Africa on intermittent preventive treatment in infants (IPTi), reporting that close to 30% of clinical malaria episodes could be avoided in African infants using this safe, accessible and simple treatment with sulphadoxine-pyrimethamine (SP). The meta-analysis included the results of a study conducted by the CISM (Macete et al 2006) that was one of the first studies on this malaria control tool.

The studies were conducted through the IPTi Consortium, an international consortium formed by more than 20 institutions in Africa, Europe and the USA, the WHO and UNICEF. IPTi is a malaria prevention strategy that consists of the administration of an antimalarial drug at different times during the first year of life, taking advantage of the Expanded Program for Immunization.

The second edition of the Global Health Lecture

At the second Global Health Lecture of the Manhiça Foundation entitled The Role of Governments in the Development of Research Capacities, which took place on February 22, 2010, in Maputo (Mozambique), speaker Graça Machel argued that human development is fundamental for economic development to take place. Referring specifically to the role of the Mozambican government in the development of research capacities, the speaker argued that the Mozambican state needs to develop policies geared towards the future and to invest more in research so that it does not depend on donations, as this dependence could distort research priorities.

Another issue raised by the speaker was the need for the government to invest in human

Launch of the phase III RTS,S/AS01E.

resource training, as the country has only a small number of researchers. "Incentives to keep the "grey matter" focused on research are an investment that will enable us to regain lost ground," she said.

Graça Machel highlighted that, parallel to education, working conditions must be more enticing and attractive to prevent brain drain. In the speaker's opinion, the government must create policies that provide incentives for businesses to invest in research. "Businesses must be clear about which cutting-edge knowledge is needed for the areas in which they operate, (...) -contractual policies are needed that enforce the production of knowledge," she pointed out.

At the end of her speech, Graça Machel encouraged the government to adopt a long-term vision, drawing up policies and strategies to ensure that all Mozambican citizens can compete on equal conditions with the rest of the world in the context of a global society.

The Manhiça Foundation holds the IV Annual MCTA Meeting

The CISM held the IV Annual Meeting of the INDEPTH Clinical Malaria Trials Alliance (MCTA) from January 18-20, 2010. Professor Fred Binka, Director of the MCTA, commented in his opening speech that the objectives of the meeting were to share ideas and eva-

Ms. Graçha Machel and Dr. Pascoal Mocumbi during the Manhiça Foundation's Global Health Lecture

luate the activities carried out by the MCTA over the last four years, to document the lessons learned, and to analyze ways in which the members of the network can continue to work together to consolidate the project in the coming years.

The representative of the Ministry of Health, Dr. Jorge Tomo, argued that the partnerships between different African countries in the area of health are of vital importance for improving governments' capacities to deal with the principal diseases brought on by poverty.

The CISM has started phase III of the vaccine against malaria

On August 7, 2009, the CISM launched Phase III of the clinical trial to test the RTS,S, vaccine, which is the most likely contender for a malaria vaccine. The inoculations have been administered at the Malavele Health Centre, at the administrative post "3 de Fevereiro", in the Manhiça District. The Phase III trial predicts the inclusion of approximately 16,000 newborn infants and children in sub-Saharan Africa. "This will be the biggest trial in the history of antimalarial vaccine trials in the African context, specifically designed to benefit African children", said Dr. Jahit Sacarlal, Head Researcher of the trial in Mozambique. He extended his gratitude once again to all the participating families and children "without whom this progress would not have been possible", and concluded that "the development of the RTS,S vaccine in Africa has strengthened the research capacities on this continent and increased our knowledge of this disease, which will continue to be crucial even after this particular trial has ended".

The phase III trial will study the safety and efficacy of the vaccine in two groups of children, aged 6 to 12 weeks and 5 to 17 months, in different geographical areas, with different intensities of transmission. The study includes 11 Research Centers in seven African countries, namely, Mozambique, Burkina Faso, Gabon, Ghana, Kenya, Malawi and Tanzania.

Executive Director of the Bill & Melinda Gates Foundation visits the CISM

The Executive Director of the Bill & Melinda Gates Foundation, Jeff Raikes, praised the role of the CISM in the search for new tools to combat the different diseases that are devastating sub-Saharan Africa, with particular emphasis on malaria. Jeff Raikes made this announcement during a visit to the CISM in March 2010, during which he had the opportunity to meet the President of the Manhiça Foundation, Dr. Pascoal Mocumbi, the Spanish Ambassador in Mozambique, Mr. Eduardo López-Busquets, and the Director of the CISM, Dr. Eusébio Macete, to discuss the center's vision regarding its programs for national and international collaboration and partnerships.

ANNEX 1: PERSONNEL

DATA MANAGEMENT DEPARTMENT

Arnaldo Nhabanga
Head of Department

Arsénio Nhacolo
Statistician

Boaventura Cuna
IT assistant

Abílio Almeida
Data clerk

Alberto Junior
Data clerk

Alice Melembe
Data clerk

Armando Matavel
Data clerk

Arminda Nhantumbo
Data clerk

Cardoso Melembe
Data clerk

Daniela Alberto
Data clerk

Edna António
Data clerk

Gonsalves Massango
Data clerk

Helena Coana
Data clerk

Helena Chavana
Data clerk

Isabel Matlhombe
Data clerk

Isabel Tsandzana
Data clerk

Laura Chijamela
Data clerk

Lee Fonseca
Data clerk

Madalena Mutevue
Data clerk

Narciso Ngalambe
Data clerk

Neli Moiana
Data clerk

Nicolau Massingue
Data clerk

Orlando Tamele
Data clerk

Romão Massango
Data clerk

Sónia Matimele
Data clerk

Almirante Mujovo
Warehouse manager

Joaquim Sitoe
Warehouse manager

INFORMATION TECHNOLOGIES

Messias Mandua
IT manager

Sérgio Tamele
IT assistant

Carlos Correia
Help Desk

LABORATORY DEPARTMENT

Hélder M. Bulo
Laboratory manager (since Feb 2009)

Luis Morais
Laboratory manager (until Feb 2009)

Óscar Fraile
Technical supervisor

Arlindo Nhamuave
Clinical analysis manager

Elias Matusse
Immunology manager

Flávio Faife
Microbiology manager

Zita Sidumo
Immunology manager

Delfino Vubil
Molecular biology manager

Dirce Moreno
Quality assurance manager

Augusto Bacar
Quality management assistant

Bernardo Vilanculo
Biosafety manager

Acrisio Joaquim
Laboratory technician

Alberto Chaguala
Laboratory technician

Ana Manhiça
Laboratory technician

Bendita Zavale
Laboratory technician

Chenjerai Jairoce
Laboratory technician

Crisóstomo Fonseca
Laboratory technician

Esperança Lázaro
Laboratory technician

Eugénio Mussa
Laboratory technician

Janeta Vilanculo
Laboratory technician

José Gomes
Laboratory technician

Laura Cumbe
Laboratory technician

Lázaro Quimice
Laboratory technician

Márcia Ubisse
Laboratory technician

Mariano Sitaúbe
Laboratory technician

Monalisa Cumbe
Laboratory technician

Nelito Jose
Laboratory technician

Pedro Dimande
Laboratory technician

Salvador Atibo
Laboratory technician

Samira Sirage
Laboratory technician

Victória Zita
Laboratory technician

Alfredo Zunguene
Microscopist

Alzenda Bata
Microscopist

Américo Matusse
Microscopist

Ana Dimande
Microscopist

António Simango
Microscopist

Augusta Tembe
Microscopist

Carlinda Tsucana
Microscopist

Cecília Zita
Microscopist

Cidália da Macuacua
Microscopist

Génia Chimene
Microscopist

Guerrelina Ribeiro
Microscopist

Guilherme Sucamer
Microscopist

Austrino Manhica
Recepcionist

Carmila Comé
Recepcionist

Ivone Munde
Recepcionist

Rita Bambo
Recepcionist

Amândio Chilengue
Warehouse manager

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Pau Balcells
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Jacinto Chilengue
Head of Finance (until Oct 2010)

Marina Espriu
Controller

Brito Gove
Senior accountant

Rafael Zunguze
Senior accountant

Abel Massingue
Accountant

Mario Djedje
Accountant

Mario Gomes
Accountant

Sidónio Zualo
Assistant accountant

Emilio Dava
Project manager

Gil Oquisso
Project manager

Sónia Marrunguja
Project assistant

GENERAL SERVICES

Delino Nhalungo
Head of General Services

Casquinha Armando
HR manager (since Oct 2010)

Abel Detepo
HR manager (until Sept 2010)

Rosária Paulino
HR assistant

Tajú Noor
HR assistant

Carmina Camal
Logistics

Constância Uamusse
Logistics

Fátima Adamo
Logistics

Humberto Poio
Logistics

Araújo Cuamba
Maintenance

Celso Matola
Maintenance

Fernando Dimande
Maintenance

Hermínio Nhacundela
Maintenance

Nelson Mabote
Maintenance

Nelson Mabote
Maintenance

Pedro Cossa
Maintenance

Raimundo Miambo
Maintenance

Silvestre Zita
Maintenance

Fausa Mandlate
Driver

Francisco Sambo
Driver

Germano Matsinhe
Driver

Joaquim Cossa
Driver

José Nhabanga
Driver

Júlio Mpfumo
Driver

Rafael Manhiça
Driver

Sebastião Ouana
Driver

Frederico Chongo
Mechanic

Sérgio Dimande
Mechanic

Silvestre Dimande
Mechanic

Amone Massango
Gardener

Alberto Inguane
Security

Alberto Macumbe
Security

Américo Dimande
Security

André Mandlate
Security

António Mazanalo
Security

Armando Lipangue
Security

Armando J. Melembe
Security

Celso Mutisse
Security

Constantino Maússe
Security

Ernesto Ubisse
Security

Fernando Duna
Security

Francisco Nhabanga
Security

Jacinto Macuácua
Security

Joaquim Machava
Security

Jorge Jeco
Security

Juca Bulande
Security

Manuel Malhongo
Security

Maria Utui
Security

Mário Timana
Security

Moisés Mucachua
Security

Pereira Sitoe
Security

Rudes Mandlate
Security

Samuel Macuácua
Security

Sebastião Muchanga
Security

Sérgio Sitoe
Security

Sozinho Tivana
Security

Xavier Langa
Security

Adélia B. Nhassengo
Housekeeping

António V. Chuva
Housekeeping

Delfina A. Macie
Housekeeping

Ester M. Mbeve
Housekeeping

Gertrudes Maluvele
Housekeeping

Guilhermina Buque
Housekeeping

Latifa Momade
Housekeeping

Nirma Nhatumbo
Housekeeping

Rabeca Langa
Housekeeping

DEMOGRAPHY DEPARTMENT

Charfudin Sacoor
Head of Department (since Dec 2009)

Ariel Nhacolo
Head of Department (until Nov 2009)

Agibo Bapú
Supervisor

Alfredo Sitoe
Supervisor

Antonio Macamo
Supervisor

Salomão Mucocana
Supervisor

Samuel Simbine
Supervisor

Sérgio Bento
Supervisor

Arsénia Mbeve
Data clerk

Farida Omar
Data clerk

Fernando Sitoe
Data clerk

Gina Firmino
Data clerk

Isabel Mabjaia
Data clerk

Julieta Massango
Data clerk

Lina Timana
Data clerk

Lucinda Soto
Data clerk

Sandra Sitoe
Data clerk

Atanásio Chirinze
Field worker

Benedito Jeco
Field worker

Benício Chongo
Field worker

Daúde Chitará
Field worker

Felisberto Mondlane
Field worker

Félix Timana
Field worker

Hilário Tsandzana
Field worker

Joaquim Machava
Field worker

Júlio Chavana
Field worker

Júlio Mulhanga
Field worker

Lisete Maolela
Field worker

Maria Sambo
Field worker

Moniz Simango
Field worker

Narciso Macamo
Field worker

Noa Mulhanga
Field worker

Paixão Chilengue
Field worker

Sérgio Xerindza
Field worker

Silvestre G. José
Field worker

Simão Gomes
Field worker

Violeta Mucocana
Field worker

Albino Chilaúle
Warehouse manager

CLINICAL SERVICES DEPARTMENT

Sónia Machevo
Medical doctor, Head of Department (from June 2009 to Feb 2010)

Sozinho Acácio
Medical doctor, Head of Department (since March 2010)

Tacilta Nhampossa
Medical doctor, Head of Department (until june 2009)

Humberto Mucasse
Coordinator

Betuel Sigaúque
Medical doctor, postdoctoral researcher

Cristian Casademont
Medical doctor

Jahit Sacarlal
Medical doctor, postdoctoral researcher

Jeremias Pita
Medical doctor

Kizito Gondo
Medical doctor, training fellow

Miguel Lanaspa
Medical doctor

Pedro Aide
Medical doctor, training fellow

Arcénio Muianga
Medical officer

Armindo Lumbelane
Medical officer

Filipe Arone
Medical officer

Madalena Ripinga
Medical officer

Manuel Muamede
Medical officer

Teófilo Luis
Medical officer

Adelina Malembe
Medical assistant

Ana Matavel
Medical assistant

Armando Tsucana
Medical assistant

Berta Juga
Medical assistant

Domingos Mazive
Medical assistant

Eduardo Sande
Medical assistant

Elsa Banze
Medical assistant

Ester Matsinhe
Medical assistant

Francisco Marengue
Medical assistant

Horácio Chaleca
Medical assistant

Jorge Uqueio
Medical assistant

Martinho Charles
Medical assistant

Palmira Migi
Medical assistant

Sérgio Roque
Medical assistant

Ângela Lugenda
Nurse

Elsa Matavel
Nurse

Esmeralda Xerinda
Nurse

Felícia Lopes
Nurse

Fortunato Romao
Nurse

Jaime Lumbela
Nurse

Joana Manhiça
Nurse

Jorcelina Rungo
Nurse

Leonild Rungo
Nurse

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Nurse

Maria Almeida
Nurse

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Nurse

Rahela Numaio
Nurse

Roque Vilanculo
Nurse

Sérgio Juliano
Nurse

Esselina Machava
Pharmacist

Esphiwa Jeco
Counsellor

Eugénia Bilana
Counsellor

Lucinda Xerinda
Counsellor

Albertina Manhica
Field worker

Alice Chitlhango
Field worker

Belinda Pelembe
Field worker

Gil Paulino
Field worker

Glória Zucula
Field worker

Ivete Chemane
Field worker

Eugrância Howana
Recepcionist

Irene Nhantumbo
Recepcionist

Isabel Nguenha
Recepcionist

Maria Siúta
Recepcionist

Maria Xirinda
Recepcionist

Teresa Chilaúle
Recepcionist

Celeste Chambal
Housekeeping

Emilia Manhica
Housekeeping

Inácia Mazutulejo
Housekeeping

Luisa Mutevule
Housekeeping

Rosa Mboa
Housekeeping

Tânia Nhatumbo
Housekeeping

TRAINING & COMMUNICATION

Teresa Machai
Head of Training and Communications

Tânia Machonisse
Communications assistant

RESEARCH AREA

Caterina Guinovart
Postdoctoral researcher

Esperança Sevene
Postdoctoral researcher

Inácio Mandomando
Postdoctoral researcher

Khátia Munguambe
Postdoctoral researcher

Nilsa de Deus
Postdoctoral researcher

Augusto Nhabomba
Training Fellow

Diana Quelhas
Training Fellow

Nélia Manaca
Training Fellow

Helena Boene
Researcher

Carlos Bavo
Researcher

Guilhermina Dinis
Research assistant

Carolina Mindu
Research assistant

Rui Guilaze
Research assistant

Lina Fiosse
Research assistant

Elpídia Pedro
Project assistant

Francisco Gomes
Field worker

Antonela A. M. Fonseca
Data clerk

Humberto Mucasse
Data clerk

Silvestre Rodrigues Cutana
Data clerk

Alves Ginja
Field worker

Cármen Correia
Field worker

Emília Matusse
Field worker

Justino Mabui
Field worker

Márcia Faife
Field worker

Marta Macamo
Field worker

Simão Saiuane
Field worker

Zefanias Nhamirre
Field worker

Celina Lucas
Nurse

Fátima Chande
Nurse

Hamina Carimo
Nurse

Jesuíta Buque
Nurse

Lídia Laço
Nurse

Maria Madalena
Nurse

Silvia Chicuecue
Project assistant

Sofia Manjate
Project manager

Atanásio Chirindza
Supervisor

Bento Nhancale
Project assistant

Anifa Mahomed Vala
Project Manager

Romão Banze
Research assistant

DIRECTOR'S OFFICE

Eusebio Macete
Director

Sheila Machevo
Executive assistant

Enric Jané
Special advisor

ANNEX 2: COLLABORATING INSTITUTIONS

Our activities would not be possible without the collaboration of the following institutions and organizations:

Aeras Global TB Vaccine Foundation (USA)

Africa Centre for Health and Population Studies (South Africa)

CDC/Kenya Medical Research Institute – KEMRI (Kenya)

Center for Vaccine Development, University of Maryland School of Medicine (USA)

Centers for Disease Control and Prevention – CDC (USA)

Centre de Recerca en Salut Internacional de Barcelona – CRESIB (Spain)

Centre for Clinical Research, Walter Reed Project, Kenya Medical Research Institute, Kisumu (Kenya)

Centre for Poverty-Related Communicable Diseases (Netherlands)

Centre Hospitalier Universitaire Vaudois, Division of Immunology and Allergy (Switzerland)

Centro de Investigação e Treino em Saúde de Chókwé (Mozambique)

Centro de Investigação em Saúde de Angola – CISA (Angola)

Contract Laboratory Services – CLS (South Africa)

Department of Genetics, Biology and Biochemistry, Università di Torino (Italy)

Department of Immunology and Infectious Disease, Harvard School of Public Health (USA)

Deutsche Forschungsgemeinschaft –DFG (Germany)

Division of Infectious Diseases, Department of Medicine, University of Freiburg (Germany)

EuroVacc Foundation (Switzerland)

Fraunhofer Institut for Biomedical Engineering – IBMT (Germany)

Fundação para o Desenvolvimento da Comunidade (Mozambique)

GlaxoSmithKline Biologicals GSK (Belgium)

HIV Prevention Research Unit, Medical Research Council (South Africa)

Hospital Central de Maputo (Mozambique)

Hospital Clínic de Barcelona (Spain)

Ifakara Health Institute (Tanzania)

Immunogenetics Research Group University of Western Australia (Australia)

Imperial College London (UK)

International Network for the Demographic Evaluation of Populations and Their Health in Developing Countries – INDEPTH (Ghana)

Infectious Disease Initiative, Broad Institute, (USA)

Institut de Recherche en Sciences de la Santé (IRSS)/Centre Muraz (Burkina Faso)

Institute for Medical Microbiology and Hygiene, Universität Regensburg (Germany)

Institute of Evolutionary Biology (Spain)

Institute of Hygiene, University of Münster (Germany)

Institute of Medical Microbiology and Hygiene, University of Saarland (Germany)

Institute of Medical Microbiology, University of Münster Domagkstr (Germany)

Institute of Tropical Medicine Antwerp (Belgium)

Institute of Tropical Medicine, University of Tübingen Medical School (Germany)

Instituto Nacional de Estadística (Mozambique)

Instituto Superior de Ciências de Saúde (Mozambique)

International Center for Genetic Engineering and Biotechnology – ICGEB (India)

International Partnership for Microbicides (USA)

Johns Hopkins Bloomberg School of Public Health (USA)

Kintampo Health Research Centre & Noguchi Memorial Institute for Medical Research (Ghana)

KNCV Tuberculosis Foundation (Netherlands)

Makerere University (Uganda)

Malaria Clinical Trials Alliance – MCTA (Ghana)

Mbeya Medical Research Programme (Tanzania)

Medical Research Council – MRC (UK)

Medical Research Unit Albert Schweitzer Hospital (Gabon)

Ministério da Saúde (Mozambique)

Ministério de Ciência e Tecnologia (Mozambique)

MRC/Uganda Virus Research Institute Programme on AIDS (Uganda)

Novartis Pharma (Switzerland)

Nuffield Department of Clinical Laboratory Sciences University of Oxford (UK)

PATH Malaria Vaccine Initiative – MVI (USA)

PneumoADIP (USA)

Pneumonia Accelerated Development and Reproductive Health and HIV Research Unit, Chris Hani Baragwanath Hospital (RSA)

Reproductive Health Research Unit, University of Wittswatersrand (South Africa)

Sanofi Pasteur (France)

Sigma-Tau Pharmaceuticals (Italy)

South African Tuberculosis Vaccine Initiative – SATVI (South Africa)

Swiss Tropical and Public Health Institute– STPHI (Switzerland)

The Walter and Eliza Hall Institute of Medical Research (Australia)

Trials of Excellence in Southern Africa –TESA (South Africa)

Universidade Católica de Moçambique (Mozambique)

Universidade Eduardo Mondlane (Mozambique)

University Teaching Hospital (Zambia)

ANNEX 3: FUNDERS

The CISM thanks the financial support by the following institutions and agencies:

AERAS Global TB Vaccine Foundation

Africa Viva Fundación

Agència Catalana de Cooperació al Desenvolupament (ACCD)

Agencia Española de Cooperación Internacional para el Desarrollo (AECID)

Agencia de Cooperación Internacional de Las Illes Balears (ACIIB)

Bill & Melinda Gates Foundation

European and Developing Countries Clinical Trials Partnership (EDCTP)

Fondo de Investigación Sanitaria (FIS), Instituto de Salud Carlos III

Fundació "la Caixa"

Generalitat de Catalunya

GlaxoSmithKline Biologicals

International Union Against Tuberculosis and Lung Disease

Johns Hopkins Bloomberg School of Public Health

Jomaa Pharma GmbH

London School of Hygiene and Tropical Medicine

Malaria Clinical Trials Alliance (MCTA)

World Health Organization

Path Malaria Vaccine Initiative (MVI)

Pathfinder International

PneumoADIP

The Hib Initiative

European Union

ANNEX 4: PUBLICATIONS

1. Aide, P., J. J. Aponte, M. Renom, T. Nhampossa, J. Sacarlal, I. Mandomando, Q. Bassat, M. N. Manaca, A. Leach, M. Lievens, J. Vekemans, M. C. Dubois, C. Loucq, W. R. Ballou, J. Cohen, and P. L. Alonso. "Safety, Immunogenicity and Duration of Protection of the RTS,S/AS02(D) Malaria Vaccine: One Year Follow-Up of a Randomized Controlled Phase I/IIb Trial." *PLoS One*. 5.11 (2010): e13838.

2. Aponte, J. J., D. Schellenberg, A. Egan, A. Breckenridge, I. Carneiro, J. Critchley, I. Danquah, A. Dodoo, R. Kobbe, B. Lell, J. May, Z. Premji, S. Sanz, E. Sevene, R. Soulaymani-Becheikh, P. Winstanley, S. Anemana S. Adjei, D. Chandramohan, S. Issifou, F. Mockenhaupt, S. Owusu-Agyei, B. Greenwood, M. Grobusch, P. G. Kremsner, E. Macete, H. Mshinda, R. D. Newman, L. Slutsker, M. Tanner, P. Alonso, and C. Menendez. "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*. 9 (2009): 61258–7.

3. Barbosa, A., D. Naniche, J. J. Aponte, M. N. Manaca, I. Mandomando, P. Aide, J. Sacarlal, M. Renom, S. Lafuente, W. R. Ballou, and P. L. Alonso. "Plasmodium falciparum-specific cellular immune responses after immunization with the RTS,S/AS02D candidate malaria vaccine in infants living in an area of high endemicity in Mozambique." *Infect Immun.* 77.10 (2009): 4502–4509.

4. Bassat, Q., C. Guinovart, B. Sigauque, I. Mandomando, P. Aide, J. Sacarlal, T. Nhampossa, A. Bardaji, L. Morais, S. Machevo, E. Letang, E. Macete, J. J. Aponte, A. Roca, C. Menendez, and P. L. Alonso. "Severe malaria and concomitant bacteraemia in children admitted to a rural Mozambican hospital." *Trop Med Int Health.* 14.9 (2009): 1011–1019.

5. Bassat, Q., M. Mulenga, H. Tinto, P. Piola, S. Borrmann, C. Menendez, M. Nambozi, I. Valea, C. Nabasumba, P. Sasi, A. Bacchieri, M. Corsi, D. Ubben, A. Talisuna, and D'Alessandro U. "Dihydroartemisinin-Piperaquine and Artemether-Lumefantrine for Treating Uncomplicated Malaria in African Children: A Randomised, Non-Inferiority Trial." *PLoS One*. 4.11 (2009): e7871.

6. Borrmann, S., W. M. Sallas, S. Machevo, R. Gonzalez, A. Bjorkman, A. Martensson, M. Hamel, E. Juma, J. Peshu, B. Ogutu, A. Djimde, D'Alessandro U, A. C. Marrast, G. Lefevre, and S. E. Kern. "The effect of food consumption on lumefantrine bioavailability in African children receiving artemether-lumefantrine crushed or dispersible tablets (Coartem) for acute uncomplicated Plasmodium falciparum malaria." *Trop Med Int Health.* 15.4 (2010): 434–441.

7. Brown, G. V., V. S. Moorthy, Z. Reed, K. Mendis, M. Arevalo-Herrera, and P. Alonso. "Priorities in research and development of vaccines against Plasmodium vivax malaria." *Vaccine.* 27.52 (2009): 7228–7235.

8. Chase, C., E. Sicuri, C. Sacoor, D. Nhalungo, A. Nhacolo, P. L. Alonso, and C. Menendez. "Determinants of household demand for bed nets in a rural area of southern Mozambique." *Malar J.* 8.1 (2009): 132.

9. Conteh, L., E. Sicuri, F. Manzi, G. Hutton, B. Obonyo, F. Tediosi, P. Biao, P. Masika, F. Matovu, P. Otieno, R. D. Gosling, M. Hamel, F. O. Odhiambo, M. P. Grobusch, P. G. Kremsner, D. Chandramohan, J. J. Aponte, A. Egan, D. Schellenberg, E. Macete, L. Slutsker, R. D. Newman, P. Alonso, C. Menendez, and M. Tanner. "The cost-effectiveness of intermittent preventive treatment for malaria in infants in Sub-Saharan Africa." *PLoS One*. 5.6 (2010): e10313.

10. de Sanjose, S., W. G. Quint, L. Alemany, D. T. Geraets, J. E. Klaustermeier, B. Lloveras, S. Tous, A. Felix, L. E. Bravo, H. R. Shin, C. S. Vallejos, P. A. de Ruiz, M. A. Lima, N. Guimera, O. Clavero, M. Alejo, A. Llombart-Bosch, C. Cheng-Yang, S. A. Tatti, E. Kasamatsu, E. Iljazovic, M. Odida, R. Prado, M. Seoud, M. Grce, A. Usubutun, A. Jain, G. A. Suarez, L. E. Lombardi, A. Banjo, C. Menendez, E. J. Domingo, J. Velasco, A. Nessa, S. C. Chichareon, Y. L. Qiao, E. Lerma, S. M. Garland, T. Sasagawa, A. Ferrera, D. Hammouda, L. Mariani, A. Pelayo, I. Steiner, E. Oliva, C. J. Meijer, W. F. Al-Jassar, E. Cruz, T. C. Wright, A. Puras, C. L. Llave, M. Tzardi, T. Agorastos, V. Garcia-Barriola, C. Clavel, J. Ordi, M. Andujar, X. Castellsague, G. I. Sanchez, A. M. Nowakowski, J. Bornstein, N. Munoz, and F. X. Bosch. "Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study." *Lancet Oncol.* 11.11 (2010): 1048–1056.

11. Diez-Padrisa, N., Q. Bassat, S. Machevo, L. Quinto, L. Morais, T. Nhampossa, C. O'Callaghan-Gordo, A. Torres, P. L. Alonso, and A. Roca. "Procalcitonin and C-Reactive Protein for Invasive Bacterial Pneumonia Diagnosis among Children in Mozambique, a Malaria-Endemic Area." *PLoS One*. 5.10 (2010): e13226.

12. Grobusch, M. P., J. J. Gabor, J. J. Aponte, N. G. Schwarz, M. Poetschke, J. Doernemann, K. Schuster, K. B. Koester, K. Profanter, L. B. Borchert, F. Kurth, P. Pongratz, S. Issifou, B. Lell, and P. G. Kremsner. "No rebound of morbidity following intermittent preventive sulfadoxine-pyrimethamine treatment of malaria in infants in Gabon." *J Infect Dis.* 200.11 (2009): 1658–1661.

13. Guinovart, C., J. J. Aponte, J. Sacarlal, P. Aide, A. Leach, Q. Bassat, E. Macete, C. Dobaño, M. Lievens, C. Loucq, W. R. Ballou, J. Cohen, and P. L. Alonso. "Insights into long-lasting protection induced by RTS,S/AS02A malaria vaccine: further results from a phase IIb trial in Mozambican children." *PLoS One*. 4.4 (2009): e5165.

14. Hutton, G., D. Schellenberg, F. Tediosi, E. Macete, E. Kahigwa, B. Sigauque, X. Mas, M. Trapero, M. Tanner, A. Trilla, P. Alonso, and C. Menendez. "Cost-effectiveness of malaria intermittent preventive treatment in infants (IPTi) in Mozambique and the United Republic of Tanzania." *Bull World Health Organ.* 87.2 (2009): 123–129.

15. Letang, E., J. M. Almeida, J. M. Miro, E. Ayala, I. E. White, C. Carrilho, R. Bastos, T. Nhampossa, C. Menendez, T. B. Campbell, P. L. Alonso, and D. Naniche. "Predictors of immune reconstitution inflammatory syndrome-associated with kaposi sarcoma in mozambique: a prospective study." *J Acquir Immune Defic Syndr.* 53.5 (2010): 589–597.

16. Mabunda, S., J. J. Aponte, A. Tiago, and P. Alonso. "A country-wide malaria survey in Mozambique. II. Malaria attributable proportion of fever and establishment of malaria case definition in children across different epidemiological settings." *Malar J.* 8 (2009): 74.

17. Manco, L., P. Machado, D. Lopes, F. Nogueira, V. E. Do Rosario, P. L. Alonso, L. Varandas, M. D. J. Trovoada, A. Amorim, and A. P. Arez. "Analysis of TPI gene promoter variation in three sub-Saharan Africa population samples." *Am J Hum Biol.* 21.1 (2009): 118–120.

18. Mandomando, I., D. Jaintilal, M. J. Pons, X. Valles, M. Espasa, L. Mensa, B. Sigauque, S. Sanz, J. Sacarlal, E. Macete, F. Abacassamo, P. L. Alonso, and J. Ruiz. "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.* 53.6 (2009): 2450–2454.

19. Mandomando, I., E. Macete, B. Sigauque, L. Morais, L. Quinto, J. Sacarlal, M. Espasa, X. Valles, Q. Bassat, P. Aide, T. Nhampossa, S. Machevo, J. Ruiz, A. Nhacolo, C. Menendez, K. L. Kotloff, A. Roca, M. M. Levine, and P. L. Alonso. "Invasive non-typhoidal Salmonella in Mozambican children." *Trop Med Int Health.* 14.12 (2009): 1467–1474.

20. Mandomando, I., B. Sigauque, L. Morais, M. Espasa, X. Valles, J. Sacarlal, E. Macete, P. Aide, L. Quinto, T. Nhampossa, S. Machevo, Q. Bassat, C. Menendez, J. Ruiz, A. Roca, and P. L. Alonso. "Antimicrobial Drug Resistance Trends of Bacteremia Isolates in a Rural Hospital in Southern Mozambique." *Am J Trop Med Hyg.* 83.1 (2010): 152–157.

21. Mayor, A., E. Rovira-Vallbona, A. Srivastava, S. K. Sharma, S. S. Pati, L. Puyol, L. Quinto, Q. Bassat, S. Machevo, I. Mandomando, V. S. Chauhan, P. L. Alonso, and C. E. Chitnis. "Functional and immunological characterization of a Duffy binding-like alpha domain from Plasmodium falciparum erythrocyte membrane protein 1 that mediates rosetting." *Infect Immun.* 77.9 (2009): 3857–3863.

22. Mayor, A., E. Serra-Casas, A. Bardaji, S. Sanz, L. Puyol, P. Cistero, B. Sigauque, I. Mandomando, J. J. Aponte, P. L. Alonso, and C. Menendez. "Sub-microscopic infections and long-term recrudescence of Plasmodium falciparum in Mozambican pregnant women." *Malar J.* 8 (2009): 9.

23. Menendez, C., A. Bardaji, B. Sigauque, S. Sanz, J. J. Aponte, S. Mabunda, and P. L. Alonso. "Malaria prevention with IPTp during pregnancy reduces neonatal mortality." *PLoS One*. 5.2 (2010): e9438.

24. Menendez, C., X. Castellsague, M. Renom, J. Sacarlal, L. Quinto, B. Lloveras, J. Klaustermeier, J. R. Kornegay, B. Sigauque, F. X. Bosch, and P. L. Alonso. "Prevalence and risk factors of sexually transmitted infections and cervical neoplasia in women from a rural area of southern Mozambique." *Infect Dis Obstet Gynecol.* 2010 (2010).

25. Montgomery, C. M., K. Munguambe, and R. Pool. "Group-based citizenship in the acceptance of indoor residual spraying (IRS) for malaria control in Mozambique." *Soc Sci Med.* 70.10 (2010): 1648–55.

26. Nair, H., D. J. Nokes, B. D. Gessner, M. Dherani, S. A. Madhi, R. J. Singleton, K. L. O'Brien, A. Roca, P. F. Wright, N. Bruce, A. Chandran, E. Theodoratou, A. Sutanto, E. R. Sedyaningsih, M. Ngama, P. K. Munywoki, C. Kartasasmita, E. A. F. Simoes, I. Rudan, M. W. Weber, and H. Campbell. "Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis." *Lancet*. 375.9725 (2010): 1545–1555.

27. Naniche, D., A. Bardaji, M. Lahuerta, A. Berenguera, I. Mandomando, S. Sanz, J. J. Aponte, B. Sigauque, P. L. Alonso, and C. Menendez. "Impact of maternal human immunodeficiency virus infection on birth outcomes and infant survival in rural mozambique." *Am J Trop Med Hyg.* 80.5 (2009): 870–876.

28. Ordi, J., M. R. Ismail, C. Carrilho, C. Romagosa, N. Osman, F. Machungo, J. A. Bombi, J. Balasch, P. L. Alonso, and C. Menendez. "Clinico-pathological discrepancies in the diagnosis of causes of maternal death in sub-Saharan Africa: retrospective analysis." *PLoS Med.* 6.2 (2009): e1000036.

29. Plowe, C. V., P. Alonso, and S. L. Hoffman. "The potential role of vaccines in the elimination of falciparum malaria and the eventual eradication of malaria." *J Infect Dis.* 200.11 (2009): 1646–1649.

30. Roca, A., Q. Bassat, L. Morais, S. Machevo, B. Sigauque, C. O'Callaghan, T. Nhamposa, E. Letang, I. Mandomando, D. Nhalungo, L. Quinto, and P. Alonso. "Surveillance of acute bacterial meningitis among children admitted to a district hospital in rural Mozambique." *Clin Infect Dis.* 48 Suppl 2 (2009): S172–80.

31. Roca, A., B. Sigauque, L. Quinto, L. Morais, A. Berenguera, M. Corachan, J. L. Ribo, D. Naniche, E. Bassat, C. Sacoor, D. Nhalungo, E. Macete, A. Schuchat, M. Soriano-Gabarro, B. Flannery, and P. L. Alonso. "Estimating the vaccine-preventable burden of hospitalized pneumonia among young Mozambican children." *Vaccine.* 28.30 (2010): 4851–4857.

32. Sacarlal, J., P. Aide, J. J. Aponte, M. Renom, A. Leach, I. Mandomando, M. Lievens, Q. Bassat, S. Lafuente, E. Macete, J. Vekemans, C. Guinovart, B. Sigauque, M. Sillman, J. Milman, M. C. Dubois, M. A. Demoitie, J. Thonnard, C. Menendez, W. R. Ballou, J. Cohen, and P. L. Alonso. "Long-Term Safety and Efficacy of the RTS,S/AS02A Malaria Vaccine in Mozambican Children." *J Infect Dis.* 200.3 (2009): 329–336.

33. Sacarlal, J., A. Q. Nhacolo, B. Sigauque, D. A. Nhalungo, F. Abacassamo, C. N. Sacoor, P. Aide, S. Machevo, T. Nhampossa, E. V. Macete, Q. Bassat, C. David, A. Bardaji, E. Letang, F. Saute, J. J. Aponte, R. Thompson, and P. L. Alonso. "A 10 year study of the cause of death in children under 15 years in Manhica, Mozambique." *BMC Public Health.* 9 (2009): 67.

34. Serna-Bolea, C., J. Munoz, J. M. Almeida, A. Nhacolo, E. Letang, T. Nhampossa, E. Ferreira, P. Alonso, and D. Naniche. "High prevalence of symptomatic acute HIV infection in an outpatient ward in southern Mozambique: identification and follow-up." *Aids*. 24.4 (2010): 603–608.

35. Serra-Casas, E., C. Menendez, A. Bardaji, L. Quinto, C. Dobaño, B. Sigauque, A. Jimenez, I. Mandomando, V. S. Chauhan, C. E. Chitnis, P. L. Alonso, and A. Mayor. "The Effect of Intermittent Preventive Treatment during Pregnancy on Malarial Antibodies Depends on HIV Status and Is Not Associated with Poor Delivery Outcomes." *J Infect Dis.* 201.1 (2010): 123–131.

36. Sevene, E., R. Gonzalez, and C. Menendez. *Current knowledge and challenges of antimalarial drugs for treatment and prevention in pregnancy.* Vol. 11., 2010.

37. Sicuri, E., A. Bardaji, T. Nhampossa, M. Maixenchs, A. Nhacolo, D. Nhalungo, P. L. Alonso, and C. Menendez. "Cost-effectiveness of intermittent preventive treatment of malaria in pregnancy in southern mozambique." *PLoS One*. 5.10 (2010): e13407.

38. Sigauque, B., A. Roca, Q. Bassat, L. Morais, L. Quinto, A. Berenguera, S. Machevo, A. Bardaji, M. Corachan, J. Ribo, C. Menendez, A. Schuchat, B. Flannery, M. Soriano-Gabarro, and P. L. Alonso. "Severe Pneumonia in Mozambican Young Children: Clinical and Radiological Characteristics and Risk Factors." *J Trop Pediatr.* 55.6 (2009): 379–387.

39. Sigauque, B., A. Roca, I. Mandomando, L. Morais, L. Quinto, J. Sacarlal, E. Macete, T. Nhamposa, S. Machevo, P. Aide, Q. Bassat, A. Bardaji, D. Nhalungo, M. Soriano-Gabarro, B. Flannery, C. Menendez, M. M. Levine, and P. L. Alonso. "Community-Acquired Bacteremia Among Children Admitted to a Rural Hospital in Mozambique." *Pediatr Infect Dis J.* 28.2 (2009): 108–113.

40. Sikora, M., A. Ferrer-Admetlla, H. Laayouni, C. Menendez, A. Mayor, A. Bardaji, B. Sigauque, I. Mandomando, P. L. Alonso, J. Bertranpetit, and F. Casals. "A variant in the gene FUT9 is associated with susceptibility to placental malaria infection." *Hum Mol Genet.* 18.16 (2009): 3136–3144.

41. Valles, X., A. Roca, F. Lozano, L. Morais, B. Suarez, F. Casals, I. Mandomando, B. Sigauque, D. Nhalungo, C. Esquinas, L. Quinto, P. L. Alonso, and A. Torres. "Serotype-specific pneumococcal disease may be influenced by mannose-binding lectin deficiency." *Eur Respir J.* 36.4 (2010): 856–863.

42. Valles, X., M. - R. Sarrias, F. Casals, M. Farnos, R. Piner, B. Suarez, L. Morais, I. Mandomando, B. Sigauque, A. Roca, P. L. Alonso, A. Torres, N. M. Thielens, and F. Lozano. "Genetic and structural analysis of MBL2 and MASP2 polymorphisms in south-eastern African children." *Tissue Antigens.* 74.4 (2009): 298–307.

43. Wells, T. N., P. L. Alonso, and W. E. Gutteridge. *New medicines to improve control and contribute to the eradication of malaria.* Vol. 8., 2009.

ANNEX 5: COURSES, TRAINING SESSIONS AND WORKSHOPS

Date	Course, training session, workshop	Place
February 2009	Microbiology diagnosis	Maputo
March 2009	Quality assurance	CISM
March 2009	Vaginal infection diagnosis	National Laboratory Services
March 2009	Quality control and biosafety	Maputo
March 2009	RDE&SBIR&eTDF	CISM
April 2009	Laboratory techniques (tuberculosis)	India
April 2009	Introduction to GCP	Online
April-July 2009	Pre-intermediate English course	British Council, Maputo
May 2009	Flow cytometry	CISM
June 2009	GCP	CISM
June 2009	MiPPAD safety training	Maputo
June 2009	MiPPAD train the trainer	Maputo
June 2009	Data management	Maputo
July 2009	Quality assurance	Uganda
July-September 2009	Pre-intermediate English course	CISM
July-September 2009	Intermediate English course	CISM
July-October 2009	Pre-intermediate English course	British Council, Maputo
July 2009	Quality management	Uganda
August 2009	Research methods	South Africa
August 2009	Liquid nitrogen and cryopreserved materials manipulation	CISM
September 2009	Clinical trial management	Maputo
September 2009	Operational research	Maputo
October 2009	Intermediate English course	British Council, Maputo
November 2009	TST reading	CISM
December 2009	GCP	CISM
December 2009	Microsoft Access	Maputo
January-February 2010	Servolab management	Berlin, Germany
January-March 2010	Introduction to GCP	Malawi
March 2010	Introduction to clinical research	Malawi
March 2010	Advanced GCP	Kenya
March 2010	Clinical trial management	Kenya
April 2010	Project Management	School of Medicine, Maputo
May 2010	GCP	CISM
May 2010	Financial management	Cape Town
May 2010	Financial reporting	Maputo
June 2010	Introduction to GCP	Maputo
June 2010	Research ethics	Online
June 2010	Research ethics	Online
June 2010	GCLP	CISM
June 2010	Biosafety	CISM
June 2010	Train the trainers	CISM
June 2010	PHC management	Maputo
June-July 2010	Electronic mobile data capture	Norway
July 2010	GCP	CISM
September 2010	Research ethics	CISM
September 2010	Clinical trial management	Malawi
October 2010	GCP	CISM
October 2010	Project Management	Maputo
October 2010	BCG vaccine handling and administration	CISM
November 2010	PHC management	Maputo
December 2010	Introduction to GCP	Online

Fundação Manhiça
Rua 12 Manhiça
Mozambique
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