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# Proceedings of the Conference on Malaria in Africa

## Practical Considerations on Malaria Vaccines and Clinical Trials

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# Preface

Since 1966, the U.S. Agency for International Development (USAID) has sponsored a program of basic and applied research with the goal of developing one or more vaccines effective in prevention of malaria. The American Institute of Biological Sciences (AIBS) joined this program in 1982, providing a variety of support services to the agency and to the network of researchers who make up the program structure. As the program progresses toward the ultimate goal, AIBS has organized, on USAID's behalf, a series of regional conferences to address the development of malaria vaccines, and to explore the potentials for conducting eventual large-scale field trials in countries where malaria is endemic.

The first of these conferences focused on Asia and the Pacific Basin and was held in Honolulu, Hawaii in April 1985. The second, which concerned Latin America, was held in Ft. Lauderdale, Florida in October 1985. The final conference, concerning malaria in Africa, convened in Washington, DC in December 1986. Collectively, these conferences have provided valuable insights into the complex problems that will be faced by planners of malaria vaccine field trials.

The final conference, attended by participants from 29 countries, brought together malaria workers from Africa, Europe, and the United States. The specific objective of the conference was to consider the prerequisites for and the constraints upon future field trials of potential vaccines that would be imposed by special circumstances in Africa. The technical presentations, discussions, workshop results, and issues discussed in this forum are reported in these conference proceedings.

In addition to the program staff who facilitated the smooth flow of the confernce AIBS is extremely grate'ful to Dr. Buck, editor of this volume, for his tireless efforts to document important information and ideas that were fostered and exchanged during this important meeting.

CHARLES M. CHAMBERS
PHILLIP E. WINTER
*American Institute of Biological Sciences*

# Foreword

For over 30 years, the United States Agency for International Development (USAID) has been actively concerned with the control of malaria. Initially, our program focused on commodity and technical assistance to national malaria control programs. After World War II, USAID became the major donor agency in this respect. With the resurgence of malaria and the emergence of drug resistance, it became apparent that new approaches were needed to address this most important of infectious diseases. Nearly 20 years ago, USAID initiated funding of research directed at development of vaccines against malaria. This effort, which began at a time when many doubted its feasibility, is now beginning to reach fruition. Applications of new technologies to problems of malaria immunity are culminating in a series of recent and current human trials of potential vaccine candidates.

USAID recognizes that the formulations now undergoing clinical trials are prototype candidate antigens on the road to discovery of a vaccine to control malaria. Although the scientific community has ample experience in developing vaccines against bacteria and viruses, we must keep in mind that *Plasmodium* malaria is a complex, challenging organism with millenia of adaptation against humans and their immune defenses. It is likely,therefore, that several more years of work lie ahead.

The investment thus far in malaria vaccine development—an investment in which other donor agencies have shared—is a large one but it represents only a small fraction of the worldwide costs of the disease and of other control efforts to date. Prudent management suggest that planning begin early for the large-scale field trials that will be necessary to guide eventual application of the vaccine. USAID has sponsored a series of three major international conferences directed at delineating regional factors that would affect future technical and program planning decisions for field trials. The conference on malaria in Africa, which is reported in this volume, was the last in that series.

From both operational and biological standpoints, Africa represents the most challenging circumstances for malaria vaccine trials. The heterogeneity of Africa in terms of host, agent, and vector mirrors the wide variety of cultural, economic, and logistical considerations. Thus Africa will be the proving ground for malaria vaccines. The proceedings of this conference will be of considerable value to USAID plans for field studies leading to field trials of malaria vaccine. More importantly, the conference fostered renewed communication among individuals from various countries who are addressing the problem of malaria in Africa.

On behalf of USAID, I thank the distinguished participants, and especially the American Institute of Biological Sciences, for their valued assistance toward reaching our common goal.

NYLE C. BRADY
*Senior Assistant Administrator,*
*Science and Technology*
*U.S. Agency for International Development*

# Introduction

These are the proceedings of the third and last in a series of regional conferences on the development and clinical evaluation of potential malaria vaccines sponsored by the United States Agency for International Development. The first conference on the state of malaria in the Asia and Pacific region was convened in 1985, the second dealt with malaria in Latin America, and the third and present with the malaria problem in Africa.

During the two and a half years since the first malaria conference in Honolulu there has been rapid progress in immunological research on malaria, both in laboratory and in field studies. The results of these investigations have changed the prospects of new malaria vaccines as the panacea that could be applied with equal efficacy to all malarious areas in a global campaign similar to the successful eradication program of smallpox.

The results of the laboratory research have had immediate benefit for clinical and epidemiological studies in many endemic areas of the world, especially in Africa. The newly defined and reproducible antigens of the sporozoite, blood stages and gametocyte of *Plasmodium falciparum* have been employed in studies of populations with stable and unstable malaria and in areas with perennial and seasonal transmission. The new tools have made it possible to identify and measure the number and location of infective and noninfective sporozoites in individual vector mosquitos, thus making it possible to fingerprint the transmission dynamics of *P. falciparum* by determining the vectorial capacity of different sibling species of *Anopheles* in the same area. These studies have shown a considerable degree of diversity in the microepidemiology of malaria, even within the same village.

With these new methods it is now possible to determine the sporozoite inoculation rate, together with the source of the bloodmeal in the mosquitos. The specific antibody responses to the geographically and seasonally changing forces of infection have been studied by age and sex of population samples under ecologically contrasting conditions. The

chain of immune responses to malaria infections and re-infections in relation to the appearance of the different developmental stages of the malaria parasite has led to the recognition of many different antigens and epitopes. Some of these appear to correspond closely to clinical and parasitologic parameters of *P. falciparum* malaria.

Finally, great strides have been made also towards the better understanding of the immune mechanisms that are responsible for blocking the transmission of malaria by inhibiting the development of the sexual stages of *P. falciparum* in the vectors.

Against this background of success and promise there are the day-to-day difficulties and obstacles of malaria control in the endemic areas, most of all the rapid spread of resistance of the available antimalarials drugs to *P. falciparum* in Africa. While this species of Plasmodium is the most important agent for severe clinical malaria and child death in Africa, there are three other species of human malaria that so far, have received much less attention by the scientific community, namely *P. malariae*, *P.ovale*, and *P. vivax*. Concomitant infections with these different malaria parasites are relatively frequent.

From all that is known to date the prospects for the use of malaria vaccines as effective tools in future strategies of malaria control programs have remained bright and realistic. However, the pace of development, evaluation and eventual commercialization of the vaccines has to be adjusted to provide answers to some of the new questions that have arisen from experimental research and from new knowledge about the epidemiology of malaria in a broad array of field situations.

This conference has brought together a group of scientists from different disciplines, clinicians, epidemiologists, entomologists, behavioral scientists, health administrators and policymakers from the nations of Africa and the World Health Organization. The conference was proceeded by a series of significant meetings and consultations on the strategy and design of clinical trials and on the epidemiologic evaluation of sporozoite and blood stage malaria vaccines. These workshops were organized jointly by the Malaria Action Program (MAP) of WHO and by the secretariat and Scientific Working Groups of the Special Program for Research and Training in Tropical Diseases (TDR) of WHO/UNDP/World Bank in Malaria Immunology (IMMAL), Field Research in Malaria (FIELDMAL), Chemotherapy of Malaria (CHEMAL), Epidemiology (EPD), and Vector Biology and Control (VBC).

The section of the proceedings describing country situation reports contains not only the original contributions of the invited speakers but also summaries of the transcribed statements on the state of malaria programs made by national delegates in different parts of the discussions during the last two days of the conference.

In addition to the formally scheduled meetings, workshops and discussions of the conference agenda, there were many spontaneous exchanges of thoughts and information between the participants during their stay in Washington, DC. Some of these informal contacts have already led to the implementation of cooperative research projects.

Finally, I have pleasure in acknowledging the considerable help given by the members of the organizing committee in preparing this conference and its agenda, i.e., Drs. C. Diggs and S. Hoffman (WRAIR); K. Western (NIAID); D. Herrington and M. Levine (University of Maryland); C. Campbell and Collins (CDC); Lopez-Antunano (PAHO); N. Rose (JHU); J. Stockard and J. Shepperd (AID/AFR); and C. Shiff, T. Bender, V. Barbiero, and J. Erickson (AID/S&T/H). Considerable credit for the success of the conference goes to the staff of the malaria project of the American Institute of Biological Sciences (AIBS), notably Ms. Dorothy Jordan, Ms. Noel Souza, and Dr. Phil Winter, who were not only responsible for the smooth operation of the logistics and administration of the conference but who were always concerned with the well-being and comfort of all participants.

Gratitude is expressed also to Drs. Beausoleil and Wurapa of the Africa Regional Office of WHO for their active support, encouragement, and scientific contributions; to Drs. Najera, Molineaux, Goriup, and Martinez of WHO, Geneva; to Dr. Lopez- Antunano of PAHO who co sponsored the conference; to Dr. Campbell and his colleagues of the Centers for Disease Control in Atlanta and in Kenya; and to Dr. Chulay and his staff of WRAIR whose help and contributions to the conference were invaluable.

The completion of the proceedings within a relatively short time would not have been possible without the continued assistance, advice, and cooperation of many individuals in the United States, in Africa, and at the World Health Organization.

Last but not least, special thanks are due to the editorial staff of AIBS, especially to Ms. Elia Flanegin for her competent, efficient, and gracious contributions as editorial associate who had to carry out her work under great constraints.

ALFRED A. BUCK
*Editor*

# OPENING SESSION

# Welcoming Address

KENNETH J. BART

I would like to welcome all of you, as well as to add the greetings of the agency's administrator, Mr. Peter McPherson. We view this meeting as an extraordinarily important one. We are pleased to sponsor it through and with AIBS. Your attendance here today suggests that all of you share our sense of importance of this meeting.

This is a rather unique time. I am sure all of you are aware that we are on the verge of having a prototype vaccine for the sporozoite stage of malaria of *Plasmodium falciparum* and a similar preparation for *Plasmodium vivax* is not far behind. The pace of the research on the merozoite stage has quickened. We find ourselves with candidate antigens that are going into primates almost faster than primates can be made available.

Preparation is under way for phase III field trials. These will be difficult. We will have to deal systematically with the varying epidemiologic situations that each of us face in designing these trials. Further, the kind of research that needs to be completed prior to bringing antigens to the field is not yet fully enunciated, and hopefully some of those questions will be clarified at this meeting.

We are in the penultimate moments prior to the selection of suitable field sites. We look forward to your thoughts and assistance in this task. The task that we are undertaking is not only complex, but there is much data that we all do not have in order to describe a path for the next several years.

Again welcome, I wish us a productive conference.

# Welcoming Remarks

BRADSHAW LANGMAID

I want to welcome you on behalf of my agency's Administrator, Mr. Peter McPherson, and the Senior Assistant Administrator for Science and Technology, Dr. Nyle Brady—both of who unfortunately are out of town.

Dr. Bart has mentioned the principal reasons for being here and he has covered many of the topics that I wanted to mention. You have a very busy and a very important agenda, so I will be brief. Until recently the only hope for those affected by malaria in malarious areas was various control measures. Now we have the opportunity of vaccines, something we have to plan for in all seriousness. The Agency for International Development (AID) has invested considerable time and effort in development of a vaccine. We have been at it for over a decade, longer than almost any other research activity the agency has undertaken. We have invested more money in malaria research than any other health research activity. We anticipate continuing this effort to a successful conclusion. To maintain this level of continuity, I think, attests to our commitment to see the development of effective vaccines through to a successful conclusion.

This is the third global conference on malaria that we have had. Last year the Latin American and the year before the Asia and Pacific areas were reviewed. Now you are to discuss Africa. I can only charge you with the effort that I know you will extend to come to a successful conclusion so we can start the planning process in Africa also. On behalf of my agency and Mr. McPherson, I wish you a busy four days and a successful conclusion.

# Opening Remarks

CARLYLE GUERRA DE MACEDO

As regional director of the Pan American Health Organization (PAHO), I am pleased to be here with you on the occasion of the third of the global series of conferences on malaria vaccines.

In 1985, the World Health Assembly expressed concern about the adverse effects of malaria on the sanitary and socioeconomic development of many tropical and subtropical areas. The member states of WHO, recognizing the need to coordinate efforts in order to forestall the deterioration of the epidemiological situation, have already started to mobilize national and international resources for the in depth review and critical analyses of the trends, causes, and risk factors affecting malaria transmission.

Within the region of the Americas, a process of stratification is under way to arrive at a better understanding of the actual situation, a determination of realistic objectives, and the selection and development of suitable and affordable methodology for prevention, surveillance, and control of the disease.

The planning of antimalarial activities is now perceived as a flexible process within the national health systems. It seeks not only to ensure the application of increasingly effective prevention and control measures but also the advancement of the progress made, utilizing all possible intrasectorial and intersectorial resources.

But there are still many gaps regarding the knowledge of the longitudinal trends of the malaria incidence. Notification to the existing malaria services does not give an adequate picture of the importance of the disease and in certain areas it is insufficient for a correct assessment of the malaria situation. Moreover, it is imperative to validate and evaluate all the available prevention and disease control measures to be applied in the field, in light of their epidemiological justification.

Immunizations are, and will remain, the main means for control of many communicable diseases. So far, the emphasis has been placed on reducing morbidity and mortality of those diseases for which vaccines are readily available, well tolerated and fully accepted.

The strategies for malaria vaccine development have allowed us to make significant progress. We have been able to identify and characterize protective parasite antigens, clone the corresponding genes and their expression in bacteria, analyze their nucleotide sequences, and reproduce the amino acid sequences of the encoded molecules. Also, the antigenic material can now be produced by genetic engineering or by chemical synthesis.

Another important breakthrough is that the most exposed protective plasmodial antigens to the immune system are now being identified and three main types of malaria vaccine are being developed:

1. A sporozoite vaccine that, if fully effective, would prevent the successful establishment of plasmodial development in the host and thus induce sterile immunity.
2. An asexual erythrocytic stage vaccine that is expected to induce immunity by restricting the replication of asexual blood stage parasites without necessarily inducing sterile immunity. Consequently, its function will be to reduce the morbidity and mortality due to malaria.
3. A transmission-blocking immunity induced by immunogens from gametocytes of the malaria parasite through the production of specific circulating antibodies. These antibodies could block transmission of the parasite through the mosquito by interfering in the sporogonic development.

Although several activities have taken place since late 1984 to coordinate specific tasks for the development and testing of the malaria immunizing agents, we think an additional effort is still needed in this regard.

Malaria vaccine research is progressing rapidly at present and there can be little doubt that the forthcoming malaria vaccines offer a justified hope for the immediate future. However, some problems and obstacles remain, we still need to carry out a series of human trials, including individuals residing in areas of natural malaria transmission. Before human trials can be contemplated, three main conditions must be met: First, the need for the vaccine should be established and its feasibility realistically assessed. Second, the regulatory authorities as well as the principal investigators and their colleagues should be fully satisfied with the results of preliminary laboratory tests in animals; these tests should provide the necessary information on the safety and efficacy of the vaccine and a clear indication of the potential benefits either to individuals, the communities, or both. Third, any human trials should be conducted under the auspices, or with the agreement of, a nationally and internationally recognized body. As you all know, biological field trials pose special problems that need to be considered in relation to contem-

porary ethical codes, such as the 1964 Declaration of Helsinki, which was revised by the 29th World Medical Assembly held in Tokyo in 1975, and the Report of the International Conference on the Role of the Individual and the Community in the Research, Development, and Use of Biologicals, which took place in Geneva in 1976.

If these conditions are met and even if the malaria vaccine or vaccines prove not to be totally successful, the basic epidemiological studies made on the dynamics of transmission, which are indispensable for the trial, will be extremely valuable for the affected countries.

On behalf of the PAHO and WHO, I want to give my warmest greetings to the distinguished scientists who are honoring our American continent with their presence.

# Opening Comments

CHARLES M. CHAMBERS

Thank you very much indeed, Dr. Bart and Dr. Antunano. And thank you too, Dr. Macedo, for your very helpful and informative opening comments. I have never had the privilege of the agenda without someone holding a bell ready to call time on me, and I appreciate Dr. Bart's kind suggestion that I might review in a little more detail some of the background that has led to this meeting.

Let me add my offered welcome on behalf of the American Institute of Biological Sciences to this, the Third International Conference on Malaria in Africa. I would like to thank each and every one of you for coming. Our great fortune in this meeting is your attendance and participation. As we go through the sessions and workshops over the hours and days ahead, I think you will appreciate that there is indeed a method to our plans. We have tried to create a mixture of participants that will establish personal bonds between people with different responsibilities in different areas of research and health care delivery. We feel these bonds are essential to taking the dramatic new results that are coming out of the laboratory into the field. One of my great pleasures in addressing a group such as this is to acknowledge the many important, even profound, contributions made to this overall effort over many recent years. Dr. Langmaid, I would like to extend through you to Dr. Brady and Mr. McPherson the sincere appreciation of our Institute and the life sciences community we represent for the consistent and unfailing leadership that has persisted administration after administration here in this capital city of Washington in focusing on this worldwide health problem of malaria. It is particularly significant in that, as you are aware, malaria is not an endemic problem in the United States. In fact, we have many challenges in working with our own government on the so-called orphan diseases and vaccines and trying to meet the need that is there. It is a tremendous credit to the Agency for International Development (AID) and its scientific community and diplomatic leadership that this program has made the progress that it has, and for that we remain indebted to you.

I would also like to recognize the invaluable participation of the Pan American Health Organization (PAHO) through Dr. Macedo and his

colleagues. This the third of a series of conferences we have held on this topic. The second was held about a year ago in Ft. Lauderdale, Florida, and involved all of the Americas, North, Central, and South; we relied very heavily on PAHO for help in organizing that meeting. In fact, we were so impressed with the level of services and support that they provided that even though this was not viewed as an Americas' meeting, we thought their expertise would still be helpful. We had originally planned to hold this meeting at the PAHO facilities. As it turned out, another one of our blessings was a tremendous growth in interest. The more we talked to people about their participation, the more they said this meeting would be even better if someone else could also come. After many committee meetings and agency concurrences, we did enlarge the participation. Unfortunately, we became too large for the "inn" at PAHO. Unlike Bethlehem, Washington was a bit larger so we were able to find another inn, this lovely Madison Hotel, centrally located here in Washington. Not to be outdone, PAHO said they would simply move their translator and interpreters right here into the hotel so we could have a meeting operating as easily as if we were in their own facilities. That is a remarkable degree of hospitality that one does not find very often and I would like to acknowledge it at this point.

The leadership that the program has received within AID, starting at the very top, has moved on through the Office of Health. Dr. Kenneth Bart is the director of that office. Others who have worked actively there are, of course, Dr. James Erickson, from whom you will be hearing shortly regarding the overall mission and scope of the AID research and implementation effort for the malaria vaccine.

This particular conference was the handiwork of a group of people, whom I would be much remiss in not recognizing. You have within your materials a brief listing and acknowledgment to those people, but I would like to identify them publicly here. These people have met several times over the past year. A year may seem like a long time, but it is certainly well within the schedule for planning a meeting of this scope.

They are Dr. Victor Barbiero, AID; Dr. Alfred Buck, AIBS; Dr. Kent Campbell, CDC; Dr. William Collins, CDC; Dr. Lawrence Cooper, AID; Dr. Steve Hoffman, Walter Reed Army Medical Center (WRAIR); Dr. Myron Levine, University of Maryland; Dr. Antunano, PAHO; Dr. Milton Rose, Johns Hopkins University; Dr. Clive Shiff, AID; Dr. James Shepperd, the AID African Desk, and colleague Dr. Joseph Stockard; Dr. Wasim Siddiqui, University of Hawaii; and Dr. Karl Western, NIH. I would like to thank all of the contributors as a group for serving as the planning committee for this meeting.

During these planning meetings, we reached out to the highways and byways to meet with you, talk with you, share our ideas and get your

input as we put the program together. Two people who spent a tremendous amount of time on the road to accomplish this were Dr. Al Buck and Dr. Clive Shiff, whom you have met and will be meeting here. Following a logical organizational approach, Dr. Buck toured West Africa and Dr. Shiff toured East Africa. That seems to have been a fairly good division of effort.

We are also standing on the shoulders of our forbearers in this particular meeting. We have learned from the two earlier meetings, one in Ft. Lauderdale for the Americas, and the premier meeting, which was held a couple of years ago in Hawaii, dealing with the problems of malaria in the Pacific rim areas. The gentleman who has served on all of our committees and was chairperson of that first meeting is Dr. Wasim Siddiqui. We look to him as the grandfather of these meetings, and are very glad to have him with us today.

Many of the materials that you have, many of the letters that you have received, the name badges, the arrangements of the hotel, etc., are, of course, the work of our staff. We are very proud of the staff who has done so much to organize this meeting and make it one you will find enjoyable. You will be reaping the benefits of this work over the next few days. Specifically, Noel Souza has been indispensable in coordinating details and logistics, handling the telephone and telegrams as well as everything that comes in and goes out. She had just joined us for the Latin American meeting and has really shown a tremendous amount of skill and efficiency in managing many of the details of this meeting. She was assisted by Liz Fergus, who is learning more about all the details for this type of meeting. They are both managed by the project director within AIBS, the person who has the day to day responsibility for overseeing the planning for these meetings. That is Ms. Dorothy Jordan, and I would like to recognize her also.

As the project begins, this new and very significant phase of moving out of the laboratory with these clinically tested antigens and into the field, we have added a new senior staff person to AIBS who will be planning and guiding the clinical testing phase. This is a person whom I hope you will have a chance to come to know much better through this meeting and someone who will definitely be staying in touch with you in the months and years ahead. I would like to introduce Dr. Phil Winter.

We also have representatives of the press with us. Throughout the meeting you may find yourself being approached and interviewed by one person or another. Ms. Laura Tangley is a senior editor of our magazine, *BioScience*, which we have distributed to you. She will be available throughout the meeting at various times. Mr. Boyce Rensberg of the *Washington Post* is expected. Ms. Nancy Tomich of *U.S. Medicine Reports* will also be with us. We hope that you have a chance to talk with

some of them and share your views about what is happening in your countries and what this particular meeting means to you.

We made a special effort to share with you the July/August 1980 issue of *BioScience*. The cover, I think, is very appropriate. It is a satellite interpretation of vegetation and water resources and shows the African continent very prominently. For those of us involved in epidemiology, the detail is impressive and the kind of information we can gain about vector propagation and things of that nature, of course, play a central role in our health care strategies.

Ms. Tangley also has a feature article in this issue. She was part of a special group that was invited to Cuba to study the sugar crop there, and the use of biotechnology and other advanced methods for that very important economic crop there.

In looking around the room and seeing so many people that are directly involved in primary health care, I am reminded of the story of the person who completed his earthly existence and moved on to his heavenly reward. As he was greeted at the gates of heaven by St. Peter, he commented, "Well, I suppose I will be out in the rear of the choir for a bit." St. Peter said, "Oh, no, no! In heaven we are all equal. It is a very, very democratic organization." The fellow seemed a bit puzzled, but he allowed if that is how St. Peter said it, that is probably the way it was. "Let me get you your wings and halo, and by the way, why don't we stop by the cafeteria." Sure enough, they went into the cafeteria and there were just common folks all in a line, and there was Moses, Mohammed, and Noah, in a line with everyone else. The fellow turned to St. Peter and said "Yes, you seem to be right, heaven is really run on a fair and democratic basis." St. Peter said, "Yes, yes, indeed." Then there was a bit of fury and an elderly man with a long white beard came rushing down the aisle, went right past the line, and got in front. The fellow turned to St. Peter and said, "Wait a minute, I thought that this was supposed to be a democratic organization." St. Peter said, "Oh, no, no, not to worry, not to worry. That is God. He thinks he is a physician."

Well, if we in our roles as primary health care providers are more than equal, it is because we have a greater responsibility and because we are faced with the challenge of taking the science in which we have all labored and bringing it forward to the benefit of mankind. That is the broad picture that has appealed to me as a working scientist, someone with many years in the classroom and the laboratory, about this particular project. In my early days of work I would see things being developed (e.g., chemotherapy techniques), and I would become quite frustrated at the many layers of bureaucracy that were involved in moving these new and fantastic discoveries out into the world to benefit mankind. As I grew older, and perhaps wiser, I learned that the checks and balances were

necessary; in our enthusiasm we scientists can be overzealous and perhaps overlook many of the secondary, yet important side effects.

Further on, I began to feel that there was a fundamental need to study the strategies of health care delivery so that the gap between the laboratory and field delivery could be reduced to the absolute minimum in terms of ethical, safe, and sound medical practices. We are learning everyday in this particular project that the laboratory has reached the stage where the products are almost now programmed by turning the dials. Not quite, but we are, of course, producing the sequenced and cloned antigens and other viral products that will be very, very useful for the entire range of diagnosis and health care delivery efforts. Many of the questions of incidence within populations, of vector propagation, and of side effects remain there, but we are finding, of course, that the laboratory is creating new tools at a rate far exceeding the health care systems' ability to absorb them.

We need to test, we need to experiment, we need to try our wings with new strategies to move from the laboratory to the field. You are participating through this conference and through the things that will follow this conference, in a grand research experiment to learn more about the methods, protocols, procedures, and administrative arrangements that will enable these new, profound, and significant laboratory products to be made available for the health and welfare of our peoples throughout the world without undue delay.

That venture, that goal, and that experience is what we are for. We have a mix of personalities and participants at this particular meeting in order to bring together people who are doing the sequencing and the cloning, who are doing the isolating and assaying, who have the intimate knowledge of the epidemiological settings and who know the physiological basis of these reactions, so that together we can develop recommendations. Those recommendations can serve as the framework for our protocols for extending the clinical and human trials into the field and eventually developing a profound revolution in health care delivery through the application of scientific knowledge.

That is the goal I leave with you and I wish you well in that endeavor. I look forward to meeting more of you, talking with you, and being with you at the end of the meeting as we can measure the progress we have made. Thank you very much.

Let us now begin the scientific sessions. I am very pleased to yield the chair, on behalf of myself and Dr. Lopez Antunano, to Dr. Phil Winter and Dr. Kilama.

SECTION I

# MALARIA VACCINES: STATE OF THE ART

W.L. KILAMA AND P.E. WINTER,
*Chairpersons*

# The AID Malaria Program

JAMES M. ERICKSON

I would like to extend my personal thanks and welcome to Washington to all of you. After 10 years as project director for this program, it is thrilling for me to see the culmination of all the research efforts as we come down to actually talking about the details of fielding vaccines in those parts of the world where malaria is endemic.

I would like to give you a brief overview of what the Agency for International Development (AID) has been doing in the field of malaria eradication and control, as well as malaria immunology. The blue-and-green book on malaria that you received in your packet was published last year, and gives a historical account of events and the relationships between malaria and the AID health programs.

AID has been directly involved in the eradication and control of malaria since about 1954, and since then we have been concerned with operational malaria control, research, and training. Malaria control has become very important now because of the worldwide resurgence of malaria. This resurgence is due to two factors: insecticide resistance and drug resistance in the malaria parasite. Throughout the period of 1954 to 1975, the agency provided approximately $1.2 billion for malaria control and eradication; this is a very large sum, especially if you consider the currency value of the 1955 dollar. The eradication period lasted from 1955 to 1970, until it was replaced with malaria control. It was during this time that AID became involved in immunological research. The scientific community was rather skeptical of the whole potential to develop antimalaria vaccines. Maybe these ideas were a little shortsighted, but in 1965 that was a fair statement.

In the book that you received, there is a specific chapter on the history of our involvement in the immunology of malaria. So I will not belabor that particular point in terms of how we got into it. I will only emphasize a few minor points. In 1966, we initiated our first program, a feasibility study with the University of Illinois. That was the only program until 1974. It was the catch-all program and was to look at in vitro cultures, antigens against the various malaria parasite life stages. But it soon became obvious that the field was too broad for one project. In 1974,

we added four additional projects. Since then, we have grown to a large collaborative network of research organizations with 17 major components.

I would like to recognize those who have made some technical breakthroughs to put us where we are today. As you know, one of the key problems in developing immunological techniques was the fact that we could not get enough parasite material to work with. There were a lot of scientists working on the in vitro culture. Until that problem was resolved, of course, there was very little that could be done.

In 1975, several laboratories experienced breakthroughs in in vitro culture. While working separately, Drs. Trager, Siddiqui, and Hayes, were able to establish long-term, stable cultures of *Plasmodium falciparum*, and thus the race began.

Up until then, it had been difficult to get started. The hybridoma technology started to become a useful tool in the early 1980s. In 1982, Dr. Hollingdale, was able to complete the in vitro cycle for the exoerythrocytic stages of malaria. This was a very important step which led to the ISI assays that are presently used in human trials.

At the same time in 1982, of course, Dr. Nussenzweig was able to isolate the circumsporozoite (CS) protein from *P. berghei* and finally from *P. falciparum*.

The progress we have seen is remarkable. What is most interesting to me is that this book, which came out in late 1985, is already out of date in terms of immunology, due to the progress in monoclonal antibody technology, genetic engineering, and synthetic chemistry. As we go through this morning's presentations, we are going to hear a great deal more about the specifics of these different antigens and materials that have emerged from these methodologies.

With the 1984 announcements of the cloning of CS genes by researchers at New York University (NYU), AID developed a triangular strategy for the acceleration of malaria research. This is basically what I want to cover now.

In 1984, the agency set down three very significant areas for expansion, one of which was basic research. A number of new technical questions became apparent at that point. We knew we had some products that looked promising, but there were many other research areas that had to be investigated. The basic research was expanded to include the exoerythrocytic stages, the sporozoite stages, and of course, most importantly, the red blood cell stages of malaria. We have accelerated the studies very quickly into the nonhuman primate models because the Food and Drug Administration (FDA) will need these data for review prior to human trials.

We have established, through our colleagues at the National Institutes of Health (NIH), a human volunteer testing facility at the University of Maryland, and Dr. Levine will be speaking to you a little later this morning about the specifics of the trial now underway. A number of people have already mentioned the several meetings we have had on field trials of malaria vaccines. Our ultimate goal is to test prototype malaria vaccines under field conditions. Dr. Siddiqui has been very much involved in setting up the Asia-Pacific meeting. You have the large green book from that particular conference held in April 1985. The Latin American conference has been mentioned already and, of course, now we are here to discuss Africa.

I would like to ask you to look carefully at another document you received in your packet—the simple little document entitled "Workshop Objectives." I will summarize my points.

At the bottom of the front page there are five specific goals, and on the top of the second page another six objectives, outlining what this meeting is trying to accomplish. It is important to realize that these are basically the same objectives with which we started in Honolulu in April 1985. Time, the state of the art, technology, and epidemiology have caught up with us. We are no longer considering only sporozoite vaccines.

I would like for you, when you look at these questions, to expand your horizons to polyvalent vaccines, which are going to be the most important for overseas use. Of course, the other species of human malaria must be considered as well. With that background in mind, I wish you good luck. I am looking forward to your recommendations, which will aid all of us in our planning and deliberations as we move forward to bring these vaccines from the laboratory to the field.

Thank you.

# Antisporozoite Vaccine: Experimental Basis and Current Status

R.S. NUSSENZWEIG

Malaria continues to be a disease of exceptionally high incidence in tropical and subtropical areas of the world. In certain areas, particularly in tropical Africa, malaria contributes greatly to the very high mortality rates among young children. For those individuals who reach adulthood and gradually acquire a variable degree of resistance, malaria is still a debilitating disease that incapacitates those afflicted for long periods.

In many areas, the beneficial results derived from current control measures, including residual insecticides and chemotherapeutic agents, have proved to be either short-lived, difficult to sustain, or only marginally effective. The reasons for this failure are multiple: the enormity of the task, in view of the broad distribution of the disease; the lack of adequate resources; the appearance of drug-resistant parasites and insecticide-resistant vectors; and the emergence of silvatic mosquitoes, which are difficult, if not impossible, to exterminate.

This situation gives a sense of urgency to attempts to develop new control measures. In spite of the foreseeable difficulties, both in development and delivery, a vaccine may provide a powerful tool for malaria control.

The development of malaria vaccines is complicated by the fact that the protective antigens are specific for each of the main stages of the parasite in the human host: sporozoites, which are present in the salivary glands of the *Anopheles* mosquitoes and are injected during the bite; asexual blood stages, which develop in red blood cells and cause the clinical manifestations of disease; and gametocytes, which are infectious for *Anopheles* mosquitoes. An effective vaccine against sporozoites would be most advantageous because it would block infection and prevent disease and transmission. Even if sterile immunity is not achieved in all individuals, the decrease of sporozoite load should diminish the severity of the disease. This view is supported by epidemiological findings, and by the lower mortality and more benign course of infection observed in animals inoculated with smaller numbers of sporozoites.

Protected immunity against sporozoites has been achieved by inoculation of irradiated parasites into rodents, monkeys, and humans.[1] The target antigens of the protective immunity have been identified by monoclonal antibodies. They consist of single polypeptides (circumsporozoite or CS proteins), which cover the entire surface membrane of the parasite and are shed when cross-linked by antibodies.[2] In every experimental model of rodent, monkey, and human malaria, monoclonal antibodies directed against CS proteins neutralize sporozoite infectivity.[3]

The CS polypeptides are likely to be involved in the initial interactions between the parasite and the host's target cells, that is, the hepatocytes. Fab fragments of monoclonal antibodies to CS proteins neutralize parasite infectivity in vivo[4] and in vitro and prevent their attachment to target cells.[5] In addition, there is a close temporal correlation between the acquisition of infectivity and the appearance of CS protein on the membrane of the parasite. Immature sporozoites (found in the midgut of *Anopheles* mosquitoes) bear little or no CS protein on their surface membranes[6] and are not infective.[7] These observations suggest that CS proteins or selected domains of these polypeptides are logical candidates for vaccine development.

CS proteins of different plasmodial species have similar structural, biosynthetic, and immunological properties. One interesting feature is that they are shed by the parasite when cross-linked by antibodies, forming a long, tail-like precipitate at one end of the parasite (CSP reaction). The significance of the CSP reaction is not clear. It is unlikely to represent a mechanism of escape from the host's immune response, since most or all parasites that have undergone this reaction lose their infectivity. An intriguing possibility is that the shedding mimics an event that normally occurs only when the parasite encounters multivalent receptors on the surface of hepatocytes. In other words, the CSP reaction could represent the premature ejection of the surface coat, triggered by a nonphysiological cross-linking signal.

CS proteins constitute one of the major biosynthetic products of sporozoites.[8] About 10% of the [$^{35}$S]-methionine incorporated into proteins by salivary gland sporozoites is found in CS proteins. However, CS proteins are not present in the blood stages of the parasite, and sporozoite-induced protection is strictly stage specific.[9] Biosynthetic studies revealed two precursors of the CS protein with higher $M_r$ and isoelectric point. Although the biosynthetic pathways are still obscure, it appears that the membrane form of CS protein is generated by sequential removal of two small basic peptides from the precursors.

CS proteins exhibit unusual immunological properties; not only do they have a strikingly immunodominant epitope, but this epitope is present several times in a single molecule. All monoclonal antibodies

against sporozoites are directed against this epitope. Preincubation of crude extracts of sporozoites with any of these monoclonal antibodies inhibits between 70% and 100% of the subsequent binding of antisporozoite antibodies found in human or animal sera; that is, most of all antisporozoite antibodies in the polyclonal antisera recognize the repetitive epitope.[10]

The structural basis for these properties was clarified by cloning and sequencing the CS genes. Only one copy of the CS gene is present per haploid genome, and the DNA sequence is uninterrupted. The central area of CS polypeptides is formed by tandemly repeated sequences of amino acids: NANP in *Plasmodium falciparum*[11,12] and DRAD/AGQPAG in *Plasmodium vivax*.[13,14] The immunodominant epitopes are located within this "repeat" domain.

On the basis of its sequence, charge densities, and secondary structure predictions, the precursor of the CS protein contains several domains between the postulated signal and anchor sequences. The larger domain contains the tandem repeats, which form $\beta$ turns, but are presumably devoid of rigid secondary structures. Two domains have charged residues and may contain $\alpha$-helical structures. One (N2) flanks the domain containing the repeats in the direction of the N- terminal. The other (C2) is closer to the C-terminus and is flanked on both sides by pairs of cysteine residues, which may form small intramolecular disulphide loops.[15] Although the N2 and C2 areas are highly polar and exposed on the surface of the CS molecule, they are very poorly immunogenic.[16]

In the case of *P. falciparum*, the immunodominant epitope is defined by only three consecutive repeats (NANP)3. This conclusion is based on studies that showed that (NANP)3 was recognized by polyclonal and monoclonal antibodies to sporozoites, and, conversely, that antibodies to (NANP)3 reacted with sporozoites and prevented their entry into hepatocytes in vitro.[17,18] A series of peptides were synthesized and used to inhibit the binding of monoclonal antibodies to extracts of *P. falciparum*. The results showed that (NANP)3, (NANP)4, and (NANP)5 strongly inhibited the binding of most of these antibodies to the antigen with almost equal efficiency on a molar basis. In contrast, the dimer (NANP)2 was a poor inhibitor.

(NANP)3 was then used as the antigen in an immunoradiometric assay (IRMA) to detect antisporozoite antibodies in the sera of humans living in an endemic area, the Gambia, West Africa. In agreement with previous epidemiological studies showing that the immune response of humans to sporozoites is age dependent,[19] it was found that the percentage of positive sera detected by the IRMA increased with age, ranging from 22% in children 1 to 14 years old to 84% in adults older than 34 years. Among many individuals older than 20 years of age, serum titers reached

as high as 1/640. The levels of antibodies to (NANP)3 in the serum of a human volunteer (G.Z.) vaccinated with x-irradiated *P. falciparum* sporozoites and protected against malaria infection had the highest reactivity and the titer reached 1/4096.[17]

Additional observations showed that most or all antibodies to sporozoites present in the human sera were directed against (NANP)3. Indeed, in these sera, a highly significant correlation ($r_s = 0.87$, $P < 0.001$) was found between the antipeptide and antisporozoite antibody titers. Moreover, the reactivity of the antibodies with sporozoites was abolished or strongly inhibited when the reaction was carried out in the presence of the (NANP)3 peptide.

Data on antisporozoite antibodies have recently also been obtained in Indonesia, using a recombinant polypeptide containing 32 repeats of NANP as antigen.[29]

The reason for the immunodominance of (NANP)3 may be simply that it is abundantly represented on the sporozoite surface. Thus, NANP appears 37 times in each CS molecule of a *P. falciparum* strain from Brazil[11] and 41 times in an isolate from Thailand (del Portillo et al., unpublished data). Based on the surface area of sporozoites, it is estimated that each parasite bears about $8 \times 10^6$ CS molecules on its membrane, provided that the molecules form a packed monolayer with the long axis perpendicular to the surface of the parasite. If these assumptions are correct, NANP would be present about $3 \times 10^8$ times on the membrane of the parasite, forming a regular array. It is well known that the polymerization of an epitope enhances its immunogenicity. For example, monomeric flagellin, which is a rather poor immunogen, becomes a very potent antigen in polymeric form.[21]

To study the immunogenicity of (NANP)3, we immunized groups of rabbits with conjugates prepared by coupling the peptide to tetanus toxoid by means of glutaraldehyde. The sera were assayed by an IRMA using (NANP)3 as capturing antigen. All samples from immunized animals were positive and these reactions were totally inhibited by the synthetic peptide. The antibodies to (NANP)3 reacted in a Western blot with the *P. falciparum* CS protein and its precursors and with the surface of glutaraldehyde-fixed sporozoites of *P. falciparum*, as determined by IFA. The sera with highest IFA and IRMA titers gave strong circumsporozoite reactions (titers of 1/50 to 1/100) upon incubation with viable parasites.

A highly significant positive correlation ($r_s = 0.87$, $P < 0.001$) was found between the IRMA and the IFA titers, suggesting that most antibodies to (NANP)3 recognized the CS protein. This was also corroborated by absorption of the sera with a *P. falciparum* sporozoite extract, which removed most of the antipeptide activity.

Immunoglobulin was isolated from the serum of one of these rabbits and tested for its ability to neutralize the infectivity of sporozoites of *P. falciparum* in vitro. The results showed that the immune IgG inhibited parasite invasion in a dose-dependent fashion. A strong effect was observed with total IgG concentrations as low as 2 μg/ml. When the antibodies to (NANP)3 were removed by absorption with peptide bound to Sepharose beads, the inhibitory effect was abolished.[17]

Taken together, these results strongly suggest that the synthetic peptide (NANP)3 represents the dominant epitope of the domain of the CS protein containing the NANP repeats, and that antibodies to the repeats recognize an uninterrupted sequence of amino acids. It is likely that the repeat domain contains sequential, and not configurational, epitopes in all CS proteins, since monoclonal and polyclonal antibodies to the repeats of CS proteins of several plasmodial species react with the denatured antigen, heated at 100 °C for 30 minutes or treated with 6 M urea and 1% β-mercaptoethanol.

In the case of *P. falciparum*, the repeat region contains a very large number of prolines and asparagines, residues frequently found in β turns. This simple type of secondary structure is formed by a few consecutive amino acid residues, and for this reason its configuration is more likely to be adopted by the synthetic peptide. In agreement with this idea, most of the antibodies to (NANP)3 react with the native CS protein.

In view of the difficulties in obtaining large amounts of antigen from the various stages of development of the parasite only genetically engineered or synthetic vaccines against malaria can be contemplated. In the case of *P. falciparum*, vaccines containing (NANP)3 are attractive candidates for further development. In this context, it is noteworthy that (NANP)3 is present in strains of *P. falciparum* from all over the world.[22,23]

A potential problem of all synthetic vaccines consisting of a peptide conjugated to a carrier protein is that they may not boost the naturally acquired immunity to the pathogen, and, more important, that these vaccines might only expand the antipeptide B memory cells but not prime antipeptide T cells. If, upon vaccination, the cellular interactions involved in the processing of the peptide-carrier conjugate lead exclusively to T-cell recognition of the carrier, then the acquired immunity to the pathogen may not be boosted by the vaccine, and, more important, a secondary immune response may not be set in motion in vaccinated individuals by the encounter with the pathogen. Under these conditions, the production of antibodies to the peptide would depend exclusively on effectiveness of the primary immunization or the secondary response upon revaccination.

One vital goal of malaria vaccination is therefore to raise T-cell and B-cell memory, which will be engaged some months or some years later by the parasite to yield protective/neutralizing antibodies. Activation of T

helper cells is also essential for γ-interferon production, which has been shown to inhibit exoerythrocyte stage development,[24] and thus may contribute to vaccine efficacy. The identification of a common dominant peptide within the CS protein sequence that induces T-cell activation has recently become a subject of intensive investigation. A prerequisite for the efficacy of such a T-cell epitope as a component of a malaria vaccine would be its recognition by the majority of individuals of genetically diverse background.

Plasmodial peptides and recombinant proteins may turn out to be considerably less effective immunogens than the CS proteins in their native configuration on the sporozoite surface. In this case, the availability of potent adjuvants for human use will be essential for optimizing the efficacy of a malaria vaccine.

To date, two candidate vaccines based on the NANP repeats have been developed. One of these, made by recombinant DNA technology ($R_{32}$stet$_{32}$) consists of a fusion protein made in *Escherichia coli* and contains 32 repeats of NANP.[25]

The other vaccine is a conjugate of a protein carrier with synthetic repeat (NANP)3, which represents the epitope recognized by antibodies in the sera of humans living in endemic areas and of animals injected with sporozoites of *P. falciparum*. Rabbits and mice injected with the NANP-conjugate make high titers of antipeptide antibodies, most of which also recognize the CS protein and neutralize parasite infectivity.[17]

These two types of antisporozoite vaccines are currently being tested in human volunteers to evaluate their safety, immunogenicity, and effectiveness. Preliminary results and details of these clinical trials will be provided elsewhere in this publication.

Because of the economic and logistic problems involved in delivering vaccines to the underdeveloped areas of the world where they are most needed, a high degree of protective immunity must be achieved by a single dose of the immunoprophylactic agent. This is a challenging proposition, particularly in the case of a malaria vaccine, which should ideally contain a mixture of antigens from the various stages of development of the parasite in the human host (i.e., from sporozoites and blood stages). Recent advances in malaria research, immunology, and biotechnology permit some measure of optimism that this can be achieved, perhaps in the near future.

## SUMMARY

Malaria affects 200 to 400 million people a year in tropical and subtropical countries. A promising candidate suitable for either a synthetic or a genetically engineered malaria vaccine has been identified. The synthetic peptide, which consists of a string of four amino acids repeated three times, is modeled on a surface component present on *P. falciparum* sporozoites when

these are inoculated into humans by mosquito bite. An immune response to the peptide may neutralize sporozoites before they are sequestered in host liver cells. The peptide reacts with antibodies in serum of randomly selected individuals living in malaria endemic areas, and with serum from a volunteer protected from infection by immunization with irradiated parasites. This peptide induces antibodies in animals; the antibodies prevent the parasite from entering human hepatoma cells, grown in culture. Gamma-interferon produced during immunization can enhance protection by its inhibitory effect on liver stages.

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# Safety and Immunogenicity of a Recombinant DNA *Plasmodium Falciparum* Sporozoite Vaccine

JEFFREY D. CHULAY

Immunization with irradiated sporozoites has demonstrated that protective immunity can be achieved in animals and humans. Sporozoite immunity is species and stage specific, and is mediated at least in part by antibodies directed against immunodominant repetitive epitopes on the circumsporozoite (CS) protein. The CS protein of *Plasmodium falciparum* is composed of 412 amino acids, 40% of which are included in 41 tandemly repeated tetrapeptides (37 NANP and 4 NVDP) of the central immunodominant region of this protein.[1] A recombinant DNA construct ($R_{32}tet_{32}$), derived from the CS protein of *P. falciparum*, was shown to be highly immunogenic in laboratory animals, and induced antibodies that mediated the circumsporozoite invasion into hepatoma cell (ISI) assay.[2] The results of additional preclinical studies, which led to the choice of $R_{32}tet_{32}$ as the first subunit malaria vaccine,[3] and the results of phase II studies of the safety and immunogenicity of $R_{32}tet_{32}$ in humans[4] are summarized here.

$R_{32}tet_{32}$ consists of 32 tetrapeptide repeats ($R_{32}$) from the CS protein $[(NANP)_{15}\ NVDP]_2$, fused to 32 amino acids ($tet_{32}$) derived from the tetracycline resistance gene of the plasmid. Initial preclinical studies indicated that $R_{32}tet_{32}$ was safe and immunogenic in laboratory animals.[2] However, $tet_{32}$ is extraneous in regard to the specific desired immune response, and a vaccine made without this portion of the fusion protein would offer theoretical advantages if it were immunogenic. We therefore produced another construct ($R_{32}LR$), which was identical to $R_{32}tet_{32}$ except that it included only the first two amino acids from the $tet_{32}$ fusion protein (leucine and arginine). When C57BL/6 mice were immunized with partially purified $R_{32}tet_{32}$ or $R_{32}LR$, they developed high antibody titers. In contrast, when C57BL/6 mice were immunized with $R_{32}tet_{32}$ or $R_{32}LR$ that was highly purified and suitable for use in humans, only the mice immunized with $R_{32}tet_{32}$ made antibodies that recognized these antigens. We therefore elected to proceed with development of $R_{32}tet_{32}$ as a vaccine candidate.

Our first human *falciparum* sporozoite vaccine (FSV-1) consists of highly purified $R_{32}tet_{32}$ absorbed to aluminum hydroxide. After preclinical testing confirmed this product was safe and immunogenic in mice, rabbits, and rhesus monkeys, FSV-1 was tested for safety and immunogenicity in 15 human volunteers. The volunteers were divided into five groups, each group receiving a different dose of FSV- 1 (10, 30, 100, 300, or 800 $\mu$g). The same dose of vaccine was administered three times at 4-week intervals.

The vaccine was generally well tolerated. Mild pain associated with the injection occurred in seven volunteers who received $\geq$100 $\mu$g. Mild tenderness at the immunization site was detected in eight volunteers, including all who received 300 or 800 $\mu$g. Five minutes after the last 800-$\mu$g dose, one individual developed sneezing and generalized urticaria, without associated hypotension or dyspnea, which resolved spontaneously after 20 minutes. This individual was the only volunteer who developed IgE antibodies directed against $R_{32}tet_{32}$.

Twelve of 15 volunteers produced antibodies that reacted with $R_{32}LR$ by ELISA. The magnitude of the antibody response, which ranged from <1 to 12 $\mu$g/ml, increased with increasing doses and never reached a plateau. Only one individual, in the 800-$\mu$g group, developed an antibody titer greater than 1:100 by ELISA and IFA. Sera from nine volunteers taken 2 weeks after the third dose of FSV-1 produced a 2+ CSP reaction. When compared with each individual's preimmunization serum, sera from 11 volunteers taken 2 weeks after the third dose of FSV-1 inhibited sporozoite invasion into hepatoma cells from 12% to 85% (median = 50%). In lymphocyte blastogenesis studies, peripheral blood mononuclear cells from 11 of 13 volunteers proliferated in response to stimulation with $R_{32}tet_{32}$. Four weeks after the first dose, the mean stimulation index of the 13 volunteers was 13.0 at the optimal $R_{32}tet_{32}$ concentration of 15 $\mu$g/ml. Prior to immunization, the mean stimulation index in the same 13 individuals at the same antigen concentration was 2.5. The proliferative response did not change after booster doses, nor was it correlated with the dose of vaccine received or with the antibody response.

Although the magnitude of the human antibody responses was lower than expected based on the preclinical animal studies (Table 1), all of the 15 human volunteers had an immunologic response to the vaccine as measured by antibody production or lymphocyte proliferation, and the concentration of antibody increased with increasing doses of the vaccine and never reached a plateau. Studies are underway to improve the immunogenicity of CS protein subunit vaccines in humans, and to determine if the immunity elicited by immunization with FSV-1 will protect against sporozoite challenge.

Table 1. Immunogenicity of *Falciparum* sporozoite vaccine (FSV- 1).

| Species | Dose (μg) | ELISA* | CSP† 0/2+/4+ | ISI‡ (%) |
|---|---|---|---|---|
| Mice | 10 | 1:6400 | 6/6/13 | 100 |
| Rabbits | 100 | 1:5800 | 6/10/9 | 97–100 |
| Humans | 10–800 | <1:100 | 21/4/0 | 12–85 |

* Maximum dilution of serum giving an OD value ≥1. Data based on a pool of 5 mouse sera or the geometric mean titer of 4 rabbits or 15 humans.

†Average number among 25 sporozoites that were negative (0), had a granular precipitate (2+), or had a filamentous precipitate (4+) when reacted with a 1:2 dilution of serum.

‡Range of inhibition of sporozoite invasion into hepatoma cells by a 1:20 dilution of serum.

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# Phase I and II Trials of *Plasmodium Falciparum* Synthetic Peptide Sporozoite Vaccine

DEIRDRE A. HERRINGTON

The purpose of phase I vaccine trials is to ensure that the vaccine in question is safe when administered to humans, and that it produces the intended immune response. The controlled settings in which such trials are carried out permit the acquisition of detailed safety data and the collection of necessary clinical specimens. Such safety and immunogenicity data can then be used to select the optimum vaccination regimen for trials of vaccine efficacy, or phase II.

We have recently initiated phase I of a *Plasmodium falciparum* sporozoite vaccine, which was developed at New York University and prepared and formulated in collaboration with the Hoffman LaRoche Company. The vaccine consists of a synthetic peptide, comprised of three tandem repeats of the repetitive amino acid subunit (asparagine, alanine, asparagine, proline) of the immunodominant portion of the circumsporozoite protein, which is conjugated to tetanus toxoid. By weight, the conjugate vaccine is about 70% tetanus toxoid. The vaccine formulation includes alum as adjuvant.

The vaccine trial is taking place at the Center for Vaccine Development, which is part of the Department of Medicine at the University of Maryland. For the past 12 years, the center has been conducting both inpatient and outpatient studies of a variety of vaccines, all in community volunteers. Volunteers hear about us through word of mouth and advertising in local papers and on college campuses. For the current malaria vaccine study we have vaccinated 45 male students, mostly from the medical and dental schools, between the ages of 20 and 30.

The current phase I vaccine trials are being conducted in a double-blind fashion. In our North American volunteer population, there is universal previous exposure to tetanus toxoid, thus we wanted to be able to compare any adverse reaction from the conjugate vaccine with reaction to tetanus toxoid alone. We also excluded volunteers who had a history of tetanus toxoid administration within the previous three years. One

"unblinded" nurse drew up each dose of vaccine or tetanus toxoid control, and kept the code describing which volunteers were recipients of the conjugate vaccine versus tetanus toxoid control. All syringes appeared identical, and contained the same volume and amount of alum. All injections were carried out by a different nurse and were given intramuscularly in the triceps muscle.

Twenty- and 50-$\mu g$ doses were given to small groups of four volunteers each and were intended primarily to test for safety. We immunized two larger groups of 18 and 19 volunteers with 100 or 160 $\mu g$ of vaccine or control, respectively, and plan to use the same doses for booster vaccinations at 1- and 2-month intervals, assuming that the boosters are tolerated well in the lower-dose groups.

The volunteers were monitored as follows: Each vaccinee was closely observed for 30 minutes postvaccination, and had his temperature, pulse, and blood pressure recorded just before, and 15 and 30 minutes after the injection. Upon discharge from the outpatient unit, the volunteers were instructed to record their temperature and pulse every 6 hours, and were asked to return for follow-up at 24 and 48 hours postvaccination. At this time they were interviewed and examined, and had their urine tested for protein.

The primary vaccinations were well tolerated. Most volunteers experienced little or no pain with the immunization, and there were no allergic reactions. Although two of the 45 volunteers did not return for their 24-hour follow-up, and one did not return for the 48-hour follow-up; all were seen at least once in the 48-hour period after vaccination. At 24 hours, half of the volunteers had mild local pain at the vaccination site, which most described as a small bruise, but it did not interfere with their usual activities. Thirty-three percent had a small amount of erythema, never more than 4 mm and usually less than 2 mm; only 7 of 43 had any induration, which again was less than 4 mm. One individual had a mild fever of 38.1 °C, associated with a mild headache and malaise, which occurred 12 hours after vaccination and lasted about 3 hours.

At 48 hours, 40% of vaccinees still experienced tenderness. Three individuals described some worsening of the pain, "as if they had been hit in the arm," but they were able to use their arms normally. Erythema was experienced by 23% of the volunteers and induration by 16%. About half felt tenderness when pressure was applied. There was no fever, and no vaccinee has had any proteinuria to date, which includes the 2-week follow-up. These safety data are summarized in Tables 1–3.

Sera and lymphocytes are being collected from these volunteers for testing the immune response to the vaccine. None of the results is available yet but antibody to the synthetic peptide will be analyzed by ELISA and radioimmunoassay, and the ability of sera from vaccinees to

Table 1. Clinical findings 24 hours after vaccination with *P. falciparum* synthetic peptide sporozoite vaccine.

| Local Pain | | Signs at Injection Site | | | | |
|---|---|---|---|---|---|---|
| Mild | Moderate | Erythema | Induration | Tenderness | Fever | Proteinuria |
| 21/43 (49%) | 0/43 | 14/43* (33%) | 7/43† (16%) | 23/43‡ (53%) | 1/44§ | 0/43 |

*≥2 mm, no individual had >4 mm erythema.
†≥2 mm, no individual had >4 mm induration.
‡One individual had moderate tenderness, remainder had mild tenderness.
§One individual with temperature of 100° F (38.1° C) for 3 hours, beginning 12 hours after vaccination.

Table 2. Clinical findings 48 hours after vaccination with *P. falciparum* synthetic peptide sporozoite vaccine.

| Local Pain | | Signs at Injection Site | | | | |
|---|---|---|---|---|---|---|
| Mild | Moderate | Erythema | Induration | Tenderness | Fever | Proteinuria |
| 16/44 (36%) | 3/44 (7%) | 10/44* (23%) | 7/44† (16%) | 21/44‡ (48%) | 0/44 | 0/44 |

*≥2 mm, 2 individuals had 5 and 7 mm of erythema, respectively.
†≥2 mm, 2 individuals had 5 mm induration.
‡One individual had moderate tenderness, remainder had mild tenderness.

inhibit invasion of sporozoites into cultured hepatoma cells will be tested elsewhere. Finally, the ability of lymphocytes to be stimulated by the peptide will be tested in a lymphocyte proliferation assay. The results of this testing will determine whether phase II trials will proceed with this vaccine.

Phase II trials of vaccine efficacy can be carried out safely and with relative ease for sporozoite vaccines. A vaccine failure will be manifested by the appearance of blood stages of *P. falciparum* following sporozoite challenge; therefore treatment can be immediately instituted minimizing any risk to the volunteer from clincial illness with *P. falciparum*. This is an important factor in studying the efficacy of such vaccines in nonimmune populations in whom there are great ethical constraints regarding *P. falciparum* experimental infections. It is crucial that the volunteers be followed for a period considerably longer than the usual incubation period, because a vaccine that is not 100% effective may permit very small numbers of viable sporozoites to penetrate hepatic cells, leading to a delay in patency as detected by blood smears. It is likely that sensitive DNA probes that are becoming available may be useful in detecting vaccine failures resulting from less than 100% vaccine efficacy.

In January 1985, we tested a challenge model for phase II trials of sporozoite vaccines in four volunteers. The study was conducted at the

Table 3: Safety studies of *P. falciparum* sporozoite vaccine following primary immunization and two booster immunizations of 20, 50, 100, or 160 μg conjugate vaccine vs tetanus toxoid control.

| | Primary Vaccination | | First Booster | | Second Booster | |
|---|---|---|---|---|---|---|
| | Vaccinee | Control | Vaccinee | Control | Vaccinee | Control |
| Local Pain | 57 | 70 | 35 | 10 | 24 | 20 |
| Erythema | 40 | 40 | 44 | 30 | 14 | 20 |
| Induration | 33 | 10 | 18 | 10 | 14 | 10 |
| Tenderness | 63 | 50 | 41 | 30 | 41 | 50 |
| Fever | 3* | 0 | 0 | 0 | 3* | 0 |
| Proteinuria | 0 | 0 | 0 | 0 | 0 | 0 |

Numbers represent percentages. Total number of volunteers was 45, 44, and 39 in the three immunization periods, respectively.

*One individual had fever following primary and second booster immunizations but not following the first booster. The fever occurring after the second booster was probably secondary to an unrelated viral infection.

Center for Vaccine Development in collaboration with the Biomedical Research Institute (which provided the infective mosquitos) and New York University School of Medicine. There are two important aspects of this model. First is the use of a suitable and safe blood supply for the in vitro cultures of *P. falciparum* and the early detection and treatment of parasitemia. Gametocytes were obtained from in vitro cultures using blood products from donors who had been extensively screened and were known to be healthy. The availability of a safe blood source must be emphasized because a mosquito could theoretically serve as a vector for viruses and other infectious agents present in the blood sources used for in vitro cultures and membrane feeding. In this model we used a volunteer supply to minimize any risk of introducing adventitious agents. The second aspect of this model is that by maximizing chances for the earliest possible detection of patency, early therapy was given and the morbidity of the infection minimized. We accomplished this by frequent and compulsive reading of blood smears.

We used the nf54 strain of *P. falciparum* because of its known chloroquine sensitivity and ability to infect mosquitoes from in vitro culture. The mosquito vector was *Anopheles stephensi*. Sporozoite challenge was accomplished as follows: A mosquito was allowed to fully feed on a volunteer. Immediately thereafter the mosquito salivary glands were dissected and microscopically examined for sporozoites. Five infected mosquitoes were allowed to feed fully on each volunteer.

Blood smears were obtained from volunteers at 12-hour intervals, beginning six days after sporozoite challenge. On day 11, parasites were found in the blood of all four volunteers. The maximum parasitemia ranged from 98 to 213 parasites per cubic millimeter of blood. At the time

patency was demonstrated, none of the four volunteers had symptoms of clinical malaria, and chloroquine therapy was immediately instituted with prompt clearing of parasitemia. One volunteer went on to develop typical symptomatology of malaria approximately 6 hours after chloroquine was administered, but recovered uneventfully after the standard oral regimen of chloroquine. We are thus confident that the efficacy of sporozoite vaccines can be studied with minimal risk and discomfort to volunteers.

## ADDENDUM

Data on the humoral immune response to this synthetic peptide sporozoite vaccine have become available since the time of the meeting. A clear dose response to vaccination was demonstrated in an ELISA, which detects serum IgG antibody against the repeating (asparagine-alanine-asparagine-proline) epitope. One of 7 vaccinees who received 20 or 50 μg of vaccine, 5 of 15 vaccinees who received 100 μg of vaccine, and 8 of 14 vaccinees who received 160 μg of vaccine developed fourfold or greater rises in IgG antibody titer following primary immunization. Antibody levels did not change significantly following booster vaccinations. Results of the ISI assay (inhibition of sporozoite invasion into hepatoma cells) and lymphocyte proliferation assays are currently pending.

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# Current Status of Asexual Blood Stage Candidate Vaccines of *Plasmodium Falciparum*

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Numerous proteins have been identified by research groups worldwide as potential candidates for an asexual blood stage malaria vaccine. Experiments have demonstrated that proteins on the merozoite surface or in the merozoite rhoptries can partially or completely protect *Aotus* (owl) or *Saimiri* (squirrel) monkeys against a lethal challenge infection of *Plasmodium falciparum*. We shall review these experiments briefly.

## MEROZOITE SURFACE COAT PRECURSOR PROTEIN

The major merozoite surface proteins have relative molecular weight (83,000, 42,000, and 19,000 [83k, 42k, and 19k]) and are enzymatically split from a glycosylated, 185–200k (gp185–200) (p195) protein found in *P. falciparum* schizonts.[1] This protein is probably the most well characterized of the blood stage antigens.[2–5] The molecule is polymorphic, having determinants conserved in strains throughout the world and determinants specific to strains found only in given geographical regions.[6–8] The polymorphism contributes, in part, to the variable molecular weight of the precursor protein reported by various research groups. Three groups have published the complete gene sequence for the protein from three strains.[9–11] Three vaccination experiments have shown that the precursor molecule can induce either partial or complete protective immunity. Researchers at the University of Edinburgh immunized squirrel monkeys with the precursor protein from a Thai strain and modified the course of infection of the *P. falciparum* Uganda Palo Alto (FUP) strain.[12] Two of three monkeys did not require drug treatment and a parasitemia of $10^7$ was reached before the infection was controlled.[9] In the second vaccination experiment, researchers at the Geneva University Hospital immunized squirrel monkeys with the precursor protein from a Zaire strain and challenged with the African FUP strain.[13,13a] Maximum parasitemias ranged from 2 to $10^7$, but none of the four monkeys required

drug treatment. In the third vaccination experiment, owl monkeys were immunized with the precursor from the Ugandan (FUP) strain and challenged with the same FUP strain. No parasites could be detected in thick blood films following challenge, and the monkeys were completely protected.[14] These three experiments show that the merozoite surface coat precursor protein can induce complete protection against the homologous strain, and partial protection against heterologous strains.

## RHOPTRY PROTEINS

A second group of protective antigens lies in the merozoite rhoptry organelles. The rhoptries are a pair of electron dense, flask-shaped organelles at the apical end of merozoites. It is believed that the contents of the rhoptries play a prominent role in the merozoite invasion process. A number of distinct proteins have been identified in the rhoptries. A 155k protein formerly described on the surface of ring-infected erythrocytes (RESA)[15,16] was recently shown to originate in the micronemes adjacent to the paired apical rhoptries.[17] As demonstrated by the Scandinavian group,[18] antibody to this protein strongly inhibited merozoite reinvasion in vitro. Researchers at the Walter and Eliza Hall Institute have cloned the gene to RESA, and, in collaboration with workers at the Centers for Disease Control in Atlanta, *Aotus* monkeys were immunized with the recombinant protein comprising the 5' repeat of RESA. All monkeys were partially protected and had parasitemias of 2–3% or less.[19] In another vaccination experiment involving a rhoptry protein, workers at the University of Geneva Hospital isolated a 41k protein with a monoclonal antibody shown to inhibit parasite growth in vitro,[20] and showed that protection could be achieved against heterologous challenge.[21] The protection was strong with a "native" protein, but weaker with the "denatured" protein.

## OTHER CANDIDATE PROTEINS

Workers at the Pasteur Institute have isolated polypeptides from the 75k region of preparative polyacrylamide gels and shown that they can partially protect squirrel monkeys.[22] Several polypeptides may be included in this preparation, including processing fragments of pf185–200. The group from Scripps Research Institute has cloned a gene for a 75k *P. falciparum* protein,[23] but it is not known if this molecule can induce protection. Similarly, the gene for the protein that binds to glycophorin on the erythrocyte surface has been identified and cloned;[24,25] however, it is not yet known if this molecule is protective. Finally, parasite-infected erythrocytes are sequestered in the deep vasculature and the molecules

responsible for cytoadherence may be important targets.[26] However, there are as yet no vaccination experiments in monkeys indicating the potential of these proteins as candidate vaccine antigens.

## STRAIN SPECIFICITY AND ANTIGENIC POLYMORPHISM

Several major questions remain unsolved. It is not known what role antigenic polymorphism will play in the malaria vaccine; however, several studies have suggested that immunity to malaria in nature is strain specific. In the early 1960s, McGregor passively transferred human IgG obtained from East Africans into nine West Africans.[27] Parasitemias in five of the nine recipients fell rapidly; however, in the remaining four recipients, the initial fall was followed by a rise in parasitemia. One interpretation of this experiment is that antigenically diverse parasites resided in the West African region, and only some of these were sensitive to antibodies in the immune sera from the East Africans. In addition, individuals residing in endemic areas can experience repeated bouts of malaria. Only at adolescence, when individuals develop antibodies to all the strains in the given area, are they semi-immune. Thus, immunity in endemic areas appears to be strain specific.

If immunity is strain specific, the prospective vaccine candidates should exhibit antigenic polymorphism. pf195 is a major block-stage antigen in which polymorphism has been partially characterized. Using a panel of monoclonal antibody, McBride classified *P. falciparum* isolates into seven distinct groups.[28] A Thai isolate was cloned by limiting dilution, and shown to contain a mixture of parasites with immunologically distinct types and falling into groups I (T9/94), (T9/105), II (T9/101), V (T9/96), and VII (T9/102). Thus, it is possible that individuals residing in this area need to be exposed to all the serotypes in this region, before full immunity is achieved.

In the monkey vaccination experiments with pf195, only partial protection was observed when monkeys were immunized with Thai[12] and Zaire[13] isolates, respectively, but challenged with the Uganda isolate. When we immunized and challenged with the Uganda isolate (homologous challenge),[14] no parasites were detected and the monkeys were completely protected. These observations indicate that circulating antibodies to conserved epitopes on proteins may be only partially protective, and that the parasite may survive in the presence of these antibodies. Rather, antibodies to the nonconserved, strain-specific epitopes may be more important in suppressing parasitemia and clinical symptomatology. Whether it will be possible to vaccinate humans with the conserved, constant regions of protective molecules (e.g., RESA), and have natural infections in endemic areas induce and boost immunity to the strain specific is not known.

## ADJUVANTS

A second problem is the identification of a safe and effective adjuvant. In 1977, we found that immunization of owl monkeys with whole, schizont-enriched preparations of *P. falciparum* induced strong protective immunity.[29] In one monkey, no parasites could be detected in thick blood films following challenge infection with the homologous strain. In subsequent studies with synthetic adjuvants, the protection was always incomplete, with parasitemias reaching 10% in some cases.[30,31a] In addition, a recent pilot experiment with merozoite surface and rhoptry proteins indicated that immunization with Freunds' complete adjuvant, but not a synthetic muramyl-dipeptide derivative, was required to induce protective antibody responses, although the antibody titers to dominant epitopes on the molecules were similar.[31] This may indicate that the protective epitopes are weakly immunogenic and that strong adjuvants will be required to potentiate antibody responses to these determinants. This is especially true in the case of recombinant proteins and synthetic peptides, which have been shown to be less immunogenic than their native protein counterparts and induce weak anamnestic responses. Thus, a safe and effective adjuvant must be identified for clinical trials.

## CONCLUSION

Direct immunization of susceptible monkeys with proteins isolated from parasites with monoclonal antibodies, or in one case with a recombinant protein (RESA), have shown that varying degrees of protection against a lethal *P. falciparum* infection can be achieved. To date the most promising candidates are pf195 and RESA (Table 1). The genes to these antigens have

Table 1. Indirect and direct evidence for protection by candidate *P. falciparum* vaccine antigens.

| Candidate Antigen | Ab Inhibits In Vitro | Protective in Monkeys | Gene Cloned |
|---|---|---|---|
| gp185–200 (pf195) | +* | +[12,13,14] | +[9,10,11] |
| RESA† | +[18] | +[19] | +[16] |
| 41k rhoptry | +[20] | +[21] | |
| p75 | | +[22] | 23 |

*Serum samples from monkeys immunized with pf–195-FUP in a pilot[31] and a subsequent full vaccination experiment,[14] in which no parasites could be detected after challenge, inhibited parasite growth by >90% in vitro (W.A. Siddiqui et al, unpublished data). Human monoclonal antibodies against pf195 strongly inhibited parasite growth in vitro.[32] (Schmidt-Ullrich R, et al. *J Exp Med* 1986; 163:179–188.)

†Ring-infected erythrocyte surface antigen.

been cloned and are being expressed in *Escherichia coli* or yeast vectors. Future work must determine whether conserved or nonconserved, strain specific epitopes on these molecules will be effective in the field. In addition, a safe and effective adjuvant suitable for human use must be identified. Nevertheless, the direct demonstration that susceptible monkeys can be completely or partially protected against a lethal infection of the human malaria parasite *P. falciparum* promises that an asexual blood stage malaria vaccine may be available soon for clinical trials.

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# Current Status of Transmission-Blocking Malaria Vaccines

J. H. E. Th. MEUWISSEN

Immunization of the vertebrate host with immunogens of the extracellular sexual stages of the malaria parasite can induce the production of specific circulating antibodies. These antibodies can block the development of the midgut stages of the parasite, when the antibodies are ingested by the mosquito during the infectious bloodmeal. Consequently, the primary objective of future transmission blocking vaccines will be to induce a reduction in the level of transmission intensity in endemic malarious areas.

Progress made in this field of studies can be summarized as follows:

1. In vitro produced fertile gametocytes of *Plasmodium falciparum* have been used for the production of monoclonal antibodies.
2. These monoclonal antibodies have been tested for their transmission-blocking capacity by membrane feeding *Anopheles stephensi* mosquitoes with suspensions of fertile gametocytes mixed with the monoclonal antibodies.
3. Macrogamete-zygote suspensions have been surface labeled with $^{125}$I, and, in this way, 230, 48, and 45 kD surface proteins have been identified by various monoclonal antibodies.
4. On Western blots of macrogamete proteins, monoclonal antibodies recognized an additional major 25 kD protein. (Table 1 summarizes the main characteristic of these three target proteins and their epitopes.)
5. Transmission-blocking antibodies occur in sera collected from people living in malarious areas. Their practical significance remains to be assessed.
6. Genomic *P. falciparum* DNA as well as cDNA libraries based on mRNA of gametocytes have been constructed. Bacterial clones selected by transmission-blocking polyvalent rabbit antimacrogamete serum produce fusion proteins.

a. These fusion proteins have not been successful in producing antisera in laboratory animals that specifically react with macrogamete target proteins in Western blots.

Table 1. Potential target proteins of transmission-blocking immunity in *P. falciparum*

| |
|---|
| *230 kD Protein* |
| Surface protein of gametes (newly formed zygotes?) |
| Absent on developing ookinetes |
| Synthesis starts by day 2 of gametocyte development |
| Not glycosylated, not hydrophobic |
| Role in transmission-blocking immunity still to be confirmed |
| *48 and 45 kD Proteins* |
| Doublet surface proteins of female gametes |
| Absent on developing ookinetes |
| Synthesis starts by day 2 of gametocyte development |
| Isoelectric point: pI = 6.0 ± 0.1 |
| Glycoproteins |
| Target of transmission-blocking immunity; likely effector mechanism |
| Inhibition of macrogamete fertilization |
| At least three different nonrepetitive epitopes are recognized by MoAbs |
| Transmission blocking epitope sensitive for the combination of SDS and DTT |
| Some evidence of antigenic diversity of one of the epitopes |
| *25 kD Protein* |
| Surface protein of activated female gametocytes, zygotes, ookinetes, and oocysts |
| Synthesis starts by day 2 of gametocyte development, but production is largely predominant following gametocyte activation |
| Isoelectric point: pI = 5.6 ± 0.1 |
| Glycosylated, acylated |
| Target of transmission blocking immunity; likely effector mechanism: lysis of maturing ookinetes |
| At least two different nonrepetitive conformational epitopes |
| Antigenic diversity of the "transmission blocking" epitope. |

$M_r$ = Relative molecular mass (apparent molecular weight) on SDS-PAGE under nonreducing conditions.

b. The fusion proteins coupled to a solid phase carrier were used for immunoabsorption of transmission blocking polyvalent rabbit serum. Some of the resulting eluted antibodies gave a positive IFA test with macrogametes, but they did not react with the target proteins on Western blots.

c. Affinity purified target proteins, coupled to a solid phase carrier and used for immunoabsorption of transmission-blocking polyvalent rabbit serum, resulted in eluted antibodies which identified bacterial clones. However, sera of mice immunized with the fusion proteins of these clones did not react with macrogamete target proteins on Western blots.

Epidemiological field studies should be initiated to collect quantitative data on transmission intensity. Parasitological, serological, and especially

entomological data, longitudinally collected in specific malarious foci, should provide a basis on which to decide if and when future malaria vaccines can be usefully applied under the prevailing circumstances for the control of malaria. With regard to transmission-blocking vaccines, there is a need for quantitative data on the size of the infectious gametocyte reservoir.

# Application of Biotechnology to Human Malarial Parasite Proteins

ROBERT T. REESE

Scientists presently studying malarial parasites have in their arsenal an ever-growing variety of techniques that we hope will not only allow us to identify, but to produce antigenic structures that will stimulate immunity. At present, monospecific antibodies (either monoclonal, or polyclonal produced against cloned expressed gene products) can be used to determine the molecular weight of the parasite protein under study (by immunoprecipitation assays or Western blots), as well as to demonstrate its localization within the organism (by immunofluorescense or immunoelectron microscopy). These same antibodies can be used to appraise the structural heterogeneity of the protein among various parasite isolets from around the world.

Recombinant DNA methods allow the cloning of gene fragments that encode portions of immunologically important proteins, and make it possible to predict a probable protein sequence from the DNA sequence. The host containing the recombinant genes can also produce the parasite (or fragment of it) in relatively large amounts. This provides a potential source of material for immunoprophylaxis once the appropriate proteins have been identified.

In addition, synthetic peptide chemistry is becoming an increasingly useful tool for analysis of the immunodominant portions of molecules, as well as production of antigens for immunization and diagnostic purposes. Increasingly sophisticated computer programs for structure prediction, used together with physical techniques such as circular dichroism, nuclear magnetic resonance, and x-ray crystallography, allow predictions of not only the secondary but the tertiary structures of molecules. Most of these tools are being used to study some of the asexual blood stage proteins as well as the circumsporozoite protein.

Monospecific antibodies have been used to demonstrate that although sporozoites may be coated with a single major protein, merozoites are not. Merozoites have a complex surface composed of both glycoproteins and nonglycosylated structures (Tables 1 and 2). They appear to be organized in two layers, the myristylated glycoproteins directly interact-

Table 1. Merozoite surface proteins not labeled with $^{3}$H-glucosamine.

| $M_r \times 10^{-3}$ | Stimulates Protective Immunity | In Most Isolates | Glycophorin Binding | Clone Available Encoding Surface Epitope | References |
|---|---|---|---|---|---|
| p83* (gp185) | + | + | | + | 1 |
| | | + | | | 2 |
| | | + | | | 3 |
| p180, p59 | | + | | + | 4 |
| p140 | | + | | + | 4 |
| p130 | | | + | + | 6 |
| p155 | + | | | | 7 |
| | + | | | | 8 |
| p75 | + | | | | 9 |
| | | | + | | 5 |
| | | + | | + | 4 |

Table 2. Merozoite surface glycoproteins.

| $M_r \times 10^{-3}$ | Stimulates Protective immunity | Lipophilic | In Most Isolates | References |
|---|---|---|---|---|
| gp190(83)* | + | | | 10 |
| gp56† | | + | – | 11 |
| gp41, 46 | | + | + | 2‡ |
| | | | + | 3‡ |
| | | | + | 12‡ |
| | + | | + | 8‡ |
| | | + | | 13‡ |
| gp34 | | + | + | 14 |
| | | | + | 2 |

*No evidence for surface location (size of native protein unpublished).

†gp50, which is found in many isolate, may be an analogue of gp56.

‡Although the molecules studied by different groups vary in molecular weight and have not all been shown to be glycosylated, they are all likely to be the same molecule.

Table 3. Polypeptides associated with *Plasmodium falciparum* infected erythrocyte plasma membranes

| Molecular Weight | Silver Stain | Membrane isolation: Metabolically Labeled* | Membrane isolation: Iodinated |
|---|---|---|---|
| >240 | + | + | – |
| 150 | + | | – |
| 96 | | – | – |
| 55 | + | + | + |
| 45 | + | | + |
| 35 | + | + | + |
| 20 | | + | + |

*Metabolically labeled from ring stage trophozoite stage with [$^{35}$S]-methionine.

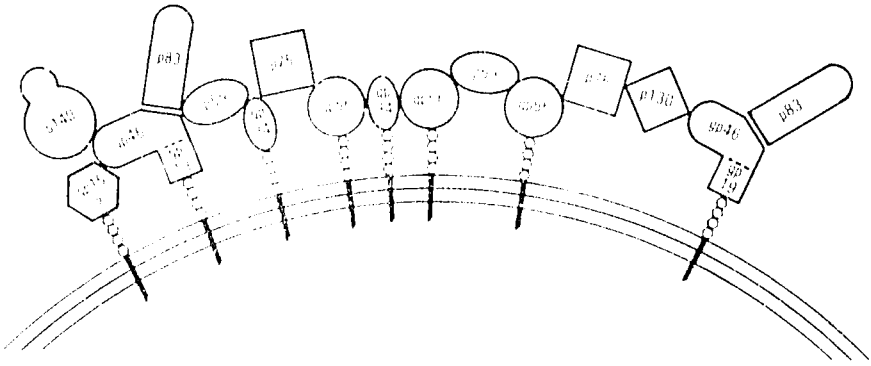


Figure 1. Model of *Plasmodium falciparum* merozoite plasma membrane. Nonglycosylated proteins p59, p75, p130, p140 and the p83 fragment of gp185 are shown as being attached to the glycoproteins in an arbitrary fashion. The glycoproteins gp15, gp19 (which appears to be a fragment of gp46), gp34, and gp56 are depicted as being linked to the lipid bilayer on the merozoite surface through the glycan which contains myristic acid at its terminus.

ing with the lipid bilayers of the merozoite, the nonglycosylated molecules probably are attached via protein-protein interaction being bound to the glycoproteins, not the lipid bilayers themselves (Fig. 1). In addition, once a merozoite enters a host erythrocyte, it dramatically alters the erythrocyte's plasma membrane (Table 3). At least eight proteins of parasite origin become associated with this membrane during the ring to trophozoite stages (Fig. 2). Gene fragments encoding portions of many of these proteins have been cloned. During the upcoming years the immunophrophylactic capacities of these molecules, used independently or in groups, should be studied.

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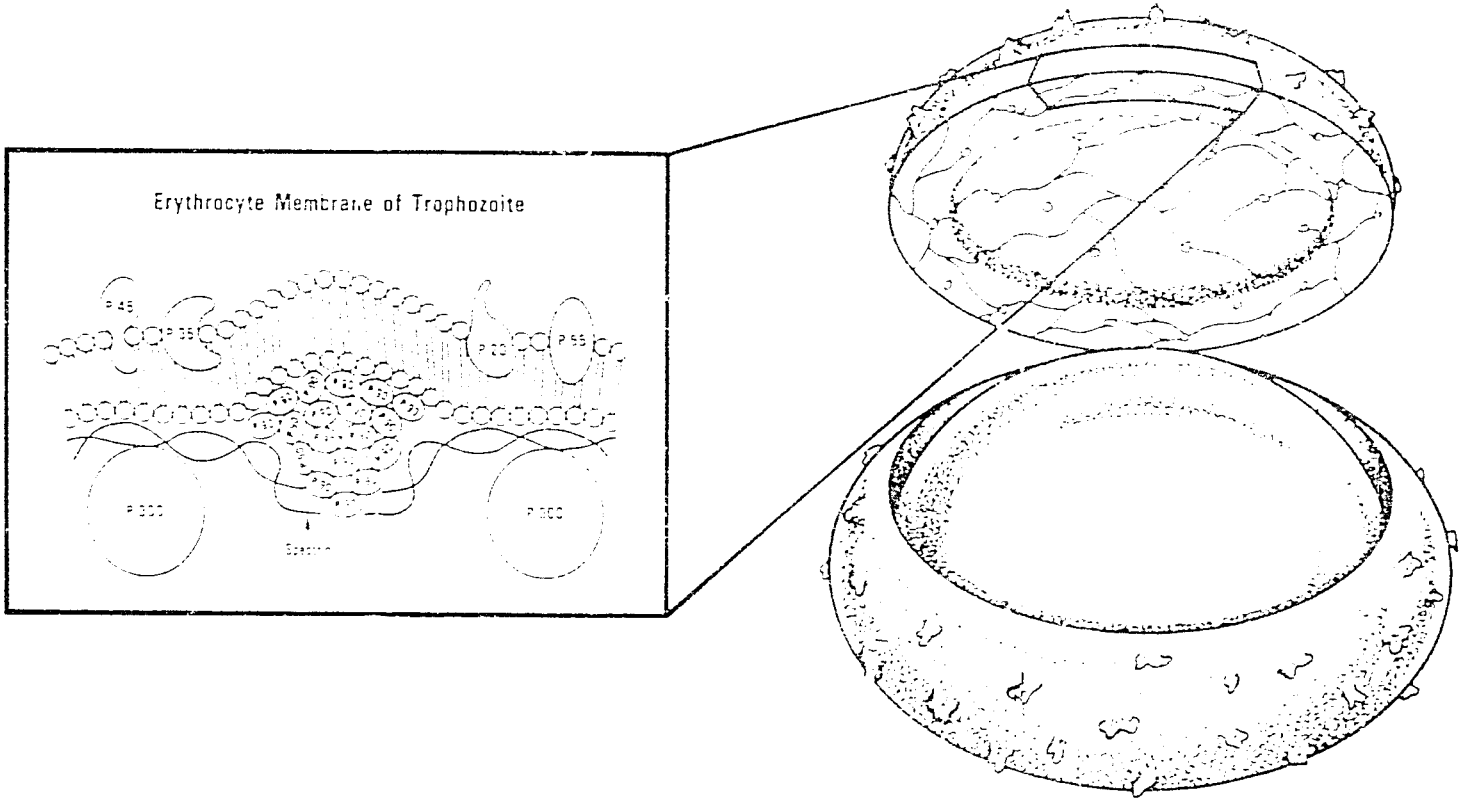


Figure 2. Model of plasma membrane of erythrocyte infected with *Plasmodium falciparum*. The knob protein (p90) and phosphoprotein pp-300 are linked to the erythrocyte membrane skeleton as is p150 (HRPA) which is not shown. At least 4 proteins (p20, p35, p45, and p55) appear to have portions that extend through the lipid bilayer into the aqueous environment outside the cell.

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# Invited Discussion

L.H. MILLER

I think a lot of the concern today about malaria is that with the high transmission in Africa of parasites resistant to chloroquine, the future may be very difficult for the children in this area. Our great hope that vaccines will have an impact in Africa is the reason this meeting is called. It is a chance for the African scientists to interact with each other and with scientists working in the more rarified atmosphere away from the real world.

The excellent reviews presented by the previous speakers give you a sense of where things are going. I think that one can conclude that there will be vaccines and that they will be effective, judging from all the work on animals that has been done to date. These vaccines will be successful in part because of a continuing commitment of funding. Without this funding, we would not be hearing anything today. Funding is from World Health Organization, Agency for International Development, foundations, and various national governments that have made a long-term commitment to malaria research and control. I hope that this commitment will continue, despite the fact that in many of these countries malaria is no longer a problem. One of the better aspects of the foreign policy of the United States and other countries is their commitment to helping an international problem.

However, I want to offer a bit of caution about the time scale. To me, the most important discovery of malaria was the fact that mosquitoes transmit the disease. Ronald Ross who made this great discovery felt that malaria could be eliminated from Africa. In fact, in the 1920s, a very austere group of the equivalent of the World Health Organization said that despite the importance of this discovery, we had little hope that this approach would be useful, and that scientists should be using drugs and working with patients and not wasting time with mosquitoes. Within a few years, of course, Mueller discovered DDT and this view of the 1920s was nonsense. So, we oscillate between those scientists who have great hope and those who say that basic research is not useful. Because science has its own pattern, one cannot make it happen faster than the discoveries will permit. In the case of controlling malaria through mosquitoes, it was

about 50 years from the discovery of the mosquito as the vector until the time when Mueller discovered DDT.

I think chemotherapy had a similar history; it was about 40 years from the time that Ehrlich started working with methylene blue as treatment until the 4-aminoquinolines were synthetized by the German organic chemists. But, things can happen rapidly or slowly. We cannot predict scientific progess. We have to accept the pace of science; we must take it as it comes. Some believe that applications happen faster today because of molecular biology. I think science moves at a similar rate today as it has in the past, at least as far as the impact of discovery on human health is concerned. Although discoveries are very exciting and important, they do not necessarily lead to an immediate application. The experiments and the research talked about today reflect work from around the world. This work will have an impact; whether it be tomorrow or in 10 years, we cannot predict. This is my warning. The tempo is unpredictable.

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# General Discussion

BREMAN: Is there any evidence that younger children, as the group who is at greatest risk, have less response to CS protein than older persons? These children live in a veritable sea of exposure, and one would expect them to have an even higher percentage of positive sera. Are there some biological reasons, or is it a question of sensitivity to the test, or are there some other reasons I have not thought of?

NUSSENZWEIG: In the study from the Gambia, which I used as an example, 40% of the children had antibodies and most of the adults. The low antibody titers were practically all found in children. As the age increased, the titers increased, too. The highest titers were found in adults over 50 years of age.

BRUCE-CHWATT: I have a question for Dr. Chulay. What struck me very forcibly was the tremendous difference between the antibody responses in Dr. Chulay's volunteers and the serum obtained from Dr. Clyde's 1973 subject, who was immunized with the sporozoites subjected to treatment by irradiation. I would not have expected the difference to be so great, especially if you remember that 5 years have passed since those experiments were conducted. What is the difference between the amount of immunizing antigen in Dr. Clyde's old experiments and the present tests?

CHULAY: That is a very difficult question to answer. In the immunization with irradiated sporozoites, heavily infected mosquitoes were allowed to feed on human volunteers. It required a minimum of 400 mosquitoes. I do not know exactly how many infected mosquitoes the volunteer got. As I recall, he got at least 800 before he was first protected. He was subsequently boosted with additional infections. I have no idea how many sporozoites were injected by the irradiated mosquitoes. There is no good way of quantifying the amount of CS protein per sporozoite. Therefore, I cannot comment on whether or not our volunteers got more or less antigen. It is very possible that the method of presentation of the antigen is critically important, and that presenting the antigen on the surface of a sporozoite may make it more immunogenic.

ROSE: To carry on that point, and perhaps extend it to the findings that Dr. Miller just discusssed, one would wonder if one of the markers that

might be used in assessing immune response in the human volunteers that have already received the vaccine is their HLA type. I wonder if either one of the two clinical trials underway was making note of the HLA haplotypes of the subjects.

LEVINE: I will respond for the Maryland trials. What we are waiting to do is to look at the serum and cellular immune responses first. In persons who are good responders, we will determine the HLA allotype. The reason for our approach is quite simple—it is fiscal. Complete HLA allotyping these days costs over $100 per test. It just does not seem appropriate to do that routinely without knowing what the extent of the immune response will be.

BRUCE-CHWATT: I have a question for Dr. Levine. We have seen quite a lot of genetic differences in mice. But, we have to go from mice to humans. I would like to know if, in the volunteers participating in the human tests, the problem of genetic constitution (e.g., the problem of HLA antigen) has been taken into account.

LEVINE: Again, our approach in our vaccine studies is to look first at the immune response, and then at the HLA allotype. If your question is also directed at the evaluation of vaccine efficacy with respect to the challenge model that Dr. Herrington described, then, indeed, knowledge of the genetic background is critical. This is based on the wealth of experience that Dr. Clyde had in his volunteer studies with induced malaria, which showed a striking difference in severity by the genetic background of the volunteers.

KAFOMY: I am impressed by Dr. Chulay's observations with aluminum oxide adjuvant. I am interested in why he feels that this observation is different from what one observes in human primates. I am aware that using aluminum oxide in squirrel monkeys requires you literally to have a one-to-one ratio of adjuvant to antigen, in order to demonstrate the immunogenicity of this protein. I am referring particularly to the 83 kD protein. I would be interested in Dr. Chulay's comments.

CHULAY: I have no experience with comparing aluminum with other adjuvants in primates with this or any other malaria antigen. As a clinician, my first concern for letting something be injected into humans is that the substance being used is safe.

Therefore, we have purposely chosen to evaluate alum as the only practical adjuvant that has been and can be used in humans. That is why we focused on it. We were pleasantly surprised when we used it in both mice and rabbits and found it was able to induce high antibody titers. We were obviously disappointed with the results that we found in humans. I do not know whether or not this will hold true for other malaria vaccines when we attempt to adjuvate them with alum in humans.

NUSSENZWEIG: I would like to cover rapidly a few points. The first one concerns genetic restriction. I am not as pessimistic as Dr. Miller in the sense of forseeing by necessity a genetic restriction of man in response to *P. falciparum* or *P. vivax* antigen. I would just like to call attention to the fact that we and others found over 90% of people making antisporozoite antibodies. Obviously, if there is genetic restriction, it is not that severe. Of course, as I pointed out there might well be a genetic restriction to the peptide to one region of the protein. That is exactly the reason why one is now moving to identify other regions that might sensitize other T cells. But the fact remains that most of the people respond to the CS antigen of sporozoites. The second comment I would like to make is in relation to alum versus Freund's complete adjuvant. The experience with Freund's complete adjuvant working and giving protection in monkeys, or whatever system you have, is strictly limited to the blood stages.

Our experience with sporozoites is quite opposite. These are different antigens. The fact is that you immunize very well with sporozoites without any adjuvant. Finally, as for the circulating antigen, again you are referring to circulating antigen of blood stages. There is just not enough sporozoite antigen in circulation.

SECTION II

# MALARIA AND MALARIA CONTROL IN AFRICA

F. DIOUF AND L. BRUCE-CHWATT
*Chairpersons*

## SECTION II

DIOUF: I apologize that I will have to speak French because I cannot speak English very correctly. At the beginning I will speak French, but we will turn over the microphone to the English-speaking people. But, at least for some time, French will be spoken while I will be presiding over the meeting.

After having listened to the statements this morning on vaccine, we will go now to the field with comments on transmission and situation reports. Some of our colleagues from Africa will be describing later the very special concept or understanding that Africans have of malaria.

As you know, malaria in Africa is multifaceted. Several epidemiological aspects are quite different from other regions of the world. We think it is extremely important to do field studies of what goes on in Africa because the problems that have been posed in the past 25 years are still the same.

Malaria eradication programs have been proposed in the past, and I think that most of you know what has happened. In most African countries where malaria eradication or control methods have been applied, the results have not been what was expected.

I do not want to monopolize the floor since we already have a 10-minute delay, so I will recognize Dr. Wurapa, who will be discussing a situation report of malaria in Africa.

# A. General Reports

## The Malaria Situation in Africa

F.K. WURAPA AND F.G. BEAUSOLEIL

### INTRODUCTION AND POLICY BASIS

Malaria is still the most important parasitic disease in Africa, with an estimated incidence of 20 million cases per year. In Africa, south of the Sahara, some 200 million people are chronically infected, and, of these, about one-third suffer acute manifestations of the disease in the course of a year. Accurate figures are not available for the number of deaths caused primarily by malaria, although it is estimated that the disease is responsible for the deaths of one million infants and young children each year.[1] The hyperendemic to holoendemic situation has remained unchanged over most of tropical Africa. A recent increase in the transmission of the disease in urban and periurban areas has worsened the situation. Concern about the resurgence of malaria in the 1970s led the director-general to propose a global malaria control strategy which was adopted by the World Health Assembly by Resolution WHA31.45 at its 31st session in 1978.[2] This resolution urged member states to reorient their antimalaria program as an integral part of their national health program and called upon the organization to provide technical support and guidance.

The situation has remained unchanged. In 1985 Resolution WHA38.24[3] was passed. It recommended that malaria control should be developed as an integral part of national primary health care systems and urged the member states concerned to:

1. Undertake an immediate review and appraisal of the malaria situation and existing control strategies in terms of their effectiveness, efficiency, and prospects of achieving and maintaining their objectives as a basis for planning the necessary modifications to maximize their contribution to the objective of health for all.
2. Comply with the seventh general program of work and plan antimalaria activities, utilizing appropriate technologies in order to contain the deterioration of the malaria problem in the future and to ensure sustained progress in control.
3. Mobilize adequate national resources for malaria control and conduct applied research on malaria.

This resolution was followed by the World Health Organization's (WHO) African Regional Committee resolutions AFR/RC35/R5[4] and AFR/RC36/R6[5] in September 1985 and September 1986, respectively. The regional resolutions recommended two strategies:

The first strategy was to emphasize the need for member states to make a steadfast commitment to combat malaria by releasing adequate resources at national, regional, and international levels.

The second strategy was to urge member states to reorient their malaria control program— with the ultimate aim of achieving malaria eradication wherever possible—as an integral part of national health programs in accordance with the guidances set out in the regional director's report,[6] and to increase their commitment (fiscal, administrative, and technical) to malaria control as part of their national development plans.

## PRESENT SITUATION

In Lesotho, St. Helena, and Seychelles malaria has either never existed or has disappeared. There is no risk of infection in these areas. These areas are also not receptive.

Reunion was certified and registered by WHO in 1979 as having eradicated the disease. The country is, however, still receptive but intensive surveillance and control measures have so far succeeded in preventing reintroduction of the disease.

Mauritius was certified and registered by WHO as having eradicated the disease. However, following a major natural disaster combined with inadequacy of the surveillance and control measures in 1975, transmission of *Plasmodium vivax* alone became established. The epidemiological situation rapidly deteriorated. However, since the reorganization and intensification of surveillance and control from the 1983 transmission season, the trend has been reversed. Transmission, which is still of *P. vivax* alone, has now been reduced to a low level and there are bright prospects of interrupting transmission.

In Cape Verde, there is risk of infection in only a few rural areas. There are intensive surveillance and control operations to prevent reintroduction of the disease in the areas where there is no transmission.

In Ethiopia, transmission has been reduced somewhat following more than two decades of control activities. There are, however, possibilities that the long drought may have been a contributory factor. Transmission has been reduced to a very low level in Mayotte. If the efforts are maintained, there are good prospects of interrupting transmission.

In Botswana, Swaziland, and Zimbabwe, transmission is seasonal and endemicity ranges from hypoendemic to mesoendemic in only

relatively small areas in each country. Epidemics are liable to occur. There have been control measures in each country during the transmission season. In Swaziland there was a major epidemic following a cyclone in 1984. In Botswana, transmission has been at a relatively low level for some time, perhaps due in some measure to the effects of the drought in the country. In all three countries reorganization and improvement of the control measures should make a significant impact.

In Sao Tome and Principe, considerable reduction in transmission was achieved after the initial spraying cycles. However, economic and financial constraints, which have made it extremely difficult to maintain the operations, have led to a deterioration of the epidemiological situation.

In the rest of the countries of the region, in the tropical rain forest and savannah areas, the situation has remained unchanged in almost all areas. Malaria is stable and endemicity ranges from hyperendemic to holoendemic levels. Transmission is extremely intense with high rates of infection and superinfection. It is estimated that each person receives 40–140 infective bites each year. The situation has not been surpassed anywhere in the world.

The principal vector is *Anopheles gambiae*, the most efficient vector in the world. The ecology and binomics of this vector are such that its control is extremely difficult. To make a significant impact on transmission it is estimated that as much as 50% daily mortality of the adult fly is necessary.

The predominant parasite species is *Plasmodium falciparum*, which accounts for well over 90% of infections, except in Mauritius where the only parasite species that is transmitted is *P. vivax*. *Plasmodium malariae* and *Plasmodium ovale* occur, but are not common.

In most countries, malaria is considered to be the most important parasitic disease and is ranked among the five most important causes of mortality and morbidity, especially among infants and young children under 5 years, and pregnant mothers. It is estimated that 5% of children die from the direct or indirect effects of the disease before attaining the age of 5 years. Malaria in pregnancy causes low birth weight, miscarriages, stillbirths, and premature births. It accounts for 15–20% of clinic attendances. Infants and young children under the age of 5 years account for up to 50% of cases. In older children and adults, the disease usually causes a transient illness with loss of vitality and reduced work output.[6]

Consequently, malaria is regarded in most countries as a major public health problem and a serious obstacle to socioeconomic development, especially in rural areas. All health authorities have indicated their desire to control the disease and finally eradicate it. However, reduction of prevalence through vector control measures is not feasible in most areas

where the disease is stable with a high intensity of transmission. In these areas, the antimalaria action that is technically, operationally, administratively, and financially feasible, especially in the rural areas, is the reduction and prevention of mortality and morbidity due to the disease through the rational and correct use of antimalaria drugs. These drugs facilitate the prompt and adequate treatment of malaria cases and the protection of vulnerable groups.

Although transmission has remained unchanged in most areas and no major control effort has succeeded, there have been some changes in the epidemiological pattern of the disease. There has been a marked reduction in rural and urban malaria-related mortality, largely attributable to the availability of antimalarial drugs. Early relief of symptoms and prevention of death has been achieved in spite of the grossly inadequate treatment in most cases. Inadequate treatment poses a serious problem to the emergence and spread of drug resistance. Although the changing environmental situation in the central urban areas has resulted in reducing the breeding of the vectors, the human crowding and other poor environmental conditions in the periurban areas have produced high rates of transmission. Another change that may have influenced transmission is the increased use of repellents, bed nets, and other personal protective measures against transmission. The last observed change that relates to transmission is the emergence and spread of drug-resistant parasite strains.

These have been the objectives of the regional antimalaria strategy:

1. Reduce malaria mortality to the lowest level possible, to the point that it ceases to be a public health problem.
2. Protect pregnant women and such other vulnerable groups as may be identified against the harmful effects of the disease through chemoprophylaxis.
3. Prevent the reintroduction of malaria into areas that are free from the disease but are receptive.
4. Reduce prevalence of the disease wherever feasible.
5. Promote and encourage the use of personal and community protective measures to the extent possible.

There are several operational goals that may be used to control malaria. The choice usually depends on the local epidemiological situation, national commitment, availability of resources, especially for maintaining the operations once they have been implemented, infrastructure of health services and technical and managerial capacity. Taking these factors into consideration, most countries in the region are attempting to reduce or present mortality due to malaria to the extent possible by the

prompt and adequate treatment of malaria cases. This is the first level of malaria control identified by WHO and is known as the *tactical variant I*. This method is recommended in areas with high prevalence of malaria, severe clinical illness and high death rates, low socioeconomic status, and limited experience in the administration of malaria programs. Although other equally effective methods for malaria control exist and can be used concurrently with the mortality prevention approach, very few countries in the region have epidemiological situations suitable for such simultaneous application.

## CURRENT SITUATION IN AREAS WITH STABLE MALARIA

An assessment of the malaria situation has not been possible in many countries because of lack of trained personnel. In Liberia, Malawi, Togo, and Zaire, which are included in the regional program for Combating Childhood Communicable Diseases (CCCD), an assessment of the situation was carried out in the limited areas where CCCD has been involved. CCCD is a bilateral cooperation program of the AID which aims at increasing child survival through control of malaria, communicable diseases, and diarrhea in sub-Saharan African countries.

The intercountry teams for malaria and parasitic diseases also collaborated with responsible national officials to assess the situation in Botswana, Mauritius, Swaziland, and Tanzania (Zanzibar).

## CURRENT SITUATION IN RECEPTIVE MALARIA-FREE AREAS

Reunion has been able to monitor the situation during the past year, and to date the intensive surveillance and control measures have succeeded in preventing the reestablishment of transmission. A WHO consultant visited the country during the year to assess the situation, which was found to be satisfactory. Cape Verde has also been carrying out a careful monitoring over the past three years and no new cases have been reported.

In the Seychelles, where there is no risk of transmission, there is constant fear about the possibility of the introduction of *A. gambiae* by aircraft from endemic areas. The government adopted a program that involves spraying aircraft arriving from endemic areas. How far this practice is justified needs to be assessed.

## REALISTIC MALARIA CONTROL STRATEGIES

Antimalaria strategies are being evolved in the countries with CCCD programs (Burundi, Central African Republic, Congo, Cote d'Ivoire, Guinea, Liberia, Malawi, Rwanda, Swaziland, Togo, and Zaire). All these strategies will be aimed primarily at the prevention and reduction of malaria-related mortality and protection of women during pregnancy. A joint AFRO/MAP (African Regional Office of WHO and the Malaria Action Program) mission collaborated with the responsible nationals to formulate a plan of action for malaria control in the Comoros.

AFRO collaborated with MAP headquarters in preliminary visits to Cameroon, Togo, and Zambia to support the development of antimalaria action as part of the Primary Health Care (PHC) systems in these countries. In light of the changing epidemiological situation of malaria in the region, the regional director has reformulated the antimalaria strategy, which emphasizes reduction or prevention of mortality due to malaria by the prompt and adequate treatment of malaria cases, and the protection of pregnant mothers with chemoprophylaxis.

## DEVELOPING AND STRENGTHENING LABORATORY DIAGNOSIS AND MONITORING

Laboratory diagnosis and monitoring has become imperative in all those countries where *P. falciperum* exists or poses a public health problem. The objective of laboratory development is assessment and monitoring of responses to standard treatment. This will enable researchers to ensure the adoption and pursuance of realistic policies and measures for adequate treatment and sound management of malaria cases because of the threat posed by the emergence and spread of drug-resistant *P. falciparum*.

Therefore, developing and strengthening laboratory work was given the highest priority during the past year. AFRO collaborated with MAP in organizing a training course for nationals in Nigeria. The intercountry teams for malaria and parasitic diseases also conducted the training of nationals in sensitivity testing, and collaborated in assessing the status of sensitivity in Angola, Congo, Kenya, and Swaziland. AFRO collaborated with the Centers for Disease Control (CDC) in Atlanta, Georgia, in organizing a course in sensitivity testing in Zaire for nationals from four CCCD bilateral countries: Central African Republic, Congo, Rwanda, and Zaire. AFRO, in collaboration with MAP and with the support of various donor governments, is in the process of developing several international courses in malariology.

## RESEARCH AND INFORMATION DISSEMINATION

The United Nations Development Program (UNDP)/World Bank/WHO Special Program for Research and Training in Tropical Disease (TDR) has continued to support a number of relevant studies on malaria in the region. AFRO has continued to disseminate information, particularly on drug resistance, during the past year to responsible national officials in the member countries.

The activities of WHO in the countries of the region during the past few years have been highly relevant to the promotion and development of action against malaria, which constitutes a major public health and socioeconomic problem in the countries of the region. Progress of antimalaria programs has not, however, been as rapid as was envisaged a few years ago; this is largely due to problems of human, material, and financial resources. There is a renewed impetus, which appears to offer some hope for the future, of organizing antimalaria action programs within each country's resources as part of the PHC system.

## VACCINES AND MALARIA CONTROL

The situation of malaria in most of the African region has not changed for the past two decades. In spite of the determination of member states to organize malaria control programs, several constraints have made the objectives illusive. Although the advent of malaria vaccines will bring many other problems (e.g., cost of procurement and cost of covering the population with required doses), it is hoped that, at least in some of the countries, the vaccine can play a synergistic role with ongoing national malaria control programs. The complexity of the malaria parasite cycle, which has made it necessary to develop three types of malaria vaccines, will be an added complication when countries plan to use these vaccines. However, the mortality and morbidity toll from malaria and its complications is serious enough to make us look with some hope to even a modest reduction in illness and death through the use of vaccines.

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# Malaria Vectors and Transmission

M. COLUZZI, V. PETRARCA, M. A. DI DECO, AND Y. T. TOURE

The malaria vectorial system in Africa south of the Sahara is probably the most powerful available anywhere to human plasmodia. The human-vector contact shows a remarkable stability and flexibility, producing extremely high inoculation rates in a wide range of geographic and seasonal ecological conditions.[1] The two most important components of this vectorial system are *Anopheles funestus* and *Anopheles gambiae*, but the stability and flexibility mentioned above should be mainly referred to the latter vector, which is in fact a complex of species and incipient species. Larval breeding in bare-edged temporary pools fully exposed to sunlight favors this group of vectors in exploiting man-made environments. Everywhere in Africa, rain-dependent pools frequently accompany new buildings, roads, and agricultural activities; and humans represent the driving force for the vector populations of the *gambiae* complex to expand, especially in relation with deforestation, desalinization in coastal areas, and irrigation. Although more mosquito vectors do not necessarily mean more malaria, the potential for transmission is certainly increasing in connection with development.

It is well established that the *gambiae* complex is composed of at least six morphologically indistinguishable sibling species. Their recognition and reliable identification can be attained only by applying the concepts and techniques of evolutionary systematics and genetics. More recent studies have shown further complexities involving chromosomal polymorphism and incipient speciation processes, which appear to be especially important in understanding the stability and flexibility of the vectorial system.[2,3]

Polytene chromosomes studies on the *A. gambiae* complex show rearrangements of the banding pattern that are all based on paracentric inversions. These occur at the homozygous state in different species (the fixed inversions utilized for cytotaxonomic identification) or constitute intraspecific chromosomal polymorphisms. Inversion polymorphisms have been recorded in all the six species of the *gambiae* complex except *Anopheles merus*, and were found to occur with particular frequency in *A. gambiae* and *Anopheles arabiensis*, the two taxa showing the widest

distribution and the closest association with man and man-made breeding places. Geographical variations in frequencies of inversions, closely related to climatic and ecological conditions, are observed in both vector species. Moreover, the adaptive values indicated by these geographic variations are consistent with the evidence obtained from studies on seasonal variations. These show that the arrangements found more frequently in humid areas increase their frequency during the rainy season, while those increasing during the dry season are prevailing in dry areas. On the one hand, the inversion polymorphism appears to be involved in the expansion of the species range towards marginal zones, and on the other hand, in the more efficient utilization of the spatial and seasonal heterogeneities of the environment in central zones.

The higher complexity of polytene chromosome differentiation has been recorded, so far, in *A. gambiae*, which can be split into forest and savanna chromosomal forms. Sometimes the latter may have the status of an incipient species. The forest form is apparently the same in all rain forest areas and intergrades with different savanna forms. The chromosome 2 standard arrangement, perhaps the oldest in *A. gambiae*, is characteristic of the forest form. The savanna forms all share one 2L inversion (2L*a*) and are further differentiated by one or more 2R inversions. A unique savanna form, characterized by the 2R*b* inversion, seems to prevail in East Africa, while in West Africa, the same arrangement occurs together with many other 2R inversions or inversion associations (most commonly *d, bc, bcd, u, cu, bcu, j, jbd, jcu*, and *jbcu*). Data obtained from laboratory colonies do not indicate the presence of mechanisms of reproductive isolation among the carriers of these 2R arrangements, all of which are potentially panmictic. However, intergradation is incomplete or even missing between some of the karyotypes, so that various chromosomally characterized savanna forms can be recognized.

Two chromosomal forms were described[2] in Senegambia in an area that includes the Gambia and part of Saloum in the north, and Casamance in the south. One of the two forms (population 1) is widespread in freshwater inland zones, characterized by the 2R*b* inversion, and can be regarded as a typical dry-savanna population of *A. gambiae*. The other form (population 2) is prevalent in the more humid, coastal, and southern zones, and is characterized by the 2R*d* inversion. In the wide central zone, where the two forms coexist, the hybridization frequency, although much lower than expected, seems sufficient to allow progressive introgression and complete intergradation. However, the presence of temporarily stable departures from the Hardy-Weinberg equilibrium, with deficits of the heterokaryotypes, indicates that the hybrid genotypes are not favored in nature in spite of their fitness or heterosis in laboratory colonies.

The situation is more complicated in Mali, where three chromosomal forms have been described[5] and denoted with the names *Mopti*, *Bamako*, and *Savanna*. The Mopti chromosomal form is characterized by the 2R polymorphism *bcu* and is prevalent in the inner delta of the Niger River and in other close riverine and/or irrigated areas that provide conditions for dry season larval breeding, particularly exploited by the 2R*bc* homozygotes. The Bamako chromosomal form, characterized by the 2R arrangements *jcu* and *jbcu*, is found along the course of the upper Niger River and its tributaries in southern Mali and northern Guinea. It appears to breed more successfully during the late part of the rainy season. The Savanna chromosomal form probably corresponds to population 1 described in Senegambia, but its 2R polymorphism, at least in southern Mali, includes other arrangements such as *cu* and *bcu* (alternatives to the typical *b* arrangement). This form is widespread in Guinea and Sudan savannas except for inundated or irrigated areas, and it appears associated mainly with rain-dependent breeding places. In Mali, a number of localities were studied where two of the chromosomal forms or all three of them coexist. Incomplete intergradation has been documented between Savanna and Mopti and between Savanna and Bamako, but not a single hybrid karyotype has been discovered so far between Mopti and Bamako. Taken alone, Mopti and Bamako should be regarded as reproductively isolated species but both show hybridization with intergrading forest-savanna chromosomal forms, which are, at least potentially, acting as a bridge for gene flow. Data obtained from studies still in progress indicate that, although each of the above chromosomal forms is more favored in certain environmental patches, competition phenomena display an important role in affecting their relative frequency and distribution.[4]

Concerning *A. arabiensis*, all populations studied so far suggest panmictic conditions. However, differences exist also in this species between northern and southern African savanna populations, with the former displaying, particularly in West Africa, a higher degree of inversion polymorphism.

The available data indicate that the diversity of the vectorial system might be affected by man-made environmental changes with relevant effects on ecoepidemiological stratification. Studies carried out in southern Nigeria[5] show that deforestation and urbanization favor the penetration of *A. arabiensis* into rain forest zones originally occupied only by *A. gambiae* monomorphic for the standard chromosome-2 arrangement (forest chromosomal form). Irrigation and especially rice cultivation in dry savanna zones of Mali and Burkina Faso tend to favor the spreading of the chromosomal form of *A. gambiae* indicated as Mopti.[3] The increasing diversity in the vectorial system is expected to involve some degree of niche compression, which generally results in a more efficient exploitation of the spatial and seasonal heterogeneities of the environ-

ment, with probable positive side effects on epidemiologically important parameters such as vector longevity and stability of total vector density. The main working hypothesis is that, in a given ecological stratum, the stability of malaria transmission increases with the increasing diversity of the vectorial system.[1] The use of insecticides is likely to have a nonuniform effect upon the various components of a highly diverse vectorial system, while situations where the system is constituted by only one chromosomally monomorphic taxon should be less difficult for a malaria control strategy based on the attack on the vector.

The experience from various antimalaria campaigns carried out in Africa generally supports the hypothesis of an inverse relationship between success of the vector control approach and degree of diversity of the vectorial system. The most promising results obtained against *A. gambiae* or *A. arabiensis* involved populations of these species that we now know to be monomorphic or nearly so. Nonuniform exposure to indoor residual spraying has been documented in the Garki project (northern Nigeria) where the use of propoxur was attempted against highly polymorphic sympatric populations of *A. gambiae* and *A. arabiensis*. The importance of the inversion polymorphism is indicated by field investigations based on parallel indoor and outdoor sampling of these populations. In both species, the chromosome-2 polymorphism appears associated with variations in endophily and endophagy. The arrangements found more often in outdoor collections are those that increase in frequency in humid environments, while the arrangements favored in dry environments are those found more frequently in indoor collections. This implies that different climatic adaptations coded by the inversions result in different house resting behavior, and consequently in nonrandom exposure of the vector population to sprayed indoor insecticides. This phenomenon may explain the failure to reduce the malaria vectorial capacity to noncritical levels by house spraying.

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# Indigenous Concepts of Malaria Control in Rural Communities

DAN C.O. KASEJE

The progress that has been made toward developing a vaccine against malaria is truly impressive. We are on the verge of a major breakthrough in medical technology, perhaps one of the greatest in medical history.

I am also impressed by the consciousness among the organizers of the conference that discussions like this one would not be complete without the effective participation of those who understand more about the intended users of the vaccine, and hence the excellent input by the speaker before me. I would like to share with you a few thoughts that you might consider more deeply later during discussions and during upcoming workshops.

The development of medical technology is only part of the whole story. The other parts have to do with the delivery system, which is made up of:

1. Health infrastructure;
2. Health personnel;
3. All that goes into making the technology developed accessible, available, acceptable, and affordable to the target recipients.

I am impressed that this meeting is aware of the need to consider the delivery system as part of its concern. Who is the target community? This group is made up of:

1. Pregnant women in areas of unstable malaria (seasonal epidemics);
2. Children under 5 years and pregnant women in areas of stable malaria.

Will they use the new technology when it becomes available? In many rural communities, vaccine coverage among children under 5 years averages 20%. These antigens have been around for many years, but the impact in terms of health studies is still minimal (even next to teaching hospitals). Does it concern us that use of a technologically developed tool determines its success? The commitment in terms of funding, research, and resource allocation should be directed towards the users. The final product needs to be acceptable in terms of:

1. Mode of administration
2. Number of doses required + duration of effectiveness
3. Complexity required of the delivery system and the logistic support.

Thus a new technology is not really a breakthrough until it is utilized widely by the target communities who are at highest risk of morbidity and mortality.

We are discussing these issues in the days of primary health care approach to health care delivery. Can the community be involved in developing the technology? Can they help to plan and implement vaccine trials?

For example, chloroquine is known to be effective and useful in pregnancy for prophylaxis, but in Saradidi it has taken a very long time to convince pregnant women to take prophylaxis. At best, the coverage was 30% when chloroquine was readily available for them in their own villages with community health workers (CHWs).

Pregnant women are among the most important groups to receive a vaccine. Although the vaccine should be administered as early as possible in the pregnancy, many women come to clinics for the first time in the third trimester.

One of the links that we may find still missing is the community link. Community involvement is necessary and crucial. Although they may not fit into our scientific molds, we will have no success without their involvement. Thus, to the needs for further work in chemotherapy and entomology, I would like to add community participation.

We must continue the search for an effective vaccine, but we must also pay attention to the mechanism that will deliver the vaccine effectively to the target communities that are at highest risk of disease and death. Working with a community to bring this about takes much longer than you would think.

To many communities where I come from, malaria is not a positive blood smear, not a set of symptoms and signs, but simply fever. This means that a number of febrile illnesses (notably acute respiratory infections and other viral infections) may be diagnosed by the patient as malaria.

Many of us who have worked in endemic areas of Africa are aware of the high rate of parasitemia among otherwise healthy schoolchildren or adults. What will be our measure of success? If we promise success and it is not experienced, we will undermine the community's faith in the vaccine.

# Chairperson's Comments

BRUCE-CHWATT: Ladies and gentlemen and dear colleagues, we are going to start the second part of our afternoon session. It is my pleasure and privilege to follow on the steps of my friend Professor Diouf, and I am particularly pleased and thank the organizers of this meeting for having allowed me to chair this part, which is connected with malaria in Africa. I have been in Africa since 1941-- for 18 years altogether, most of the time working on malaria. To me, it is going back to the good old, bad old days when everything was young and fresh and green and beautiful. To you, or to most of you, life is still like that, and I wish you the very best of luck in whatever you are going to do after this meeting.

We are opening this afternoon's session, ladies and gentlemen, with Dr. Fivawo, who is going to talk about indigenous concepts of malaria control. I would like to stress the importance of this approach. We have been told this afternoon that a large majority of the population of Africa suffers from malaria, and their concept of malaria, their way in which they regard malaria, is of extreme importance for our attempt to relieve the brunt of this disease on the African population. Dr. Fivawo.

# Indigenous Concepts of Malaria and Malaria Control in Tanzania

MARGARET FIVAWO

The first scientist who reported indigenous people's knowledge of malaria in east Africa was the famous German scientist Robert Koch. In 1897, Robert Koch traveled from Dar es Salaam, in what was then Deutsch Ostafrika, to western Usambara. Here he found out that the disease that he had been called to investigate—a disease that seriously attacked many German settlers—was malaria. The settlers acquired the disease while traveling between Dar es Salaam, which is on the coast, and their newly established homes in the mountains. A few days after arriving in the mountains, many settlers became seriously sick from what they believed to be an acclimatization disease caused by the sun's strong rays.

Robert Koch reported that the native people called the disease *Homa ya mbu* which literally means mosquito fever. He reported further that the natives said they contracted the disease after being stung by lowland mosquitoes. To avoid the disease, the Sambaa people avoided staying late or spending nights in the lowland plains. When they came down from their mountain homes to trade, they made sure that they were back in the mountains by sunset, hence avoiding bites by the malaria-causing mosquitoes of the lowlands. The lowland dwellers, the Bondei, were known to be more tolerant of the *Homa ya mbu*. The Bondei were also known to have effective medicines to cure malaria.

On the question of immunity, Robert Koch had the following to say:

> Of the thirty porters who participated in the journey, not a single one fell sick, but they were all men from the coast. If the mountain dwellers have no immunity to malaria, which seems to be the case, it follows that they find no opportunity in the mountains to acquire immunity the natural way; in other words, malaria is not present in the mountains.[1]

Clyde and Msangi found in eastern Usambara that there were differences of malaria infection between the tea plantation laborers who came from different parts of the country. The Bena, who came from Njombe in the southern highlands where there was no malaria, had the

lowest levels of immunity, while the Sambaa, who were indigenous, had more malaria parasites than the Bondei laborers.[7] Clyde showed the distribution of malaria in Tanzania during the early 1960s when there were still isolated areas without malaria.[8] The current malaria situation in Tanzania is serious, with no single district being malaria free. Chloroquine resistance is also reported from various parts of the country.

Tanzania's policy of malaria control aims at protecting the most susceptible groups of the population, children under 5 and pregnant women. Chloroquine prophylaxis has been provided to these susceptible groups for a number of years through the maternal and child health (MCH) program. A study carried out in 1984 in the Muheza district, with the objective of assessing the local concepts of malaria control in a rural community, showed that the population had a good knowledge of malaria and that efforts were taken by the community members to control the disease.[9] The Muheza district is in a region of hypoendemic malaria with few areas of lower endemicity at high altitude. Data were collected from two village communities using a basic questionnaire and interviews with several key persons. The questionnaire was administered to mothers of children under age 5. The key persons included local health personnel, traditional birth attendants, healers, and elders of the villages. The majority of the mothers had themselves used chloroquine prophylaxis during pregnancy. These women were also responsible for taking their children to the MCH clinic for vaccinations as well as giving the weekly malaria prophylaxis to their little children. Some of the findings of the study are presented in Table 1.

Table 1 shows that malaria was mentioned as the most prevalent illness by about half of the respondents, the second most prevalent being diarrhea. The awareness of the villagers agreed with the disease reports from the village dispensary statistics, as well as those from the district hospital, which showed that malaria is the most commonly reported disease for inpatients and outpatients.

Elders reported that malaria is more frequent and more severe now than during their own youth and childhood (the elders were all over 60 years). Traditional medicine practitioners use the term *nyongo* to refer to malaria and they too reported an increase in nyongo cases in recent years. These local medicine men know herbal medicine, which they prescribe for treatment of malaria. One such herb, which is popularly known as a cure for malaria, is known under the local name of *zugwa*, and is identified under the botanical name *Colens kilimandschari*. Chemical screening for the active ingredients and medicinal value of the plant has yet to be done.

Response to a question on lethality of diseases showed that 85% of the respondents considered malaria to be one of the lethal diseases (Table 2). Regarding the cause of malaria, 46% mentioned mosquitoes. (Table 3).

Table 1. Distribution of respondent opinions concerning the prevalent illnesses in the village.

| | Most Common Illness: | | Second Most Common Illness: | |
|---|---|---|---|---|
| | Number | Percent | Number | Percent |
| Malaria | 147 | 49.0 | 43 | 14.3 |
| Fevers | 49 | 16.3 | 17 | 5.7 |
| Diarrhea | 44 | 14.7 | 72 | 24.0 |
| Abdominal problems | 19 | 6.3 | 44 | 14.7 |
| Measles | 11 | 3.7 | 7 | 2.3 |
| Headache | 8 | 2.7 | 27 | 9.0 |
| Anemias | 3 | 1.0 | 3 | 1.0 |
| Vomiting | 2 | 0.7 | 18 | 6.0 |
| Coughs | 1 | 0.3 | 15 | 5.0 |
| Asthma | 1 | 0.3 | 4 | 1.3 |
| Mchango* | — | — | 2 | 0.7 |
| Other | 9 | 3.0 | 31 | 10.3 |
| Don't know | 2 | 0.7 | 2 | 0.7 |
| No reply | 4 | 1.3 | 15 | 5.0 |
| Total | 300 | 100.0 | 300 | 100.0 |

*Mchango is convulsions, usually in children, associated with malaria and other high fevers, probably cerebrospinal meningitis among them.

Table 2. Distribution of respondent opinions concerning deaths due to local illnesses.

| Illnesses | Not very easily | Not easily | Not sure | Easily | Very easily |
|---|---|---|---|---|---|
| Measles | | 6.0 | 0.3 | 13.7 | 80.0 |
| Diarrhea | | 2.0 | 0.3 | 19.7 | 78.0 |
| Convulsions | 0.7 | 3.0 | 1.7 | 20.7 | 74.0 |
| Zonga | 0.7 | 10.3 | 4.3 | 49.3 | 35.3 |
| Malaria | 0.7 | 12.7 | 1.3 | 62.0 | 23.3 |
| Tuberculosis | 0.3 | 10.3 | 1.0 | 55.0 | 23.3 |
| Djins | 2.7 | 27.7 | 5.7 | 43.0 | 21.0 |
| Fever | 5.0 | 69.0 | 1.7 | 19.3 | 5.0 |
| Headache | 10.0 | 62.7 | 1.0 | 28.7 | 1.7 |
| Bronchitis | 5.7 | 79.7 | 1.3 | 11.0 | 2.3 |

Data shown as percent distribution.

What is important to note about the responses to the question of the cause of malaria is the complete association of the disease with the environmental conditions, rather than magical or some supernatural causes. This would make the disease more easy to control with an appropriate strategy than would be the case if the disease were related to magical etiologies.

The majority of the respondents considered malaria diagnosis to be easy (Table 4). Even though their diagnoses may be incorrect at times, the

Table 3. Distribution of respondent opinions concerning what causes malaria.

| Causes | Number | Percent |
|---|---|---|
| Mosquitoes | 139 | 46.3 |
| Cold | 76 | 25.3 |
| Dirty water | 38 | 12.7 |
| Pollution | 17 | 5.3 |
| Heat | 7 | 2.3 |
| Change of weather | 5 | 1.7 |
| Dust | 2 | 0.7 |
| Excessive work | 2 | 0.7 |
| Other | 8 | 2.7 |
| Don't know | 2 | 0.7 |
| No reply | 5 | 1.7 |
| Total | 300 | 100.0 |

Table 4. Distribution of respondent opinions regarding how easy it is to diagnose malaria.

| Difficulty | Number | Percent |
|---|---|---|
| Very easy | 255 | 85.0 |
| Very difficult | 35 | 11.7 |
| Not sure | 10 | 3.3 |
| Total | 300 | 100.0 |

Table 5. Distribution of respondent opinions concerning the symptoms of malaria.

| | Numbers | Percent |
|---|---|---|
| Fever, chills, pain in joints | 70 | 23.0 |
| Weakness in joints | 60 | 20.0 |
| Fever and chills | 59 | 19.7 |
| Fever | 48 | 16.0 |
| Vomiting | 47 | 15.0 |
| Other | 5 | 1.7 |
| No reply | 11 | 3.7 |
| Total | 300 | 100.0 |

villagers seemed to be able to distinguish different types of fevers. The mentioned symptoms of malaria range from combinations of symptoms to fever, weak joints, or vomiting (Table 5). In small children, malaria symptoms include fever, withdrawal from play, crying, and refusal to eat. The course of action that follows the home diagnosis varies between administering a single aspirin tablet, chloroquine, or herbal medicine. Some younger mothers seek the opinion or advice of elder women before taking any step; sometimes the husband gives the advice.

Chloroquine is considered a powerful antimalaria medicine by villagers, except for some side effects—mainly itching, which is reported to follow a few days after using chloroquine to treat malaria. The prophylactic use of chloroquine during pregnancy is reported to be effective. However, some women report that large dosage of the drug can induce abortion, and young girls often use chloroquine to induce illegal abortions. Therefore, chloroquine is generally used by the pregnant women with some precaution. Patients often use biomedicine, which they store in their houses or buy from local stores, as well as herbal medicines, simultaneously or one after another.

When the *mzugwa* is used, the leaves of the plant are boiled and drunk as tea three times a day. If the fever persists the following day, it is assumed that the disease is not malaria and professional assistance is sought. More complicated preparations are used to treat severe fevers and other diseases. Villagers also use herbal repellents to protect themselves against mosquito bites. *Mvumbasi (Ocimum suave)* is popularly used for smoking the inside of houses at night. The fresh branches of the plant are also used on the walls of the houses and around the sleeping places. The plant has been proved to have strong insect repellant activity.[5]

Other protective measures that are practiced by community members include screening of house windows and the use of mosquito nets during sleep. However, the popularity of these two measures is limited to the few who can afford to pay for the screens and the bed nets. There is no doubt that, given low prices, many more people would use these control measures. Environmental control is practiced by keeping the grounds around the house free of tall grass and water-holding areas. The shell of the coconut is used as firewood, which takes care of this common item that could serve as a good holder of rainwater and breeding place for mosquitoes.

An evaluation of malaria in a sample of the village population showed high parasite levels in the small children under age 5. The highest percentage of those with negative parasitemia was from those in the 15 and above age group (Table 6). This malaria evaluation was done in early March, before the start of the big rains; after the rains, the parasite levels were even higher.

There can be no doubt that this rural population would welcome a malaria vaccine. However, it would be more beneficial if use of the malaria vaccine would be integrated into the vector control and personal protection measures that are already practiced by the population. The integrated approach would facilitate effective control of the vector that constitutes a nuisance pest, as well as strengthen general health education and the control of other diseases that are prevalent in the communities.

Table 6. Number and percent distribution of parasitemia levels in Tongwe Village, March 1984.

| | | Number of parasites/200 white blood cells | | | | | |
|---|---|---|---|---|---|---|---|
| | All Levels | 0 | 1-100 | 101-200 | 201-400 | 401-800 | 801-1600 |
| All cases | 136 | 41 | 66 | 9 | 11 | 5 | 4 |
| Percent | 100 | 30 | 49 | 7 | 8 | 4 | 3 |
| Infants: | 28 | 2 | 11 | 2 | 8 | 3 | 2 |
| 0-11 mos | 17 | 2 | 6 | 0 | 6 | 2 | 1 |
| 12-23 mos | 11 | 0 | 5 | 2 | 2 | 1 | 1 |
| Percent | 100 | 7 | 39 | 7 | 29 | 11 | 7 |
| Children: | 54 | 9 | 34 | 5 | 3 | 1 | 2 |
| 2-4 yr | 35 | 62 | 1 | 3 | 3 | 1 | 1 |
| 5-9 yr | 19 | 31 | 3 | 2 | 0 | 0 | 1 |
| Percent | 100 | 17 | 6 | 39 | 6 | 2 | 4 |
| Adolescents and adults: > 15 yr | 54 | 30 | 2 | 1 | 20 | 1 | 0 |
| Percent | 100 | 56 | 39 | 4 | 0 | 2 | 0 |

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# General Discussion

BRUCE-CHWATT: I am going to call Professor Coluzzi in a second. Dr. Kaseje, would you like to carry on after Dr. Fivawo because you said you are going to be very brief. Perhaps you would like to extend your contribution.

KASEJE: Yes, I would. Thank you very much, Mr. Chairman. Ladies and gentlemen, mine is going to be very brief. I am really impressed by the progress that has been made towards the development of malaria vaccine. I believe that many of us are excited to be involved in this. I believe that this is going to be one of the major medical break-throughs in this decade. I am mostly impressed by the fact that the organizers of the workshop considered it necessary to include people like Margaret Fivawo, who understands the communities that we try to produce these technologies for. I will simply share with you a few thoughts that might need to be taken into account as part of the process of our work in trying to solve the problem of malaria.

The development of medical technology like malaria vaccines, I would like to suggest, is but part of the story. The whole story is larger than just the medical technology. The other parts of that story must include the delivery system, the infrastructure, the personnel, and other things that go with bringing the technology to the users, and, of course, the community of the users that we have heard about from Dr. Fivawo. The new technology has to reach the people, in other words, be accessible, available, acceptable, and affordable to them. Without doing that, I believe the results of a breakthrough will be seriously limited.

Now, who are the target communities for these particular vaccines? I believe that those at risk of developing disease as a result of malaria infection are target communities. These are the people who live in areas where malaria is endemic and who are susceptible. Children under five and pregnant women have been mentioned.

How can we make sure that the technology developed will be utilized? We all know that the population coverage that we are usually able to achieve by new technologies has been extremely low in rural areas. It is a fact well known to many of us that even with the vaccines available today, like, for example, measles, only about 20% of many populations are covered. These vaccines have been available for many

years. Therefore, the development of an effective malaria vaccine will not immediately solve the problem of malaria. This, therefore, means that the commitment, from major funding organizations and donor countries, that has been mentioned should not be limited to the scientific aspects of the development of the vaccine alone, but should also include the search for an effective mechanism that would bring that particular result of technology to the people that will use it. I think we have to look for a mechanism that would make it possible for this particular vaccine and any other vaccines like it to be not only accepted, but to be part of the medical care system within the communities.

Dr. Fivawo mentioned some of the aspects that might be taken into account, like talking to the healers and community leaders. I believe that the process will not just end there. It will be necessary to make the communities we work with part of the discussion. We are now in the era of primary health care and, I think, we are saying that primary health care communities will not only passively participate in the projects we have developed, but they will have to be actively involved in the process of providing health care. In any new health technology, community participation in health care will be extremely important. As we consider vaccine trials in communities, it will be very important that we also consider the ways and means by which communities can be involved in the process as early as possible. So, many of us will agree that simply developing new technologies is not enough. I think the vaccine is not useful until it has reached the people that are at risk, and need to be protected.

All of this may not be easily understood by many of us. That is why I am saying that the commitment to research and continued funding must include the search for appropriate mechanisms that will make these malaria vaccines part of community action. People must be involved in their own programs; they should not be projects that are imported from outside. Although I have not provided a lot of answers to my questions, I hope I have sufficiently sensitized the participants of this conference to subjects that are often forgotten. Thank you very much, Mr. Chairman.

BRUCE-CHWATT: Dr. Kaseje, I would like to congratulate you on stressing so clearly and so pertinently a point that will be one of the main aspects of our future action. I thank you very much.

We still have some time for discussion. I recall that the problem of mortality due to malaria, especially among the younger age groups, has been mentioned today by Dr. Diouf. I know that one of my friends and colleagues here was very involved and very interested in the problem. I would like to call on you to present it. Dr. Gerald Breman of CDC, who was in Africa for a number of years, has some very definite ideas on this problem.

BREMAN: I have some ideas, Professor. We are having a meeting on the great hope of the future. I wish I had some data to present. The malaria vaccine that is going to help decrease morbidity and mortality makes it incumbent upon us to know what those measures are before we begin the interventions. The problems that have occurred are basically definitions. What is malaria in a continent where over 99% of the diagnoses are made without parasitologic confirmation, and where fever is frequent? From that evolves the question: "What is malaria- associated mortality?" We have, as Dr. Wurapa presented before, some numbers. The official numbers are approximately 5–7 million cases of fever reported that go down as malaria and the estimated figure that 1 million deaths occur per year. But where do these figures come from? Most people are interested in the 1 million deaths, a figure that has remained the same for the past 20 years, despite the fact that the population has increased. This is important because, when we don't have the data, people will not consider that malaria is so important if they are involved in planning on the international, national, and sometimes even local bases. We are struggling against other programs that merit support as well.

There is one country I know in the Caribbean with only *Plasmodium falciparum* that has been heavily supported by foreign donors for the past 25 years, which has never reported malaria mortality. For whatever reason at this particular point, that country is having tremendous difficulty getting support for malaria control because other programs are basically stronger in terms of presenting relevant data concerning the toll of the disease.

What is known? Well, in 1952, our chairman did a most fascinating investigation in Lagos based on the study of all persons dying. He estimated the toll due to malaria in children less than one year old as 12.5/1000 and for those from 1 to 4 years as approximately 7/1000. This was an urban population. There were those with directly associated deaths and those where the deaths were indirectly associated with malaria. As I understand it, this was the basis upon which the 1 million figure was derived and has been maintained over the years. There have been a few other studies in the intervening years which keep the malaria based mortality pretty much in the same order. One would have thought that this would be decreasing because of better health services, higher standard of living, nutritional programs, and health education.

The overall infant mortality seems to be changing. It is unclear as to where that is changing because the closer you look, the more often you find that there is a heterogeneity, not only across Africa, but even within countries. Recently in the program that Dr. Wurapa mentioned—the CCCD Program, an AID-sponsored African Child Survival Combating Communicable Diseases Program—a closer look was taken at the data from Liberia,

Zaire, and Togo, using classic demographic methods. The assumption was that it was not worthwhile to find disease-specific mortality retrospectively, but perhaps it was overall mortality that would be the best indicator. The rates of infant mortality range from 33 to about 190/1000. What we learned from these population-based retrospective studies was that: they are expensive, are difficult to validate, and have enormous bias depending on the population you choose. It is almost impossible in a retrospective study to find a disease-specific cause. Even neonatal tetanus, where a baby dies before 10 days, is tough to evaluate. But, what we found is that, in some communities, the infant mortality is probably dropping considerably.

More recently, there have been two interesting studies. I hope we will hear one tomorrow. Dr. Greenwood in the Gambia has done some fascinating prospective studies looking at a group of children in rural areas with special epidemiologic conditions. I will not talk about this because Dr. Greenwood is here. But, a look at a population in Kinshasa, Zaire, with parasitologically confirmed malaria and with the clinical diagnosis made on the wards, including hospital record reviews, by board-certified pediatricians and clinicians, indicated that a view at the overall mortality is not going to identify trends for disease-specific mortality. Over the past 6 months in Kinshasa, Zaire, the mortality of children under 5 years has approximately tripled, going straight up; while the overall infant mortality, if you just look at that, has stayed the same. The hypotheses is that drug resistance may be related to this, although there are a number of other factors that may be involved. What is the overall mortality in Kinshasa when you look at the figures with all the qualifications of it being a hospital-based study? They are of the same order as the Bruce-Chwatt findings about 20 to 25 years ago. If you apply these more recent studies to all of Africa, you are not really far from the 1 million deaths that we have heard about in the past considering the population has doubled, etc.

The methods are very, very imprecise. We need innovative approaches—approaches of the type that are being done in the Gambia. While prospective studies are the most accurate, they are also the most costly and time consuming. We need more studies where just a cohort of births are followed either at a hospital or in a community and then see what happens to those and try to define what is going on. Perhaps the easiest for our colleagues is simply hospital-based surveillance where people come in and where we can measure the severe clinical cases and deaths due to malaria and whether the trends are changing. So, innovation using the data that are available, getting support for this is extremely important because otherwise we will not have the baseline data that is needed when the interventions come in. Let me just say that one thing I did not know of until about three or four years ago was the number of

episodes of fever children have per year. If we do not know that, how can we measure the ultimate outcome of what the vaccines are doing, irrespective of the epidemiologic indices, serologic parameters, and the rest. We found that the number of episodes of fever per year varies from 4–15 episodes. Thank you.

BRUCE-CHWATT: Thank you very much, Dr. Breman. You may recall that not very long ago Dr. Wurapa and also Dr. Diouf mentioned a problem of drug resistance. Apart from the phenomenon of drug resistance that is related mainly to the parasite and, secondly, to the response to the parasitic infection, there is a totally human ecological aspect, namely the failure to take the drugs. The problem of compliance is and may be a very serious problem due to a number of factors. Professor Salako has been involved for a number of years in the study of one of these factors. I would like to call on Professor Salako to tell us something about his findings.

SALAKO: Thank you, Mr. Chairman. I want to show a slide to back up what Dr. Wurapa had described to us about the spread of resistance to chloroquine in Africa. These data were obtained from the literature and from WHO publications (Fig. 1).

This is the appearance of chloroquine-resistant *P. falciparum* in Africa. The first place in Africa to report this was the Comoros Islands in 1978; and then Kenya and Tanzania in 1979; Madagascar in 1980; Uganda in 1981; and the Sudan, Zaire, and Zambia in 1982; in 1983, Burundi, Gabon, Malawi, and Mozambique; Angola, Namibia, and Ruwanda in 1984; Cameroon and the Congo in 1985; and just this year in the last month or two, resistance was described from Benin.

Now if you look at the map, you will see that this spread has been almost complete from the east to west, but somehow there has been a jump after Cameroon. One would expect Nigeria to follow but there has been a jump over Nigeria to Benin, which is just west of Nigeria. We do not know whether we are missing it in Nigeria, or whether this is a general jump.

Over the past several years, I have been monitoring sensitivity to chloroquine in Nigeria. The data from last year's work show that for chloroquine, we see full sensitivity to the drug. Mefloquine, an alternative to chloroquine is already showing primary resistance. The slope of the curve is so much less than that of chloroquine. In fact, among 12 patients, two distinctly have parasites that were resistant to mefloquine, even though mefloquine had not been used at all in that community, and quinine, which is related to mefloquine, had not been used for over 40 years. So mefloquine may not, in fact, be the answer to chloroquine resistance in West Africa if and when it arises.

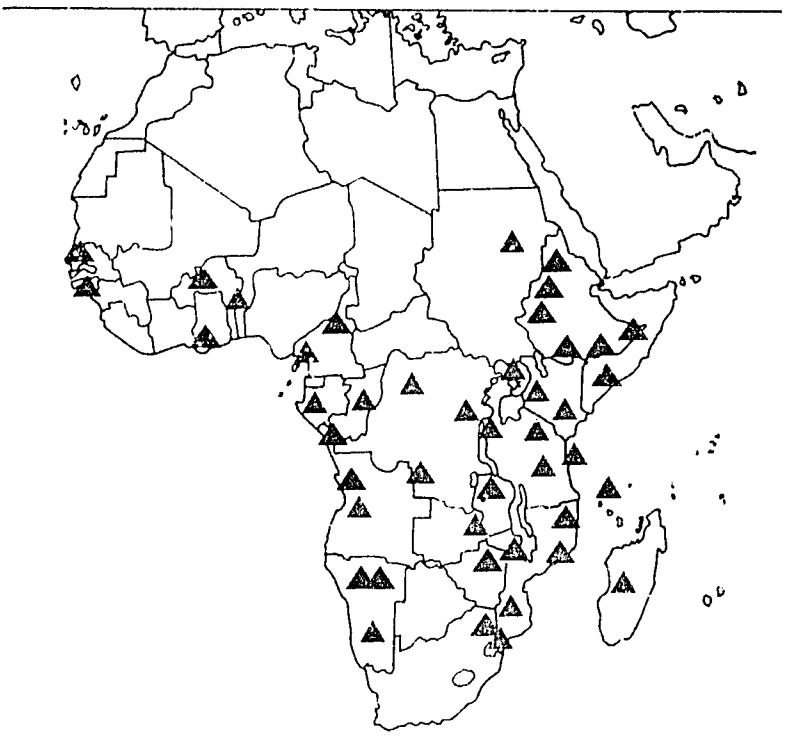

Figure 1. Distribution of *Plasmodium falciparum* chloroquine resistance in tropical Africa (From World Health Organization, No. 84771.)

We are interested in monitoring sensitivity to chloroquine because there are many patients in Nigeria who do not tolerate the drug at all. Because of the side effect of itching in about 15–20% of the population, many patients do not take the full therapeutic dose of this drug. We have studied this reaction and found a kinetic basis for it. In fact, some of these patients who itch very badly from chloroquine metabolize and distribute the drug differently from normal patients. The pure drug is more tightly bound to the skin than the metabolite compared with patients who do not itch. This form of kinetic difference may, in fact, be responsible for this. The other drugs that are available for use like pyrimethamine-sulfadoxine, amodiaquine, and so on, are not really better than chloroquine in our population and so we really are going to be in some difficulty if chloroquine resistance does come. This is my contribution. Thank you.

BRUCE-CHWATT: Thank you very much for your contribution. Any other discussants? Yes, go ahead, Dr. de Cieta.

DE CIETA: Thank you, Mr. Chairman. I am from Sao Tome and Principe, and much was spoken about resistance, but little has been said about my

country, perhaps because it is very small. But as far as resistance is concerned, we recently found that there were cases of *P. falciparum* malaria fever that did not respond to 3-day chloroquine treatment. This led us to suspect that there was resistance to the 4-aminoquinolines.

Tomorrow we will address the resistance problem in Sao Tome and the situation resulting from an incomplete malaria eradication program. Dramatic results were obtained initially for a 3-year period, and after that, we saw an epidemic outbreak of malaria.

Drug resistance appeared when there was a loss of immunity. We cannot speak of definitive resistance, but rather of relative resistance. I simply wanted to draw your attention to this, and I hope to come back to this later in the discussion. Thank you.

Bruce-Chwatt: I have one question for you. Has WHO sent someone there to examine the degree of resistance with in vitro testing?

De Cieta: Well, WHO has not yet sent anyone. Recently the Portuguese Red Cross sent a mission, which worked with the European Economic Community, but, as far as I know, it has not conducted in vivo studies for chloroquine. The mission carried out a large-scale chemoprophylaxis campaign, especially for children up to 15 years of age, but I do not think that it has performed either in vivo or in vitro tests. We have informed WHO of the situation in Sao Tome and sent them a preliminary report on the tests that we conducted.

Bruce-Chwatt: Thank you, Dr. de Cieta. I would highly recommend you speak to Dr. Wurapa, since he does represent the regional office. You can perhaps ask him to send another mission to perform field trials. Thank you very much. Any other discussants? Yes.

Taylor: My name is Taylor from Zimbabwe, Ministry of Health. In the line of drug resistance, I do not think that the situation has really been made clear enough because, as far as Africa is concerned, there is no alternative to the use of chloroquine. We are neither fortunate enough in Africa to have the health infrastructure that is available in Southeast Asia, nor the financial resources that allowed them to use alternative drugs quite readily when chloroquine resistance appeared. Now we heard Dr. Wurapa talking about the control or, let us say, the reduction of morbidity and mortality due to malaria by the use of drugs in rural areas. Although this is not going to be an effective strategy for very long, it is the principle strategy that is recommended and available at the moment. What we have seen in the last four or five years is a dramatic spread of chloroquine resistance in Africa to the extent where in some countries it rapidly rose to 60–70% of the malaria cases. We are fortunate at the present time that chloroquine is still beneficial in controlling morbidity, even though it does not necessarily effect a cure.

What concerns me is how long before we reach the situation in Southeast Asia where chloroquine does not work at all. We know that the precarious financial situation in many countries is unlikely to allow the widespread mass use of other drugs like fansidar, to which we are also experiencing resistance. We hear that there is mefloquine resistance already in Nigeria, and that is also a very expensive drug and unlikely to be widely available. So, what I am trying to paint is a very gloomy picture, and we are not likely to have the time to wait for a vaccine to be developed either.

One area that I think has not been emphasized enough is the potential use of vector control. This really has been very underplayed, particularly by WHO, for Africa. There are many constraints, but I think it is something that really needs to be reevaluated, not necessarily with the view of looking for the cessation of transmission, but looking at reducing the number of malaria cases to a level where they might possibly be manageable through the use of drugs. These measures are not necessarily national-scale vector control programs, but local projects that are manageable within the resources and the constraints of the country. Thank you.

BRUCE-CHWATT: Thank you very much, Dr. Taylor. Yes, Dr. Haridi. I would like to remind you that you have to give your name and the country where you come from before you start.

HARIDI: Dr. Haridi from Sudan. I would like to put the question to Professor Coluzzi if he could give us a comparative analysis, if possible, about the vectorial capacity of the two important vector species, namely *Anopheles gambiae* and *arabiensis*, also the reaction of the two species to control measures, particularly insecticide treatment.

BRUCE-CHWATT: Thank you very much, Dr. Haridi. That is a challenging question to Professor Coluzzi.

COLUZZI: You are right, Mr. Chairman. I think broadly speaking we are confronted with two vectors both closely associated to humans and in which anthropophily is one of the main characteristics. However, there are differences in anthropophily, no doubt, which are very obvious in areas where we can study sympatric populations of *A. gambiae* and *A. arabiensis* confronted with the situation of choice between animals and humans as a blood source. This is probably related to a different mechanism of speciation of the two species. They both speciated with a close association to humans, but the working hypothesis, also considering the present distribution of the two species and the chromosomal evidence, is that *A. arabiensis* was speciated by following humans into arid,

savanna areas with domesticated animals associated to humans. So, the association with humans was also an association with domesticated animals that were present.

*Anopheles gambiae* has probably a completely different specia- tion process that was developing in forest areas. We can say this hypothetically, based on important evidence, because the most primitive karyotypes that we find in *A. gambiae* are identical to the karyotype found in *A. quadrimaculatus*, and which is also closely related to *A. melas* and *merus* karyotypes, which have probably the oldest speciation processes. So the most primitive karyotype of *gambiae* is the forest type, not the savanna one. All indications from the chromosomal status show us that *A. gambiae* is starting from the forest and not from the savanna as we would expect. If this is true speciation of *gambiae*, it is a very recent process because we cannot assume the presence of *A. gambiae* in the forest except with shifting cultivation, and some agriculture in the forest. However, this hypothesis is quite convincing if one considers the high degree of anthropophily of *A. gambiae*. This is so because association with a small group of people in the forest doing shifting cultivation was much more closely related to humans than to any alternative for bloodmeal. This is completely different from what was happening with *A. arabiensis* in the savanna area. Any *A. gambiae* that was changing its biting behavior to an animal in the forest, let us say an antelope, was in danger of losing contact following the manmade opening of the forest.

This is a partial explanation, in terms of evolutionary thinking, of the difference between these two vectors, both of which are closely related to humans. In many situations *A. arabiensis* has no choice for its survival except man. This happened for instance in urban areas as I mentioned in my presentation. *A. arabiensis* can be completely anthropophilic in this situation. Where we see a difference between the two species is particularly in nomadic people, where many animals surround the small huts in which the families live and sleep. When we collected *A. gambiae* and *A. arabiensis* inside these huts, we found that *A. gambiae* was capable of traveling through the herd without biting cows, keeping an anthropophily of close to 90%. This was found in a study in northern Nigeria. In contrast, *A. arabiensis* can lower its anthropophily to a level of 20–30%. So there is a considerable difference between these two species, but this becomes obvious only in a situation in which animals, particularly cows, outnumber humans, and are distributed in such a way that the animal is approached before the mosquito reaches humans.

We should expect that the control of *A. arabiensis* is less easy and is more subject to zoophily and, possibly exophily. We have observed *A. arabiensis* populations that were highly exophilic. Some of these populations have been studied by Dr. Reeding in the Sudan, and I had the

opportunity to study some of these populations recently in Madagascar. I was really surprised to find *A. arabiensis* zoophilic and exophilic, and raised an open question in the high plateau in Madagascar. If these *A. arabiensis* are highly zoophilic and exophilic, it either has been the result of the insecticide pressure in the lowlands, or it is a peculiar chromosomal type of *A. arabiensis*. We hope to solve this problem by another study in Madagascar.

BRUCE-CHWATT: Thank you very much, Professor Coluzzi.

HOFFMAN: Hoffman from Walter Reed. It seems from what we have heard that most people here would be thrilled if 5–10 years from now we had a malaria vaccine that gave 50% protection under the conditions of exposure in East Africa. Therefore, if we are really serious about control, then, as Dr. Taylor mentioned, we will have to incorporate vector control measures and probably better delivery of chemotherapy. I have a question for Dr. Coluzzi and then, perhaps, for Dr. Kaseje, which may be expanded to others.

For Dr. Coluzzi, I wonder whether the efforts to subspeciate the *Anopheles gambiae* complex can be used in any way to tailor control measures, such as source reduction, which would make more efficient use of the available resources. And throwing back Dr. Kaseje's statement that we have to work on bringing the technology to the community, I wonder whether under the conditions that we have now he feels that communities such as those, perhaps, in the Kisumu area could be organized to utilize the available measures better for the local control of malaria, particularly aiming at source reduction and vector control.

BRUCE-CHWATT: Thank you very much. Professor Coluzzi, would you like to answer very briefly, please?

COLUZZI: Well, I would say that up to now, the splitting of the *A. gambiae* complex has particularly helped in the understanding of epidemiologic patterns, but not so much in fighting these vectors. It was also quite helpful in understanding why residual indoor spraying was unsuccessful in certain areas and more successful in others. What is clear from the data we are accumulating is that there is a very obvious relationship between chromosomal variability of *A. arabiensis* and *gambiae* and the success of indoor spraying. So indoor spraying was successful essentially in fighting the populations of *A. gambiae*, which were less variable chromosome carriers, and more marginal in genetical terms.

The other point that is emerging is that some of these populations should be considered very carefully with regard to source reduction in vector control programs, just because of the danger of further adaptations. I was mentioning in my presentation particularly the situation in the urban areas, and particularly the forest areas. I think the problem should

be handled very carefully. Some research is needed, first of all, to clarify further the problem and then to act with a program that has a major impact on source reduction, which in the urban environment is expected to be quite successful.

BRUCE-CHWATT: Thank you very much, Professor Coluzzi. Would Anyone else like to take part in this discussion? Dr. Kaseje, please.

KASEJE: Should I go on with the second question? Thank you very much, Mr. Chairman. The answer to that question is yes. It will be important to involve the communities, examples that were given around Kisumu in Kenya, in utilizing the available means in combating malaria. In the area of vector control, one is a little cautious and I may be corrected here. When we are involving the community in taking action, it is important that the action taken should be effective, because it is the result of that action that will reinforce belief and trust in it. It is that process that will help them to internalize those activities and make them part of their own life style. But if the actions that they take as a result of our interaction with them fails, in other words the consequences are not sufficiently effective for them to realize, then that has a way of undermining not only what we are trying to introduce, but also other things related. And so, if perhaps source reduction as we know it today may not be effective in sufficiently reducing the mosquito population to have an impact on transmission, then it may be a fairly dangerous method to follow at this particular time.

BRUCE-CHWATT: Thank you very much, Dr. Kaseje.

MFONFU: Dr. Mfonfu from the Cameroon. I have a question for Professor Coluzzi. In Cameroon and the extreme north around Lake Chad, studies have been carried out in that area and they have shown that transmission of malaria is perennial. This area is an inundated area and many dams have been built for the cultivation of rice. In your presentation, you said that the construction of dams for the cultiva- tion of rice did not have any effect on malaria transmission. The entomologic studies showed that in this area the vectors were *A. gambiae* and *A. funestus*. Well, you did not mention *A. funestus* in your presentation. The authors of the studies state that *A. funestus* maintains the transmission of malaria in the dry season in the dams and that *A. gambiae* increases the incidence of malaria during the rainy season. I want to know what you think about it. Thank you.

COLUZZI: Dr. Coluzzi. I was probably mentioning *A. funestus* too rapidly. Of course, *A. funestus* plays a very important role in, mainly, the Sudanese and Guinea savanna areas, and particularly where more permanent bodies of waters are present at the end of the rainy season. What

I did not say was that dam and rice cultivation does not affect malaria transmission; it is just the opposite. Probably, I was misunderstood. It is not the big body of water that is producing *A. gambiae*, but the more marginal areas of the dam and the irrigation canals that originate from the dam. As far as rice cultivation is concerned, I was probably misunderstood, because I stressed the point that rice cultivation, in spite of increasing tremendously the density of the mosquitoes, does not increase simultaneously the transmission potential. These results originate from studies carried out by French investigators in the Bobo Dioulasso area in Burkina Faso, which show clearly that in spite of a very high density, there is less transmission. Time does not permit to analyze the details now. There will probably be comments by Dr. Carnevale. Thank you, Mr. Chairman.

BRUCE-CHWATT: Thank you very much. Profession Kilama is not present. We still have five minutes, I would like to give a conclusion of what we have had this afternoon.

First of all, we heard the remarkable description of a panorama of African diseases, especially malaria, in relation to a number of factors, namely population increase. We know that the population of tropical Africa is increasing at an average rate of 3% per annum. In some countries, the increase is 4–4.5% per annum. Therefore, the problem of food in relation to the population is increasingly important. We know to what extent the growing of food contributes to deforestation, and to what extent the deforestation has an effect on the whole ecology of these countries. We have seen that any rural development, by increasing irrigation, brings in its wake the increased breeding of malaria vectors in the same way as increased irrigation produces increase of schistosomiasis in many parts of Africa, so there is no one single factor. All the factors are intertwined.

In a particular way, one factor is dependent and creates the other problems. We have also heard from Dr. Kaseje how important is the involvement of the population as such, namely, the socioeconomic development to allow for the involvement of primary health care. We have heard far too little today about the primary health care and its role in malaria control. Perhaps we will discuss it tomorrow or the day after. But, I would like to add my own personal view on the trend from malaria eradication to malaria control.

I was involved in 1956 in the basic laws of malaria eradication, the Sixth Expert Committee of Malaria in Rome, and I realize to what extent we were unaware of the problems that we were going to face. Then, in 1979, when the trend changed slowly at first and then faster and faster, from malaria eradication to malaria control, quite a number of people thought that malaria control was simpler than malaria eradication—that malaria control was a "poor man's malaria eradication." They were

completely and utterly wrong. Malaria eradication technically speaking was much, much easier than malaria control. In malaria eradication you went by the book. You had a rigid set of technical means that you had to use in the field and you hoped for the best. However, in malaria control, you have to adapt your technical means to the epidemiology of the area, the human ecology of the area, the availability of well-trained staff, and the response of the population.

So, malaria control, in my view, is much more difficult than malaria eradication because it has to be adapted to local conditions. People who know how to do it are very, very few, and that is the reason why training of malaria staff at all levels is the only way to produce what we expect to be, namely, primary health care, which will take malaria control within its orbit. We also know to what extent the number of technical problems that arose during the past 20 years have affected malaria control, namely resistance to insecticides, virtually all insecticides, and the problem of the impact of insecticides on ecology of the country, resistance of *P. falciparum*—thank God, that is the only one for the time being—to our best antimalaria drugs. The attempt to decrease the impact, the pressure, the selection pressure on *P. falciparum* brings me to the very odd problem of malaria prophylaxis versus treatment of malaria. Let us not forget that malaria prophylaxis was in a way, something like malaria eradication. You used the drug chloroquine at that time for all populations and for all age groups. Now, we have second thoughts about it. This type of careless use of drugs produces resistance. Therefore, we have to decrease the selection pressure and we have to use the drugs for those people who are actually down with an attack of malaria. But, what do we know about malaria, about those who have malaria? What is the difference between malaria disease and malaria infection? This brings me to the problem of DNA probe. People want to use the DNA probe to know more about the malaria infection. The answer is very simple. If you use the DNA probe for the whole population of Africa, you will find out what many of you could guess, that 99.9% of the people are already infected. What is this knowledge going to give you? Very, very little. There is a moral problem, a moral dilemma. What right have we got to eliminate from malaria chemoprophylaxis those who have a clinical effect of malaria or whose clinical effects have not been diagnosed by a physician?

We want to prevent selection pressure on the parasite but at the same time we forget the human element involved. How to solve this problem, I do not know, but I would like to stress this moral dilemma to you. If we are going to give treatment of malaria only to those who have been confirmed as suffering from acute malaria, we are omitting completely the 60–70% of infected persons who have no or only a few clinical signs and

symptoms. Technical problems of this nature are going to be more and more involved in our future planning.

There are two things that have to go together. In the first instance, we have to improve our technical methods of malaria control by means of improved knowledge. In the second instance, as Dr. Kaseje rightly pointed out, we have to increase as fast as possible the socioeconomic status and knowledge of those African populations, especially in rural areas, on whom malaria control by primary health care will depend. As we are talking about improvement of technical knowledge, I would like to finish here with a little story. I have two boys—both of them are doctors now. My younger son once asked me, "Daddy, tell me how stars are formed." I said to him, "Really, Bobby, I do not know." Then he said, "Daddy, what is the function of a messenger DNA?" I said, "Bobby, frankly, I do not know." Then he said to me, "Daddy, you don't mind my asking you all those questions?" I said, "No, of course not, Bobby, this is the only way to learn."

On this story, we will perhaps close our session this afternoon.

# Reference Center for Drug Resistance of Malaria in West Africa[a]

T. R. GUIGUEMDE

Until 1983, chloroquine resistance of *Plasmodium falciparum* was limited to Madagascar and to parts of East Africa (Kenya, Burundi, and Tanzania). In 1984 chloroquine resistance was noted in the eastern parts of Zaire and in Angola. More recently (March 1985), it was also observed in Cameroon and in the Central African Republic (CAR), where it assumed epidemic proportions with rapid spread from the foci of Bangui and Limbe, near Douala. Until then no case of clinical chloroquine resistance had been described from West Africa.

For the nations of West Africa, the eventual spread of drug resistance has become a major public health concern. This concern became apparent in the deliberations and research projects presented at the 25th Technical Conference of OCCGE, Bobo Dioulasso, 15–19, April, 1985. The experts of the member states and the scientific council of OCCGE proposed the creation of a surveillance system for drug resistance to *P. falciparum* in the OCCGE countries. Accordingly, in November 1985, the council of ministers of the eight member countries established the Reference Center for Drug Resistance to Malaria (CRC/OCCGE) with headquarters at the Centre Muraz. Justification for creation of such a center came rapidly with the recognition of the first 10 cases of clinical chloroquine resistance in Cotonou, Benin.

We are presenting in this paper the objectives, organization, operation, and surveillance methods as advocated by the member states.

## OBJECTIVES OF THE CENTER

The reference center has the following targets:

1. Establish in each of the member states a team capable of performing in vivo and in vitro tests of drug resistance as needed for national surveillance, using standardized methods.

[a]Translated from French; see French summary at the end of this paper.

2. Assure adequate staffing and continuing training of these teams.
3. Maintain the role of technical council for the national teams.
4. Suggest appropriate treatment regimens for malaria to prevent or limit the further spread of drug resistance in West Africa.
5. Collect the results of drug sensitivity studies of the subregion and disseminate this information to the member states of OCCGE.
6. Collaborate with centers in overseas (Centre National Francais de la Chimiosensibilite and the malaria branch of CDC in Atlanta) to get information on the latest technical developments and of surveillance of resistance among nonimmune travelers to Africa.

## CHOICE OF THE CENTRE MURAZ AS REFERENCE CENTER

The reasons that have guided the experts of member states and the scientific council in their choice of the Centre Muraz as reference center for surveillance of drug resistance are as follows:

1. The center has qualified technical personnel to perform the tests needed to determine the level of drug resistance (Drs. Guiguemde, Gbary, Ouedraogo, Douchet and a number of laboratory technicians experienced in malariometric studies).
2. The center has the necessary laboratory equipment.
3. The center has had considerable experience in the field of malariology, in particular in studies of drug resistance in Africa. Between 1982 and 1986 the following tests have been performed: 768 in vivo tests over 7 days of observation, 30 in vitro tests (WHO macro test), 92 in vitro WHO microtests, 82 in vitro semi-microtests (Le Bras), and 40 in vitro tests using radioisotopes (Le Bras et al).
4. The center can provide teaching and training courses for its member states, as well as at the international level (e.g., Joint International Course in Malariology, CIDESCO-OCCGE).
5. The center is already participating in collaborative studies with foreign research institutions.
6. Its location in the heart of west Africa facilitates its role as coordinator between the national teams of surveillance.

## SURVEILLANCE STRATEGY

### Passive Surveillance

During the past years, the emergence of chloroquine resistance in Africa was detected among the nonimmune parts of the population (expatriates

and children under 5) by reduced efficacy of chemoprophylaxis or by treatment failures in clinical malaria. The nonimmunes are the very targets of passive surveillance.

At the national level, physicians and health agents should be encouraged to prepare blood smears from each patient at day 0 (before treatment), and again at day 7 after treatment with chloroquine administered at a dose of 25 mg/kg body weight over 3 days. Each treatment failure and each malaria attack in a patient who has received adequate chemoprophylaxis should be reported to the national surveillance team.

At the CRCP/OCCGE level, the center maintains contact with foreign institutions and will inquire about all patients with a diagnosis of drug-resistant malaria who have had a recent travel history to West Africa. This type of passive surveillance will only detect clinical resistance, i.e., drug resistance at an already elevated level.

## Active Surveillance

It appears that resistance to chloroquine appears only after a progressive decline in drug sensitivity. The objective of active surveillance is the early detection of this decline before clinical resistance has developed. Hence, there is justification for using tests that employ low doses of 5–10 mg/kg of chloroquine.

*Peripheral Sentinel Centers.* In each country villages will be selected for study that have a dispensary staffed by a registered nurse (infirmier d'etat) and a primary school. These villages will be representative of different epidemiologic patterns of malaria (e.g., urban and rural areas, forest zones, the sahel, and savanna.) For reasons of feasibility, the in vivo tests to be used must be simple, using low doses of 5–10 mg/kg of chloroquine. They will be carried out as follows: on a Monday (day 0) the nurse will prepare thin blood smears from 100 pupils of the primary school. Thereafter, the children are weighed. One half of them will receive chloroquine at a dose of 5 mg/kg body weight and the other half 10 mg/kg. On the following Monday (day 7) a second blood smear is taken. All slides will be sent to the National Surveillance Center for staining, reading, and interpretation. In case of suspected drug resistance, the central surveillance team will visit the place to verify the results. The peripheral sentinel teams should repeat their investigations one or two times per year, if possible.

*National Centers.* The national teams will carry out in vitro tests in combination with in vivo tests whenever there is suspicion of drug resistance from the results of the tests performed by the sentinel teams, and whenever requested by a physician in case of clinical resistance.

*Reference Center of OCCGE.* If the need should arise the CRCP could participate in the in vitro studies of the national surveillance teams in their respective countries.

## ORGANIZATION AND OPERATIONS

The structure of the surveillance system of drug resistance in the member states is depicted in Figure 1.

### Peripheral Sentinel Centers

The nurses of these centers are trained by the central surveillance teams in the methods of simplified in vivo tests, including preparation of blood smears, data collection, etc. Their work is being supervised by the central team.

### National Centers

A physician or pharmacist is the team leader. The other three or four team members have either similar qualifications or are laboratory technicians experienced in malariometric studies. The national team is trained by the Central Reference Center in the methods of in vivo and in vitro tests. It prepares and executes the national program of surveillance of drug resistance.

### Relationship Between CRCP of OCCGE and National Centers

The national centers collect and submit national surveillance data to CRCP and disseminate the information received by CRCP to other countries. These centers must regularly distribute information and train the national teams in new surveillance techniques by CRCP. Furthermore, the national centers must supervise national surveillance activities by CRCP, and prepare standardized treatment regimens for malaria for the member states, taking into account the level of drug sensitivity of the malaria strains in the subregion through periodic meetings with those responsible for the national malaria parasite control programs.

### Relationship Between CRCP of OCCGE and Cooperative Centers

CRCP and the cooperative centers exchange information on drug resistance in Africa and in other regions of the world, and technology transfer of new surveillance methods for drug resistance in malaria.

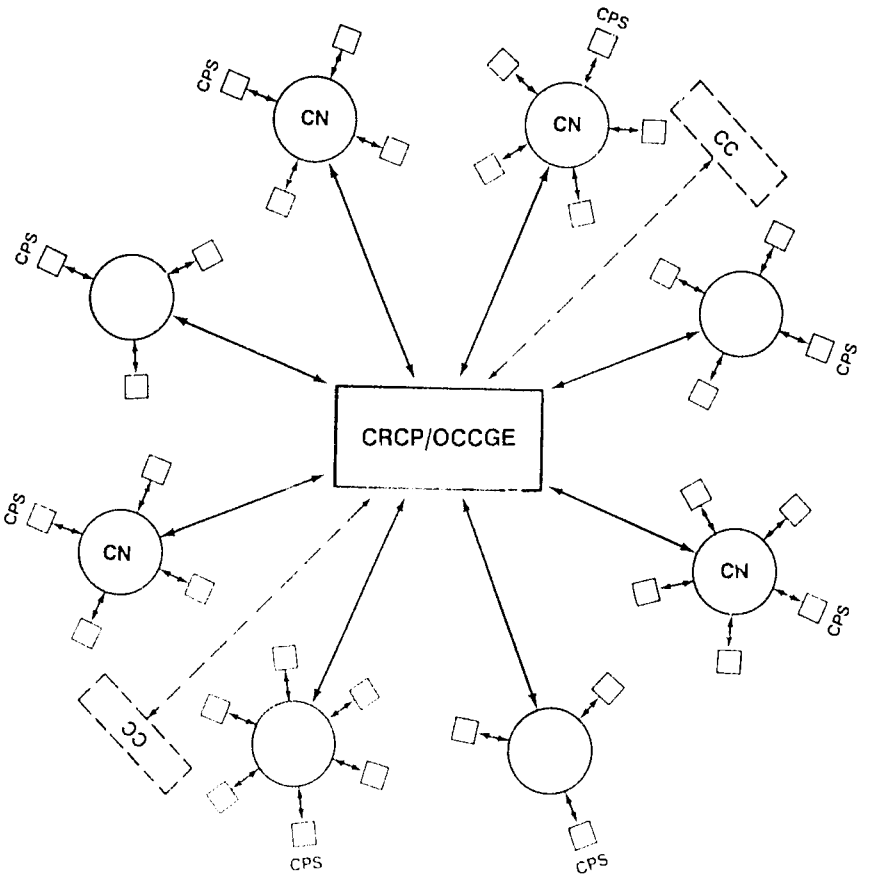


Figure 1. Schema of the surveillance system for drug resistance in malaria of the West African countries of OCCGE. There are a minimum of three peripheral centers (CPS) per country, and they carry out in vivo tests. National centers (CN) carry out in vitro and in vivo tests and are responsible for training technicians. The Malaria Resistance Reference Center (CRCP) coordinates the work of the national centers. Cooperative centers (CC) include, among others, IMET of France and the Centers for Disease Control of the United States.

## CONCLUSIONS

Confronted with the catastrophic spread of drug resistance to malaria parasite, the member states of OCCGE decided to establish a reference center for drug resistance in order to coordinate the surveillance program in the subregion.

The careful studies of drug resistance carried out by expatriate teams using different methods of measurement did not produce results that

would permit a homogeneous assessment of drug resistance of the various plasmodial strains studied. It became necessary to integrate the surveillance activities into the national programs of the countries in such a way that they would be part of a continuing and coordinated system for the entire subregion.

The planned strategy of surveillance must, most of all, be feasible (i.e., based on active surveillance at the peripheral level using simplified in vivo tests, and on continuing passive surveillance at all levels of the national health services). This initiative which initially concerns only member states of OCCGE could eventually include all countries of west Africa. To this end the support of all nations and international organizations would be greatly appreciated.

## RESUME

Face a la catastrophe que constituerait la propagation de la chimioresistance du paludisme, les Etats-Membres de l'OCCGE (Organization de Coordination et de Cooperation pour la lutte contre les Grandes Endemies en Afrique de l'Ouest), ont cree au sein du Centre Muraz, le Centre de Reference pour la surveillance de la chimioresistance du Paludisme (C.R.C.P./OCCGE).

Ce centre a pour vocation la formation technique des equipes nationales a la même methodologie des tests de chimiosensibilite et la coordination des activites de surveillance. La strategie de surveillance adoptee se veut avant tout d'être faisable: surveillance active de premiere ligne par des tests *in vivo* simplifies a doses faibles (5 ou 10 mg de chloroquine/kgp), surveillance passive continue a tous les niveaux des centres de sante des etats.

La structure du systeme de surveillance est la suivante:

1. Des Centres Peripheriques Sentinelles (C.P.S.) dans chaque pays.
2. Un Centre National par pays.
3. Le C.R.C.P./OCCGE.
4. Des Centres collaborateurs etrangers (Centre National Francais de la chimioresistance, Malaria Branch du CDC d'Atlanta).

Cette initiative qui concerne d'abord les etats de l'OCCGE est appelee a englober l'ensemble des etats de l'Afrique de l'Ouest. Pour ce faire vivement mande.

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## B. Country Situation Reports

# Malaria in Cameroon

DANIEL MFONFU

According to the health statistics of 1977 through 1979 of the Directorate of Studies, Planning, and Statistics in the Ministry of Public Health, the reported annual incidence of malaria was 11.6% and the mortality rate was 4/100,000 inhabitants. The analysis of these data shows that malaria is the principal cause of morbidity in the 0-to-14-years age group. It is the second most frequent cause of death after measles for this age group.

Several World Health Organization (WHO) publications described the presence of strains of chloroquine-resistant *Plasmodium falciparum* on the African continent. Studies carried out in Cameroon up to January 1985 revealed no case of malaria resistance to chloroquine.

In February 1985, however, the first cases of chloroquine-resistant malaria were diagnosed in two French expatriate children living in Limbe who were receiving the drug as chemoprophylaxis (Charmot 1985; Sansonetti et al. 1985). The appearance of chloroquine-resistant *P. falciparum* malaria has caused fear and confusion as to the treatment of malaria in Cameroon.

Considering the socioeconomic progress made in the country in 25 years, and taking into account the considerable improvements of the health delivery system manifested by the development of health infrastructures, training of personnel, adequate supply of drugs and primary health care, it is anticipated that malaria has been regressing.

Studies carried out in Cameroon over the last 10 years have shown that the prevalence of malaria is decreasing. However, new trends in the health statistics and the emergence of resistance to chloroquine have made malaria a public health priority in Cameroon.

## OBJECTIVES AND METHOD

To understand the present situation of malaria in Cameroon, we analyzed the national disease data. The objectives in analyzing the national disease data were the following:

1. Revise the epidemiological maps of malaria established in 1957 and 1969;

2. Study the geographical distribution of the reported malaria cases in 1984 and 1985;
3. Analyze the results of malaria surveys carried out in Cameroon;
4. Compare data obtained from malaria surveys since 1957 in order to study the evolution of the disease;
5. Make a review of data on the emergence of chloroquine-resistant *P. falciparum* in Cameroon;
6. Make a review of studies on the sensitivity of *P. falciparum* to chloroquine and other antimalarial drugs;
7. Prepare an epidemiological map of the prevalence of malaria in Cameroon.

The method involved analyzing the results of various malaria studies that were available from the Service of Epidemiology, Ministry of Public Health.

## GEOGRAPHIC DISTRIBUTION OF MALARIA ENDEMICITY IN CAMEROON: 1957 AND 1969

### Languillon (1957)

Languillon and associates visited only sectors in the zones without insecticide treatment. The study, which started in 1955, divided Cameroon into four major regions:

1. Forest region of the South;
2. Mountainous region of the West;
3. Savanna region;
4. Sahelian region of the North.

With the exception of the Bamileke area, the regional average of the malaria indices was above 50%, hence malaria was hyperendemic.

In the savanna and the sahelian regions, as well as in the mountains of the west, the principal vectors are *A. funestus* and *A. gambiae*.

In the forest *A. mouchetti* and *A. nili* maintain malaria transmission in natural sites and *A. gambiae* is responsible for malaria epidemics.

### Delfini and Abane

Delfini and Abane established an epidemiological map of malaria in Cameroon from data collected from 1962 to 1969. Their study divided the country into eight zones:

*Zone 1: Humid Forest.* The epidemiology of malaria in this region was influenced by an antimalarial campaign that took place from 1954 to 1961, and from 1954 to 1964 in the pilot zone in and around Yaounde. It is thus necessary to distinguish between zones with and without previous treatment.

In the area without previous antimalarial program, the level of malaria transmission lies between mesoendemic and hyperendemic. The species of *Plasmodium* are *P. falciparum*, *P. malariae*, and *P. ovale*, in order of importance. The confirmed vectors are *A. gambiae* and *A. funestus*.

In the area with previous treatment (pilot zone of Yaounde), malaria has been stable and mesoendemic but is showing a tendency to increase.

*Zone 2: The Coast.* Malaria is hyperendemic. *P. falciparum* predominates and is followed by *P. malariae* and *P. ovale*. *A. gambiae* and *A. funestus* are the confirmed vectors.

*Zone 3: The High Plateaus of the West.* The climactic variations due to differences in altitude cause great fluctuations in the prevalence of malaria. It ranges from very low, to mesoendemic on the hills, to hyperendemic in the valleys.

*Zones 4–8: Central Savanna, Adamaoua, Benoue Basin, and Mandara Mountains.* Malaria is hyperendemic in zones 4–7 and mesoendemic in zone 8 (an inundable savanna).

## GEOGRAPHIC DISTRIBUTION OF REPORTED MALARIA CASES: 1984 AND 1985

Although systematic passive reporting of cases is insufficient, it shows seasonal and evolutive trends of diseases and health problems as seen in Figure 1.

In all, 817,965 cases of malaria with 329 deaths were reported in 1984 and 799,738 with 327 deaths in 1985.

The annual incidence was 81/1000 cases with a mortality of 3/100,000 population. Of the 1176 cases that would be expected from making extrapolations of data collected during the past two years, 935 (79.5%) were actually reported. Malaria has remained a major cause of morbidity in all age groups.

## REVIEW OF MALARIA SURVEYS

### Minkama and Nalassi

The communities of Minkama and Nalassi, in the Lekie Division of the Central province, were studied by Ripert and associates in June 1975 during the rainy season.

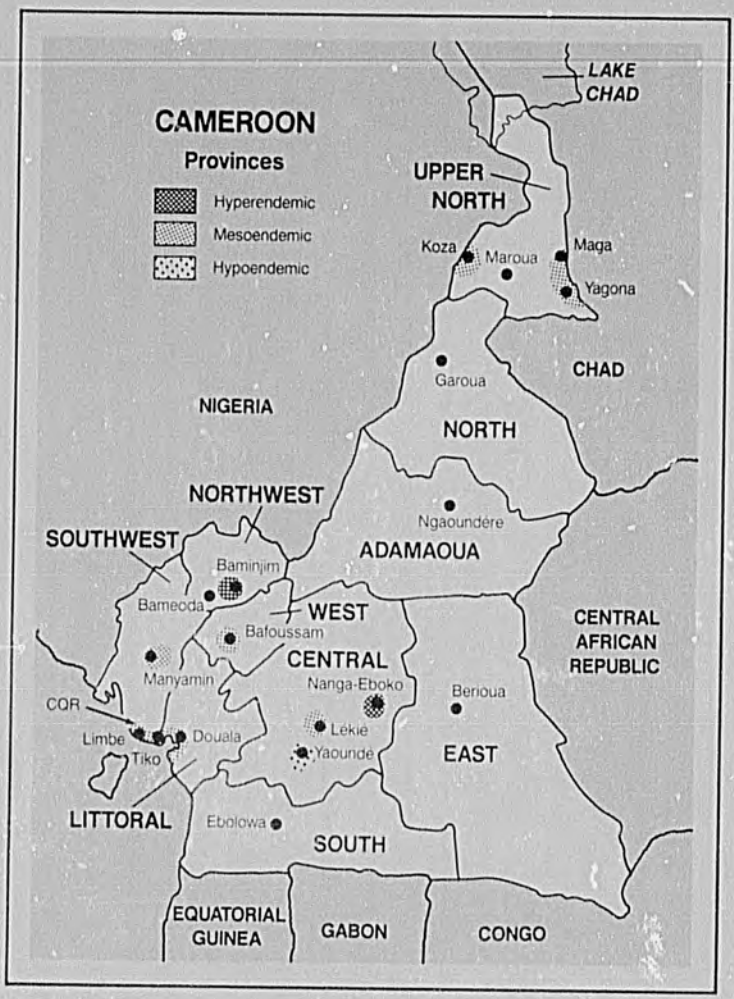


Figure 1. Epidemiologic map of malaria in Cameroon, based on field studies. CQR, chloroquine resistance of *P. falciparum*.

Examination of thick smears showed malaria parasites in 20.8% of the slides. 95.7% of these slides were positive for *P. falciparum* and 4.3% for *P. malariae*. The parasite rate of children up to 9 years of age was 42.2%. With the use of an immunofluorescent test, antibodies were found in 43.7% of the blood samples in the study area.

## Koza

A survey of the Koza region in Mayo-Tsanga in the Extreme-North province was carried out by Ripert and associates in April 1979, at the end

of the dry season.

*P. falciparum* was the only species of *Plasmodium* found in the area. The parasite rate in children 2–9 years of age was 40.8%, and the spleen rate was 35.6%. The immunofluorescent test performed with an homologous *P. falciparum* antigen was positive in 38.4% of the children in this age group.

Malaria cases are more numerous at the end of the rainy season, but a new spell of the disease is also noticed at the beginning of the year while the outside temperature is especially high. Malaria, now mesoendemic in the study area, has decreased. This seems to be related to the increased use of chloroquine by the residents.

### Yagoua and Maya

Data from the rice-field regions of Yagoua and Maga in the Mayo-Danay Division of the Extreme-North province were collected by Couprie and associates in April 1981, at the end of the dry season.

The results of the studies, carried out in the irrigated regions of the Logone Valley, showed that *P. falciparum* is the only malaria species found in the area.

The plasmodial index of children aged 2–9 years was only 8.5% at the end of the dry season; this contrasted to the spleen index, which was 34.8%. These figures suggest that malaria is mesoendemic in the region. *A. gambiae* is the predominant vector in the rainy season and *A. funestus* in the dry season.

### Manyemen

The forest region of Manyemen in the Meme Division of Southwest province was studied by Cornu and associates in December 1983, at the beginning of the dry season.

The prevalence was 18.7% for *P. falciparum*, 10.5% for *P. ovale*, and 1% for *P. malariae* in the villages of Mungo Ndor and Kokobuma. The parasite index of children aged 2–9 years was 38.2%, and the spleen index 26.6%, with a mean Hackett score of 1.6. Malaria is thus mesoendemic in the region. The immunofluorescent test using *P. falciparum* antigen was positive in 66% of the children in this age group. *A. gambiae* is the main vector of malaria in the area.

### Bamendjin Dam

The Bamendjin Dam area in the Mezam Division, North-West province was surveyed by Atanga et al. (1979). They found that 25% of the

population had parasitemia with *P. falciparum*, while 80% had a positive indirect immunofluorescent test for malaria. The local vector is *A. funestus*.

### Nanga-Eboko

Nanga-Eboko in the Upper Sanaga Division, Central province, was visited by Ngnintedom-Bavoua from September 1985 to February 1986. *P. falciparum* was the only species diagnosed. The plasmodial index in 2- to 9-year-old children was 30.3% and the spleen index 23.7%. Malaria is mesoendemic in this town of the forest region.

### Bafoussam

Bafoussam in the Mifi Division, West province, was studied by Tchinda Abel from September 1985 to February 1986. *P. falciparum* was the only species of plasmodium found in the area. The parasite index in 2- to 9-year-old children was 13%, while the spleen index was 2.5%.

### Yaounde

The city of Yaounde was sampled by Merlin and associates in September 1985. Only *P. falciparum* was found in the population sample studied. The parasite index of 2- to 9-year-old children was 9.2% and the spleen index 8.3%. Yaounde is in a hypoendemic zone. The crude prevalence rate of IF antibodies in the general population was 37.8%. Self-medication with chloroquine in subcurative doses is practiced widely and used by about 90% of the children with fever.

### Limbe, Tiko, and Douala

The recent survey in Limbe, Tiko and Douala was carried out by Merlin and associates in July 1985. Only *P. falciparum* was identified. The parasite index in 2- to 9-year-old children was 14.1%; the spleen rate was 32.6%. The prevalence of IF antibodies was 50.8%.

Self-medication of fever with chloroquine is practiced by 90–97% of the local residents. This high rate of chloroquine use in nontherapeutic doses might have been responsible for the emergence of chloroquine resistance in Limbe.

### Mbenega, Essong, and Nkolossan

Mbenega, Essong and Nkolossan in the Lekie Division, Central province, were visited by Moyou and Avenec in 1984. *P. falciparum* was identified

in 27.7% and *P. malariae* in 2.8% of the population. The total parasite rate was 30.1%, and the gametocyte rate 3.9% for the three villages.

### Maga

Maga in the Mayo Danay Division was studied by a team of OCEAC in October 1985. Inside the project zone with its well-constructed villages, the plasmodial index in 2- to 9-year-old children was 23.2% and the spleen index 6.4%. In contrast, in population samples living outside the development zone, the plasmodial index was 40.1% and the spleen index 20.9%.

## CHANGES IN MALARIA ENDEMICITY: 1957–1985

Languillon (1957) found that with the exception of the Bamileke region, malaria was hyperendemic in Cameroon. The analysis of the results of the malaria surveys revealed that the prevalence of malaria has significantly decreased since 1969 (Fig. 2).

Malaria is now hypoendemic in Yaounde and mesoendemic in the other regions studied. Factors that have contributed to the decline of the prevalence of malaria include:

1. Widespread use of antimalarial drugs;
2. Improved housing;
3. Urbanization combined with vector control in towns;
4. Better environmental hygiene through health education and improved waste disposal and water supply;
5. Better health delivery systems;
6. Treatment of irrigated agricultural areas by the Department of Agriculture.

## REVIEW OF DATA ON THE EMERGENCE OF CHLOROQUINE RESISTANCE OF *PLASMODIUM FALCIPARUM*

Charmot (1985) and Sansonetti and associates (1985) reported the first cases of chloroquine-resistant *P. falciparum* malaria. The cases involved two French children under correct chemoprophylaxis with chloroquine in Limbe (February 1985).

Racourt and associates (1986) reported the case of a 56-year-old French male on vacation to Douala who visited Limbe in April 1985. On

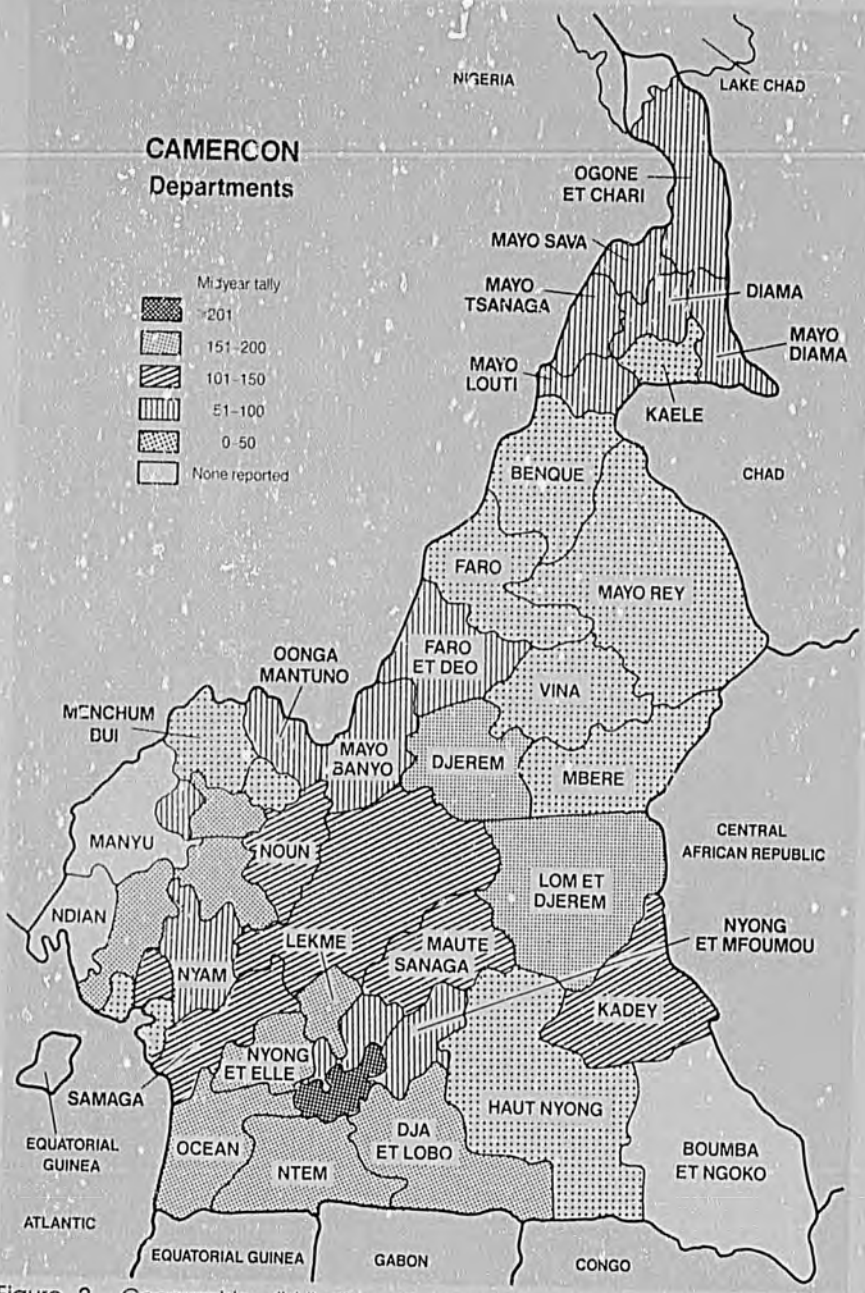


Figure 2. Geographic distribution of reported malaria cases in Cameroon 1984–1985 for each department in the country's Ministry of Public Health. Tally of the declared incidence of malaria per 1000 inhabitants is shown for each department. Cases reported in 1984 totaled 817,965; in 1985, there were 799,738. The average annual national incidence was 81/1000 inhabitants.

his return to Paris, he developed fever; chloroquine-resistant *P. falciparum* malaria was diagnosed. The patient later died of his disease.

## REVIEW OF STUDIES ON SENSITIVITY OF *P. FALCIPARUM* TO CHLOROQUINE AND OTHER ANTIMALARIAL DRUGS

The analysis of these studies revealed the following conclusions:

1. Confirmed the existence of chloroquine-resistance *P. falciparum* in the Limbe area;
2. Showed that *P. falciparum* is still sensitive to amodiaquine;
3. Suggested that there might be a chloroquine-resistant strain of *P. falciparum* in Garoua;
4. Revealed that chloroquine resistance is limited to the region of Limbe.

Most of the authors agree that the chloroquine resistance observed in Cameroon is related to the abusive consumption of this drug in all febrile illnesses.

## EPIDEMIOLOGICAL MAP OF MALARIA

From malaria surveys and studies on sensitivity of *P. falciparum* to chloroquine carried out in Cameroon, I tried to prepare a preliminary epidemiological map of malaria in the country. The map will be modified as other results of studies are received at the Ministry of Public Health.

## STRATEGY FOR MALARIA CONTROL

### Chemotherapy

Chemotherapy programs include the diagnosis and treatment of patients with confirmed malaria. Systematic presumptive treatment of all cases of fever with a single dose of 10mg/kg of chloroquine constitute the first important step in the national antimalarial program. This is widely applied in the primary health care.

### Chemoprophylaxis

The chemoprophylaxis program concerns the high-risk groups of children from 0 to 5 years old, pregnant women, and nonimmune individuals who

visit Cameroon for a short stay. WHO advises chemoprophylaxis only for pregnant women and nonimmune individuals on short visits to endemic areas.

### Vector Control

Vector control is done through basic measures of sanitation, individual protection by putting light wire lattice on windows and the use of mosquito nets and insecticides. Based on these measures, the government has launched a campaign of hygiene and sanitation, which seems to be successful.

### Vaccine Against *Plasmodium*

In perspective, the production of a vaccine against *plasmodium* would enhance the control of malaria and would greatly decrease the incidence of malaria.

## CONCLUSION

My review of available data on malaria in Cameroon show that:

1. Malaria has remained a public health problem in Cameroon.
2. The prevalence of malaria has significantly decreased in the entire country. Malaria is now mesoendemic according to studies.
3. There exists chloroquine resistance of *P. falciparum*. At present it is still limited to the zone of Limbe.

In order to update the epidemiological map of malaria in Cameroon, malaria and entomological surveys and studies on the sensitivity of *P. falciparum* to antimalarial drugs are encouraged.

# The Malaria Situation in Ethiopia

AWASH TEKLEHAIMANOT

Malaria is widely distributed in about 70% of Ethiopia and is the country's foremost health problem. Its impact on socioeconomic development is deeply felt and well recognized. It is estimated that 64% of its 42.2 million inhabitants reside in potentially malarious areas. The varied physical features of the country create a wide range of climatic conditions that represent different levels of malaria endemicity. There are two main seasons of transmission, following the big and small rains of June through August and May through April, respectively. Table 1 shows demographic data and vital statistics for the country.

Malaria control, which was initiated in certain parts of the country about 25 years ago, is presently extended to cover a significant proportion of the country. In spite of these efforts, the prevalence of malaria is still high due to changing epidemiological characteristics. The construction of dams, irrigation schemes, large-scale population movements in the form of labor and resettlement in agricultural developing areas, are some of the few factors that at present are influencing the fluctuations in malaria transmission (Fig. 1).

## NATIONAL POLICY ON MALARIA

The government, recognizing the impact of malaria on health and socioeconomic development, has made long-term commitments to controlling the disease. The organization is therefore adequately staffed and sufficiently budgeted to carry out nationwide activities.

These are the organization's objectives:

1. Maintaining the efforts of those administrative regions of the country that have eradication operations.
2. Extending control activities to other malarious areas to prevent or reduce mortality and morbidity.
3. Conducting operational research to improve existing control methods and strategies.
4. Promoting and enhancing community participation and intersectoral collaboration.

Table 1. The demographics and vital statistics of Ethiopia.*

| |
|---|
| Area: 1,250,000 km$^2$ |
| Overall population: 42,000,000† |
| Age distribution (yr):† |
| <1 = 3.6% |
| 1–4 = 14.2% |
| 5–9 = 15.9% |
| 10–14 = 12.5% |
| <15 = 46.2% |
| >15 = 53.8% |
| Geographic distribution† |
| Rural = 88.7% |
| Urban = 11.3% |
| Crude birth rate: 46/1000 population |
| Crude death rate: 18.1/1000 population |
| Infant mortality rate: 142/1000 live births |
| Life expectancy at birth: 47 yr |

*Data from *Comprehensive Health Services Directory, 1983–84*, Ministry of Health, Ethiopia.

†Data from nationwide census of May 1984.

5. Ensuring step-by-step functional integration with the basic health services through integrated primary health care system.

## CONTROL APPROACHES

The organizational setup is well established and has one central, 15 regional, and 69 sector offices, with a total of 1600 different catagories of permanent employees who are distributed throughout the country. Control measures include application of indoor residual insecticide, environmental management and larviciding, treatment of cases, and, in some instances, administration of chemoprophylaxis to vulnerable groups.

### Residual Spraying

Spraying (DDT, 75% wettable powder at 2 g/m$^2$) is carried out selectively based on epidemiological considerations. The localities in the operational areas are stratified into high, medium, and low levels according to their endemicity or malariogenic potential and receive "two spray rounds, one spray round and zero spray round" of residual insecticide per year, respectively.

Mass blood surveys that are carried out every year during the main transmission seasons (September through November) and parasitological data that are generated from the Malaria Detection and Treatment Posts

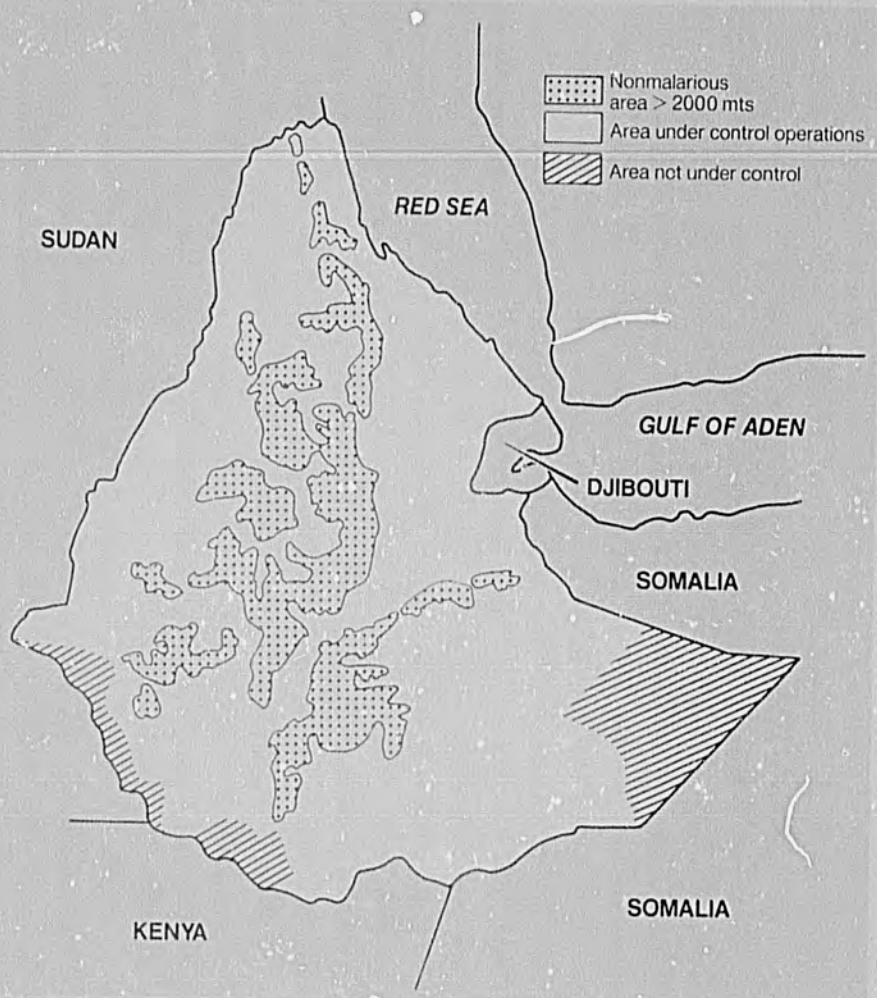


Figure 1. Distribution of malaria in Ethiopia and areas under control operations.

(DTPs) provide the epidemiological basis for classifying the localities into the two-, one-, and zero-spray round categories. In 1986, a total of 1,092,042 unit structures in 5360 localities were sprayed with residial insecticide and 3,512,001 people were directly protected through such measures.

## Environmental Management and Larviciding

A combination of control strategies are employed whenever feasible, particularly in permanent breeding sites. Currently there are 10 towns with a total population of 436,108 that are utilizing environmental man-

agement and larviciding techniques in conjunction with focal spraying. This approach was found to be effective in reducing the prevalence of malaria and the level of transmission.

### Chemotherapy

Antimalarial drugs are used as a supplement and/or complement to spray operations in most of the areas. The general drug-use strategy recently has been revised as a result of the growing concern about the emergence of chloroquine-resistant *Plasmodium falciparum*. All confirmed cases as well as clinical cases (if diagnostic facilities are not available) are treated with a standard dose (25 mg/kg) of chloroquine and with 45 mg of primaquine. Since the prevalent species is *P. falciparum*, primaquine is used as a gametocytocidal drug.

### Diagnostic Services

Great emphasis is being given to the establishment of a network of diagnostic facilities to provide treatment of malaria cases. Presently, the program has 1 central, 15 regional, and 50 field laboratories. The field laboratories are located in areas where health stations are not available and thus facilitate timely examination of blood slides. These field laboratories are expected to serve as entry points for other primary health care activities.

The main role of the central laboratory in Addis Ababa is to carry out supervision and quality control on the work efficiency and diagnostic skills of the regional and field laboratories. All slides diagnosed as positive and 5% of those diagnosed as negative by the regional and field laboratories are cross checked for accuracy at the central laboratory.

Additionally, 630 malaria DTPs are established throughout the country to collect blood smears and to treat all febrile cases presumed to be malaria. These posts could be either health stations of the Ministry of Health, clinics of other governmental and nongovernmental agencies, or individuals from rural and urban community associations.

### Chemoprophylaxis

The provision of population-based chemoprophylaxis is not practiced in the regular operational areas of the control program. However, depending on local situations, chemoprophylaxis (5 mg/kg) is provided to migrant workers, new settlers, construction workers, and army units for limited periods of time.

### Biological Control

The organization is opting for a comprehensive vector control approach that integrates chemical, environmental, and biological control methods. The possibility of using biological control agents such as *Bacillus thuringensis israelensis* and larvivorous fish is being considered. The organization is currently involved in the evaluation of larvivorous fish for use as biological control agents. So far, laboratory assessment of the larvivorous capacity of three species of fish indigenous to Ethiopia, *Alphanius disper*, *Aplocheilichthys antinorii*, and *Oreochromis niloticus* is completed and encouraging results are obtained.

Field trials with these three species of fish will resume. The fish project is funded by the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases.

## ASSESSMENT AND EVALUATION OF CONTROL MEASURES

### Seasonal Prevalence Surveys

During the peak transmission season, which generally extends from September to the end of November, random blood smears are collected from representative samples of localities selected from three epidemiologically stratified areas. The objective of the survey is to assess the efficacy of the control measures taken and to monitor the malaria situation in those localities where spraying operations are withdrawn.

In 1985 a total of 2264 localities from different parts of the country were selected, from which a random sample of 249,981 blood films was drawn; out of these, 9582 were positive (Table 2). By far, *Plasmodium falciparum* was the predominant species, representing 73% of the total cases. The overall prevalence rate for the entire sample for the season was 3.5%. All age groups were infected; the highest number of cases was in the 10-and-above age group. The age-specific prevalence rates for the under 1, 0–4, 5–9, and 10 and over age groups was 2.9%, 3%, 4.1%, and 3.2%, respectively. While it will be important to note that the data were collected from operational and nonoperational areas (localities that are not sprayed), this information nevertheless clearly indicates that malaria is still a serious health problem in Ethiopia.

### Detection and Treatment Posts

The 630 DTPs distributed throughout the country monitor the malaria situation. The DTP staff adequately trained and supplied with all essen-

Table 2. Malaria prevalence surveys conducted during transmission season of 1981–1985.*

| Year | Slides Examined | Positive | % Positive | Plasmodium Species Distribution Pf | Pv | Pm | mix | Age Distribution of Positive Cases (yr) <1 | 1–4 | 5–9 | 10+ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1981 | 367,781 | 19,165 | 5.21 | 11,130 | 7,974 | 125 | 64 | 162 | 3,320 | 3,476 | 12,207 |
| 1982 | 374,375 | 6,736 | 1.80 | 5,107 | 1,591 | 81 | 43 | 128 | 1,257 | 1,318 | 4,033 |
| 1983 | 365,627 | 9,074 | 2.48 | 7,090 | 1,961 | 81 | 58 | 161 | 1,872 | 1,911 | 5,130 |
| 1984 | 392,360 | 8,185 | 2.09 | 6,212 | 1,983 | 70 | 80 | 105 | 1,415 | 1,587 | 5,078 |
| 1985 | 249,981 | 9,582 | 3.83 | 7,053 | 2,471 | 124 | 66 | 128 | 1,821 | 1,952 | 5,681 |

*Data from National Malaria and Other Vector-Borne Diseases Control Service, Ethiopia.

Table 3. Results of analysis of blood films collected by detection posts from fever cases, 1981–1985.*

| Year | Slides Examined | Positive | % Positive | Plasmodium Species Distribution Pf | Pv | Pm | mix | Age Distribution of Positive Cases (yr) <1 | 1–4 | 5–9 | 10+ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1981 | 45,286 | 15,964 | 35.25 | 3,982 | 11,981 | 35 | 34 | 107 | 1,432 | 1,651 | 12,774 |
| 1982 | 50,166 | 13,573 | 27.06 | 5,744 | 7,795 | 84 | 50 | 87 | 1,132 | 1,534 | 10,820 |
| 1983 | 100,501 | 29,437 | 29.29 | 10,255 | 19,173 | 85 | 76 | 167 | 2,088 | 2,942 | 24,240 |
| 1984 | 214,192 | 59,583 | 27.82 | 29,755 | 29,896 | 192 | 260 | 318 | 4,455 | 5,712 | 49,098 |
| 1985 | 300,653 | 101,578 | 33.79 | 45,845 | 55,495 | 619 | 381 | 558 | 6,931 | 9,932 | 84,157 |

*Data from the National Malaria and Other Vector-Borne Diseases Control Service, Ethiopia.

tials. A total of 300,053 febrile cases were treated through the DTP during 1985 (Table 3).

### Monitoring Outbreaks

The program has established a surveillance mechanism for monitoring the occurence of outbreaks in different parts of the country. During the peak transmission seasons, all sector and zone offices are required to submit weekly reports on the status of malaria in their respective areas. The sectors receive their reports from DTPs, peasant and urban associations, and other governmental and private organizations.

The program has issued specific guidelines detailing the steps and actions to be taken by all concerned in the event that malaria outbreaks occur. As a result of the fast and efficient reporting system, flare-ups are often extinguished rapidly. In 1985, of the 80 outbreaks that were reported, 72 were confirmed to be due to malaria. The outbreaks occurred in 742 localities with a population of 299,199. From a total of 26,563 blood slides collected, 4988 were found to be positive. The distribution of the

742 localities was 53 in nonoperational areas, 544 in zero-round areas, 57 in one-round areas, and 88 in two-round categories.

### Monitoring Drug Resistance

Chloroquine-resistant *P. falciparum* isolates are widely spread in the East African region, and this development is of concern to our program. The central, regional, and field laboratories are utilized to monitor such development. A combination of monitoring systems are used based on technical capabilities and facilities available.

At the field level, the field laboratories follow up *P. falciparum* malaria cases by taking blood smears at day 0 (at time of initial treatment) followed by a second blood slide at day 7 (post-treatment). This procedure, which is referred to as a "modified in vivo test," is simple, inexpensive, and is easily carried out by microscopists and technicians. Yet, the data that are generated from such sentinel posts are good indicators of treatment failures in the respective areas.

The second level of monitoring is conducted at the regional levels, where the WHO 7-day in vivo tests are carried out. In addition, there is a small research unit attached to the main laboratory in Addis Ababa. The unit has trained manpower with facilities for continuous in vitro malaria culture and uses both in vivo and in vitro testing methods to conduct antimalarial drug sensitivety studies in Addis Ababa and in the field. The location of the laboratory in Addis Ababa, a malaria-free city, is convenient for the study since the possibility of reinfection can be excluded.

Recent studies have indicated the presence of chloroquine-resistant *P. falciparum* isolates in areas bordering Somalia, Kenya, and Sudan (Fig. 2). Containment measures, which included chemotherapy (chloroquine, primaquine, Fansidar) and residual spraying, were carried out in those specific areas. Further studies are being conducted to determine the prevalence of *P. falciparum* resistance isolated in the problem areas. The project is supported by WHO and has received modest funding from the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases.

### Entomological Activities

An eight-person entomology team is assigned to each zone and is responsible for the overall entomological activity. Each team carries out routine susceptibility studies as well as bioassay tests to monitor the efficacy of spraying. In addition, each team is responsible for supervising sector personnel in their entomological surveys and source reduction activities. The entomology section has the facilities of an insectory to

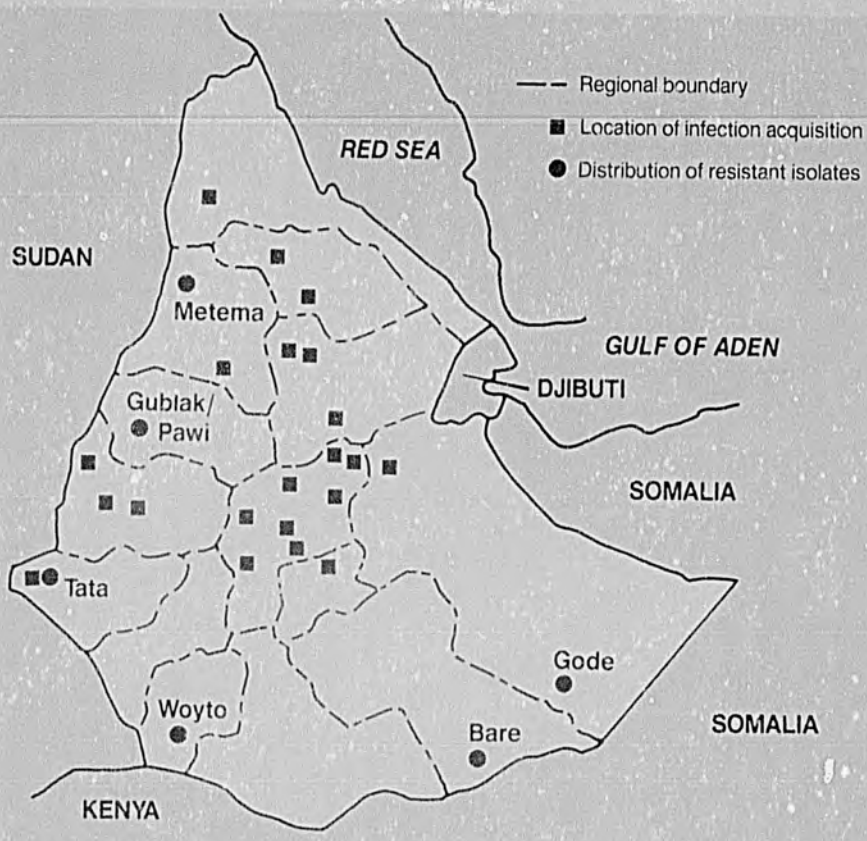


Figure 2. Distribution of *Plasmodium falciparum* resistance to chloroquine in Ethopia. Data from the National Malaria and Other Vector-borne Diseases Control Service.

carry out its research activities. There are 42 *Anopheles* species in Ethiopia, with *A. gambiae* as the main vector and *A. funestus*, *A. pharoensis*, and *A. nili* as secondary vectors.

In areas where agricultural pesticides are intensively used, periodic DDT susceptibility tests are conducted. So far, none of the vector mosquitoes developed resistance towards DDT.

## MALARIA CONTROL AND PRIMARY HEALTH CARE

Antimalaria activities, as part of primary health care services, have been recognized and fully accepted by the program for a number of years. The actions initiated and the advances made in community participation so

far, are very encouraging and progressive.

There is strong community participation and multisectoral cooperation in the following activities:

1. Residual insecticide application;
2. Blood slide collection and treatment of fever cases;
3. Monitoring, reporting, and control of outbreaks;
4. Environmental management and larviciding.

Community involvement in spray operations, which began in 1979 in one administrative region, gradually expanded. By 1985 most of the spraying activities were carried out through community participation in 14 regions. Intersectoral collaboration and community participation had a positive impact on the quality of the work in terms of better coverage and minimum replastering of sprayed houses. This is attributed to improved community awareness of the program's objectives.

It is well recognized that a comprehensive and multidisciplinary approach is needed in order to deal effectively with the control of vector-borne diseases. A collaborative committee representing various sectors such as Ministry of Health, Water Resources Development Commission, Addis Ababa University, and the Ministry of Industry is established to facilitate an integrated approach to the control of water-related, vector-borne and snail-borne diseases in irrigated agriculture and in other water development projects. The Malaria and Other Vector-borne Diseases Control Program (MVDCP) is the permanent secretariat of that committee. This intersectoral collaborative committee is recognized and supported by the WHO's panel of experts on environmental management (PEEM).

## CURRENT DEVELOPMENTS

The organization, which used to have vertical program characteristics, is presently reorganized to include the control of other vector-borne diseases and is thus known as Malaria and Other Vector-borne Diseases Control Program (MVDCP). In addition to malaria, the control of schistosomiasis, trypanosomiasis, and onchocerciasis are given first priority and basic survey studies are being undertaken. The last two diseases are endemic in the settlement areas. It is believed that MVDCP, which will be cost effective, will bring about a positive impact on the socioeconomic development of the country.

# Malaria and Maternal and Child Health Care in Côte d'Ivoire[a]

G. IMBOUA-BOGUI AND M. DIAWARA

Malaria is endemic throughout the territory of the Côte d'Ivoire, and is one of the most important reasons for seeking consultation at primary health care (PHC) clinics. In a recent study at M'bengue in the savanna, 80% of the children under 4 years were found to have parasitemia. In the forest zone, the authors reported that 54% of the children in this age group had malaria parasites in the rainy season as compared with 12% during the dry months.

Malaria is the leading cause of morbidity and mortality in young children. During the rainy season, 77% of children with febrile illnesses had malaria parasitemia as compared with 35% in children without fever, according to a study in Buyo by the authors.

With the broad objective of reducing child mortality, the African regional program "Combat of Communicable Childhood Diseases" (CCCD) was established. It includes malaria as one of its target diseases. The program, financed by the U.S. Agency for International Development (AID), supports research to improve the efficacy of control methods and strategies. Malaria control is based on routine administration of antimalarial chemotherapy to all febrile children under the age of 4.

The specific research objectives of the project are as follows:

1. To determine the frequency of clinical malaria in 0- to 4-year-old children who are attending the maternal and child health (MCH) clinic in Agboville;
2. To evaluate the efficacy of antimalarial treatment administered by the mothers to their children at home.

This paper reports the results of the first part of the study, which was carried out during the dry season, from March to May 1986. The second part is scheduled for the rainy season.

[a]Translated from French.

## MATERIAL AND METHODS

Agboville is the administrative seat of the department with the same name. The town is situated at a distance of 90 km from Abidjan. The MCH clinic is responsible for both disease prevention (immunization, health and nutrition education, prenatal and well-baby clinics), and curative medical services (treatment of current illnesses of mother and child). A physician or nurse is responsible for administering chloroquine to the children; this is done after taking a blood sample and completing a health interview with the mother of the sick child. On the basis of clinical and parasitologic examinations, the children are classified into three disease categories, i.e. malaria, malaria associated with another febrile disease, and fever of unknown origin.

## RESULTS

### Prevalence of Malaria

Of the 191 children (0 to 4 years) who had fever, 42 children (22%) were found to have parasitemia with levels >1000 plasmodia per cubic millimeter of blood. These parasitologically positive cases comprise children who had clinical malaria alone or together with another concomitant febrile disease.

### Reasons for Attending an MCH Clinic

We tried to identify reasons that had motivated the mothers to bring their children to the MCH clinic in Agboville.

The mothers mentioned an average of two health conditions (1.7) per child (Table 1). Of these, fever was the main reason for the consultations. It was listed by 40% of the mothers as the single reason for clinic attendance and was found in 69% (132/191) of all clinic consultations, either alone or accompanied by other clinical signs and symptoms.

### Principal Causes of Morbidity

Systematic physical and parasitologic examinations (thick blood smear and white blood counts) allowed us to diagnose the illnesses of the children seen at the MCH clinic.

Table 2 shows that the frequency of clinical malaria in the sample was 47% (90/191). Of these, 25 cases (27.8%) were confirmed parasitologi-

Table 1. Illnesses listed by mothers as reason for clinic visit.

| Reason for Clinic Visit | Cases | Percent |
|---|---|---|
| Fever | 132 | 40.1 |
| Cough | 48 | 14.6 |
| Diarrhea | 32 | 9.7 |
| Vomiting | 15 | 4.6 |
| Anorexia | 14 | 4.2 |
| Cold | 12 | 3.6 |
| Pyoderma | 9 | 2.7 |
| Abdominal pain | 6 | 1.8 |
| Dyspnea | 6 | 1.8 |
| Weakness | 5 | 1.5 |
| Loss of weight | 4 | 1.2 |
| Ascariasis | 3 | 0.9 |
| Immunization | 17 | 5.2 |
| Conjunctivitis | 4 | 1.2 |
| Other conditions | 22 | 6.9 |
| Total | 329 | 100.0 |

Table 2. Distribution of children with fever by clinical and parasitologic diagnosis.

| Clinical Diagnosis | Clinical Cases | Thick Blood Smear | |
|---|---|---|---|
| | | Positive | Negative |
| Malaria | 90 | 25 | 65 |
| Respiratory diseases | 35 | 8 | 27 |
| Febrile diarrhea | 31 | 5 | 26 |
| Skin infections | 10 | 1 | 9 |
| Rhinopharyngitis | 3 | 2 | 1 |
| Candidasis | 3 | 0 | 3 |
| Conjunctivitis | 2 | 0 | 2 |
| Measles | 3 | 0 | 3 |
| Anemia | 2 | 0 | 2 |
| Intestinal parasites | 2 | 0 | 2 |
| Colic | 2 | 0 | 2 |
| Otitis | 1 | 0 | 1 |
| Rectal prolapse | 1 | 0 | 1 |
| Pertussis | 1 | 1 | 0 |
| Myositis | 1 | 0 | 1 |
| Dentition | 1 | 0 | 1 |
| Lymphadenitis | 1 | 0 | 1 |
| Not determined | 1 | 0 | 1 |
| Total | 190 | 42 | 148 |

cally, as compared with 16.8% (17/101) among those who had other concomitant diseases, i.e. pneumonia, diarrhea, and staphylococcal skin infections.

Table 3 shows that only 25 cases (28%) of the clinical diagnoses of malaria made by physicians were confirmed parasitologically. We conclude that malaria, whether based on clinical diagnosis or on the result of parasitologic examination, plays an important role in the clinic attendance of sick children.

### Effect of Prior Home Treatment on the Validity of the Parasitologic Results

Of the 191 cases studied, 149 (78%) of the mothers stated that they had treated their children at home with either modern or traditional medication before coming to the MCH clinic. Table 4 shows the distribution of the children with fever by the type of medication received from their mothers.

Table 3. Clinical and parasitological diagnoses of malaria.

| | Clinical Diagnosis | | |
|---|---|---|---|
| Smear | Malaria | Other DX | Total |
| Positive | 25 | 17 | 42 |
| Negative | 65 | 84 | 149 |
| Total | 90 | 101 | 191 |
| Percent Positive | 27.8 | 16.8 | |

Table 4. Distribution of children with fever according to the type of prior home treatment and by the results of parasitologic examinations.

| | Blood Smear | | |
|---|---|---|---|
| Treatment | Positive | Negative | Total |
| Chloroquine | 9 (20.5)* | 35 | 44 |
| Chloroquine plus other modern antimalarials | 2 (50.0) | 2 | 4 |
| Chloroquine plus traditional medicine | 1 (33.3) | 3 | 4 |
| Traditional medicine | 11 (30.6) | 25 | 36 |
| Traditional and modern drugs | 0 | 1 | 1 |
| Quinine | 0 | 6 | 6 |
| Other modern drugs | 2 (15.4) | 11 | 13 |
| No treatment | 12 (28.6) | 30 | 42 |
| Not known | 5 (11.9) | 37 | 42 |
| Total | 42 (22.0) | 150 | 192 |

*Percentage shown in parentheses.

Was home treatment effective in reducing the frequency of parasitemia as determined by microscopic examination of thick blood smears? To answer this question, we have regrouped the data on home treatment into three categories: treatment containing chloroquine, treatment without chloroquine, and no treatment. The results are shown in Table 5. The difference in the rate of parasitemia between the groups was statistically not significant and was not affected by the type of home treatment received before the first clinic visit.

We regrouped the data again into two groups, i.e. those who had received antimalarial drugs and those who had not. These results are depicted in Table 6.

No differences in the malaria parasitemia rates were noted, regardless of whether the home medication contained an antimalarial drug. We conclude that the self-administered antimalarial treatment by mothers to their children had no effect on parasitemia.

## Efficacy of the Treatment Given by Mothers

Of the 191 children in the clinic, only 51 (25%) had received chloroquine at home. A matrix comparing the chloroquine dose that the 50 children treated by their mothers actually received with the correct dose calculated from the body weight of the child is shown in Table 7.

**Table 5.** Results of parasitologic examinations for children by type of home treatment for fever before clinic visit.

| | Thick Blood Smear | | |
|---|---|---|---|
| Treatment | Positive | Negative | Total |
| With chloroquine | 12 (23.5)* | 39 | 51 |
| Without chloroquine | 12 (26.[illegible]) | 38 | 50 |
| No treatment | 12 (28.6) | 30 | 42 |
| Total | 36 (25.9) | 107 | 143 |

* Percentages shown in parentheses.

**Table 6.** Results of parasitologic examinations of blood smears of children with fever by treatment with and without antimalarials prior to clinic visit.

| | Thick Blood Smear | | |
|---|---|---|---|
| Treatment | Positive | Negative | Total |
| Antimalarial | 12 (21.1)* | 45 | 57 |
| No antimalarial | 25 (27.2) | 67 | 92 |
| Total | 37 (24.8) | 112 | 149 |

* Percentage shown in parentheses.

Table 7. Efficacy of the chloroquine treatment given by mothers.

| | Chloroquine Dose Received, *mg* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Required* | 20–29 | 30–39 | 40–49 | 50–59 | 60–69 | 70–79 | 80–89 | 90–99 | 100–109 | Total |
| 20–29 | | | | | | | 1 | 1 | | 2 |
| 30–39 | 3 | | | | | | | | | 3 |
| 40–49 | 2 | | | | | | | | | 2 |
| 50–59 | 1 | | | | | | | | | 1 |
| 60–69 | 6 | | | | | 1 | | | | 7 |
| 70–79 | 3 | | | | | | | | | 3 |
| 80–89 | 3 | | | 3 | | | | | 1 | 7 |
| 90–99 | 3 | | | 3 | | | | | 1 | 7 |
| 100–109 | 3 | | | | | | | | 1 | 4 |
| 110–119 | 2 | | | 1 | | | | | 1 | 4 |
| 120–129 | | | | 1 | | 1 | | | 1 | 3 |
| 130–139 | 1 | | | | | | | | 1 | 2 |
| 140–149 | 1 | | | | | | | | | 1 |
| 150–159 | 1 | | | | | | | | 2 | 3 |
| 160–169 | | | | | | | | | | 0 |
| 170–179 | | | | | | | | | 1 | 1 |
| 180–189 | | | | | | | | | 0 | 0 |
| 190–199 | | | | | | | | | 1 | 1 |
| Total | 29 | 0 | 0 | 8 | 0 | 2 | 1 | 1 | 10 | 51 |

* 10 mg of chloroquine per kilogram of body weight.

Of the 50 children who were treated with chloroquine, the dose was too high in 5 cases (10%), correct in 1 case, and too low in 44 cases (88%). The average dose of chloroquine actually received was 49 mg instead of 93 mg, which would have been the effective dose based on a standard of 10 mg/kg of body weight. The difference between the two average doses is statistically highly significant.

The results show that the chloroquine doses given by the mothers to their children were too low to have had an effect on parasitemia. However, the doses administered by the mothers were actually those recommended by the midwives for malaria chemoprophylaxis of their newborns at the time of delivery (i.e., "one tablespoonful three times a week on Monday, Wednesday and Friday"). The recommended dose would be adequate for newborns weighing 3–4 kg.

### Duration of Home Treatment

Table 8 depicts the number of children treated before visiting the MCH clinic by the type and duration of the treatment received. Of 101 mothers who stated that they had treated their children, only 91 remembered the exact length of treatment.

Table 8. Distribution of cases by the type and duration of treatment received before child visited MCH clinic

| Days Duration | Treatment | | | |
|---|---|---|---|---|
| | Modern | Traditional | Combined | Total |
| 1 | 33 | 5 | 2 | 40 |
| 2 | 12 | 8 | 2 | 22 |
| 3 | 7 | 6 | | 13 |
| 4 | 4 | 2 | | 6 |
| 5 | 1 | 1 | | 2 |
| 6 | 0 | 0 | | 0 |
| 7 | 1 | 5 | | 6 |
| 8 | 1 | 0 | | 1 |
| 9 | 0 | 0 | | 0 |
| 10 | 1 | 0 | | 1 |
| Total | 60 | 27 | 4 | 91 |

The average duration of the treatment with modern drugs was 2 days, compared with 3 days for traditional treatment. This difference is statistically significant. When the mothers were giving traditional medication, they tended to treat their children longer than with modern drugs.

### Length of Delay of Clinic Visit

Table 9 presents a distribution of the children with and without home treatment by the number of days they had suffered from fever before the visit to the MCH clinic.

The average delay in seeking medical help in the MCH clinic was 3.5 days for those who had received home treatment as compared with 2.4 days for children who had not. This difference is statistically significant and indicates that mothers who treat their children at home wait on the average 3 days before consulting the MCH clinic.

### Relationship Between the Duration of Febrile Illness and Length of Home Treatment

Table 10 shows the relationship between the onset of home treatment and the duration of fever for 86 children whose mothers were interviewed for the exact information. The mean duration of illness was 3.4 days compared with 2.4 days for the length of treatment given. These results indicate that mothers tend to wait before treating their children. There is a difference of one day between the onset of illness and the beginning of treatment.

Table 9. Delay of MCH clinic visits for sick children by status of home treatment.

| Day with Fever Before MCH visit | Treated Yes | No | Total |
|---|---|---|---|
| 1 | 11 | 7 | 18 |
| 2 | 34 | 12 | 46 |
| 3 | 20 | 9 | 29 |
| 4 | 12 | 5 | 17 |
| 5 | 4 | 1 | 5 |
| 6 | 2 | 0 | 15 |
| 7 | 15 | 0 | 2 |
| 8 | 1 | 0 | 1 |
| 10 | 2 | 0 | 2 |
| Total | 101 | 34 | 135 |

Table 10. Distribution of children with fever by duration of illness and length of home treatment.

| Length of Treatment | Duration of illness (days) 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 8 | 19 | 6 | 2 | 1 | | 1 | | | | 37 |
| 2 | | 12 | 6 | | | | 2 | | | | 20 |
| 3 | | | 5 | 3 | 2 | 1 | 2 | | | | 13 |
| 4 | | | | 6 | | | | | | | 6 |
| 5 | | | | | 1 | | 1 | | | | 2 |
| 6 | | | | | | | | | | | 0 |
| 7 | | | | | | | 6 | | | | 6 |
| 8 | | | | | | | | 1 | | | 1 |
| 9 | | | | | | | | | | | 0 |
| 10 | | | | | | | | | | 1 | 1 |
| Total | 8 | 31 | 17 | 11 | 4 | 1 | 12 | 1 | 0 | 1 | 86 |

## CONCLUSIONS

The study has shown the following:

1. Malaria has remained the major cause of morbidity in MCH clinic visits of 0- to 4- year-old children.
2. By continuing to follow a midwife's advice concerning the choloroquine treatment of infants at birth, mothers are administering doses that are too low to affect parasitemia in older children.
3. Mothers delay the first clinic visit by an average of 3 days, especially when they provide traditional home treatment to their children.

4. Mothers do not start home treatment of their sick children immediately. There is a delay of about 1 day between the onset of fever and the beginning of treatment.

On the basis of these results, the following recommendations are made:

1. Educate and inform the mothers about the need to give chloroquine at doses of 10 mg/kg of body weight in all cases of acute fever regardless of its nature.
2. Educate, inform, and train health workers in prescribing and dispensing malaria chemotherapy at a single dose of 10 mg/kg of body weight.
3. Create village or community health units to administer effective antimalarial (presumptive) treatment as early as possible.

# Malaria in Nigeria

L. A. SALAKO

Malaria has been for a long time one of the great public health problems of the world. Although it is now present in an endemic form only in the tropical and subtropical regions of Africa, Asia, and South and Central America, it has not always been so localized in its distribution. At the beginning of this century, malaria was distributed practically worldwide, covering large parts of southern Europe, the Asian part of the Soviet Union , and the southern parts of the United Stated, in addition to the regions that are now still endemic for the disease. The general improvement in public health and socioeconomic conditions in this century led to the disappearance of the disease in most of Europe and North America so that by the end of the World War II, its presence in these continents was confined to small foci in their southern parts.[1]

As a result of the global malaria eradication program launched by the World Health Organization (WHO) in the 1950s,[2] the malaria distribution changed substantially. By 1970 the disease had been eradicated from Europe, the Soviet Union, the United States, and even in some parts of southern Asia including most of India,[3,4] all these areas represented approximately 25% of the pre-eradication malarious areas. In the years that followed, the countries from which malaria was eradicated, with the exception of those of Asia, remained free of transmission. In Asia, a combination of factors, including importation of malaria to a previously free area, reduced commitment to the eradication activities, and increasing resistance of mosquitoes to the available insecticides have led to the reappearance and spread of malaria.

In the WHO eradication program of the 1950s and 1960s, Africa was not a target region. This was because at that time there was *(a)* inadequate knowledge of the incidence of the disease in the region, and its effect on morbidity and mortality; *(b)* uncertainty about the effect of eradication measures on premunition; *(c)* inadequate knowledge of vector behavior in the region; and *(d)* lack of infrastructural facilities, skilled manpower and economic resources needed for prosecuting such a program. Therefore only pilot epidemiological and control programs were recommended for tropical Africa.

Since the 1950s, a large number of surveys of malaria in various parts of Africa have been published, and there are now comprehensive and reliable data on the malaria situation in several countries covering different geographical zones in the continent. These data have enabled us to study the epidemiological characterization of the continent with respect to malaria and to subsequently develop strategies for controlling the disease in the region.

Malaria in Africa is a remarkably enormous problem. More people are exposed to the risk of malaria in tropical Africa than in all other parts of the world. The intensity of transmission is higher in Africa than in any other malarious areas of the world. Possibly over 90% of the estimated 200 million malaria- infected people in the world are in Africa.[3] Whereas in most other malarious areas of the world *Plasmodium vivax* is the most common plasmodial species, in Africa the most common species is the highly virulent *Plasmodium falciparum*. It is therefore not surprising that more people die of malaria in Africa than in all other parts of the world put together. A conservative estimate of malaria mortality in Africa would put the figure at one million per annum, and these are predominantly children under the age of 5.

## EPIDEMIOLOGY

Nigeria is a West African country extending from the Gulf of Guinea on the south to the southern reaches of the Sahara Desert on the north. It is entirely situated north of the equator between latitudes 4 and 14°. It is bounded on the south by the Atlantic Ocean, on the west by the Republic of Benin, on the east by the Republic of Cameroon, and on the north by the Republics of Niger and Chad. Nigeria is the most populous country in Africa, with a population of about 100 million, which is approximately 25% of the population of black Africa. The country spans at least five geographical zones which are the coastal, mangrove forest, and tropical rain forest zones in the south, and the savanna and sahel zones in the north.

There are two main climatic seasons—the wet and the dry seasons. The wet season in the south extends from May to October but although most of the rainfall occurs during this period, a little rainfall is usually recorded in the other months. By contrast, the annual rainfall in the north is practically limited to the months of June, July, August, and September. As a rule total annual rainfall is a lot greater in the south than in the north.

## MALARIA TRANSMISSION

The initial large-scale surveys of malaria in Nigeria were undertaken just after the Second World War. Since then, several other surveys have been conducted at intervals up to the present time.[5–14] These surveys show that malaria is holoendemic in all parts of Nigeria. Although transmission occurs all year round with a high intensity, the rate is higher in the wet than in the dry season. This high transmission is strikingly demonstrated by the Garki project[13], which found that the vectorial capacity, which represents the likelihood of transmission of the parasite, was about 1000 times the threshold for maintaining the endemicity, and the infective mosquito bite per person per year was about 100 times the critical value required to maintain the endemicity. With these very high transmission figures, it is not surprising that in the same study the cumulative incidence of patent parasitemia was 100% over an 18-month period for *P. falciparum* in children between 1 and 8 years old. These remarkable transmission figures place malaria in Nigeria on an unsurpassed pedestal and make the task of its eradication, using currently available methods, a daunting and indeed practically impossible one, even when pursued with the greatest enthusiasm and total commitment.

## PREVALENCE

Several studies have shown a high parasite rate all over the country, but the actual rates are determined by a variety of factors. One of these is whether urban or rural communities are involved. In general, parasite rates are higher in rural than in urban areas. In a survey of asymptomatic school children in urban Lagos in 1956, a parasite rate of 48–50% was found.[9] A similar survey in the same area 15 years later showed a parasite rate of 33%.[10] On the other hand, in a semirural suburb of Lagos a parasite rate of 92% was found among school children in 1951,[6] and 20 years later the rate was 80.5%.[10] The malaria prevalence among school children in and around Lagos, the nation's capital, did not therefore appear to have changed to any great extent in the 20 years between 1950 and 1970. Other studies have investigated the parasite rates in rural communities in different parts of the country.[8,11,13,18] The picture of malaria in Nigeria that emerges from these studies can be summarized as follows:

The malaria situation in Nigeria has changed very little in the last 40 years. The whole of Nigeria is malarious—to a hyperendemic degree in most parts. Parasite rates vary considerably in any area according to the time of year and the age group involved. Variations also occur between different parts of the country, especially between north and south. In

infants, the average crude parasite rate is usually below 50%. The rate actually varies with the age studied; the rate is low in the first 6 months of life and increases fairly rapidly in the next 6 months to 60% or more at 12 months. The parasite rate in the first 6 months is consistently higher in the north than in the south. The parasite rate is at its highest in preschool children; rates of 90% or more are found in this group in the maximum transmission season. In children between the ages of 5 and 10 years, the parasite rate remains high but falls from a high of about 90% in the 5–7 years age group to 60–75% in the 8-10 years age group. The 11–15 years age group has an average parasite rate between 20% and 50%, and in adults, this has usually fallen to between 10 and 30%. The crude parasite rate is usually higher in the wet than in the dry season (Fig. 1) but this seasonal variation is more marked in the north than in the south.

In all parts of Nigeria, *P. falciparum* is the predominant species causing infection; during the wet season about 96% of the crude parasite rate is due to this species. *P. malariae* is the next most common. In children up to 10 years, its rate can be up to 30%, usually as a mixed infection with *P. falciparum*. The *Plasmodium malariae* rate is usually higher in the dry season than in the wet when *P. falciparum* is at its highest. *Plasmodium ovale* is seen occasionally and *Plasmodium vivax* very rarely.

The density of parasitemia is highest in the preschool children. By school age, children in all parts of the country have an asymptomatic parasite density that is usually not more than 200 per $\mu$l.

In surveys in which the gametocytes were examined, gametocytes of *P. falciparum* were seen in 10% of examined infants, 20% of children aged 1–4, and 12% in older children. In adolescents, the gametocyte rate was usually below 5% and this fell further still in adults to 1% or less.[5] In a more recent study by us,[18] a gametocyte rate of 16–17% of the specific *P. falciparum* rate was found among school children aged 6–10 in a village in the southwestern part of Nigeria (Table 1).

## MORBIDITY

Although the high percentage of asymptomatic malaria parasitemia is of considerable epidemiological interest, it is the morbidity and mortality associated with plasmodial infections that make malaria the great public health problem that it is.

It has been estimated from hospital records in Nigeria that 8–9% of hospital attendances were due to malaria,[5] that there was usually a sharp increase in hospital outpatient attendances with the onset of the wet season, and that the increase was due predominantly to the number reporting with malaria.[8] Although malaria is usually diagnosed in most

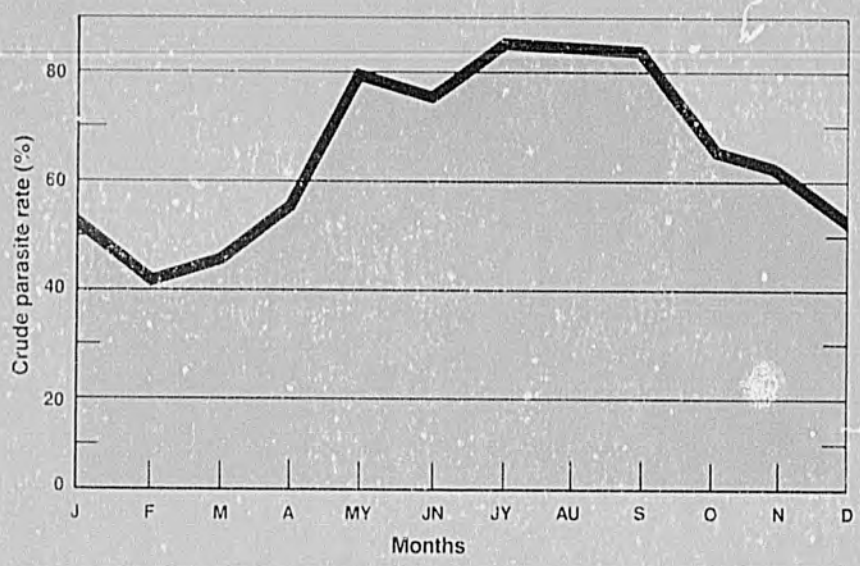


Figure 1. Crude malaria parasite rate in primary school children aged 6–12 years from a rural area in southwestern Nigeria in a 12-month period.

Table 1. Malaria parasite rate amongst children aged 6–10 years in Ijaye, southwestern Nigeria.

| | Month of Survey | |
|---|---|---|
| | May 1985 N=1100 | July 1985 N=1150 |
| Number with Parasitemia | 869 | 996 |
| *P. falciparum* alone | 610 | 959 |
| *P. malariae* alone | 109 | 21 |
| *P. falciparum* plus *P. malariae* | 150 | 16 |
| *P. falciparum* gametocytes | 125 | 167 |
| Overall malaria parasite rate | 79.0% | 86.6% |
| *P. falciparum* as % of total parasitemia | 7.5% | 97.9% |
| *P. malariae* as % of total parasitemia | 29.8% | 3.7% |
| *P. falciparum* gametocytes as % of *P. f.* rate | 16.4% | 17.1% |

health institutions in the country on clinical grounds alone, this diagnosis is not always correct. Thus, it was shown in a large teaching hospital in Ibadan, southwestern Nigeria, that only 37.4% of 500 cases of children diagnosed as suffering from acute malaria on clinical grounds actually had parasitologically proven malaria.[12]

In a longitudinal study of malaria at the University College Hospital, Ibadan, over the past 6 years, a total of 6,132 children aged 6 months to 12 years who presented with a pyrexial illness diagnosed clinically as malaria were screened for parasitemia. Only 2930 (47.8%) of them were

positive for asexual forms of the malaria parasites and could therefore be confirmed as suffering from malaria. The causative agent was *P. falciparum* in 97% of cases. About 3% had mixed *P. falciparum* and *P. malariae* infection while less than 1% had *P. malariae* alone.[18] These findings show that considerable morbidity is caused by plasmodial infection in Nigeria and further demonstrate the predominant role of *P. falciparum* in malaria in the country.

Mortality rate due to malaria is difficult to determine since the deaths occur mostly in children below age 5 and many of these occur outside the hospitals. It would therefore serve no useful purpose to use urban hospital data to estimate malaria mortality rate in a country that has no register of births and deaths and where 75% of the people go from cradle to grave without ever seeing the inside of a hospital. Suffice it to say, however, that mortality from malaria, especially in rural preschool children would be quite high.

## VECTORS

*Anopheles gambiae* s.l. and *Anopheles funestus* are the predominant vectors. The vector density per room per day shows a relative stability of *A. funestus* population with a slight dip in the months before, and compensatory increase in the months after, the rains. By contrast, *A. gambiae* shows an explosive increase with the rains. Vector density is generally higher in the north than in the south. In the south the mosquitoes show only slight seasonal variation in their infectivity rates. This contrasts markedly with the situation in the north where the sporozoite rate of the mosquitoes is practically zero during the later parts of the dry season. In both the north and the south, the sporozoite rates for *A. gambiae* are slightly higher than those for *A. funestus*. In the wet season, the sporozoite rates for both A. gambiae and *A. funestus* are higher in the south than in the north.[8]

## PARASITE SUSCEPTIBILITY TO DRUGS

Since 1979, monitoring of the susceptibility of *P. falciparum* to antimalarial drugs in Nigeria has been undertaken using in vivo and in vitro techniques.[14–18]

The in vivo tests are done using either the WHO standard field test in which patients are followed up for 7 days or the extended field test in which observation is continued for 28 days.[19] In some modification of the extended field test for drugs with long half-life (e.g., mefloquine-sulphadoxine-pyrimethamine), observation was continued for 35 days.

In vivo test results show full sensitivity of *P. falciparum* to chloroquine, amodiaquine, sulphadoxine-pyrimethamine (Fansidar), quinine and mefloquine-sulphadoxine-pyrimethamine (Fansimef). On an average, parasite clearance occurs in 1.7 days and fever clearance in 2.5 days with the rapidly acting drugs like chloroquine and amodiaquine. With the more slowly acting drugs like quinine, Fansidar and Fansimef, the average parasite and fever clearance times were 2.1 and 2.7 days, respectively.

*In vitro* sensitivity tests have been done using the microtechniques or macrotechniques based on the petridish candle jar method of growing the parasites.[20,21] Sensitivity has been tested against chloroquine, amodiaquine, mefloquine, and quinine (Figs. 2 and 3). These tests show that *P.*

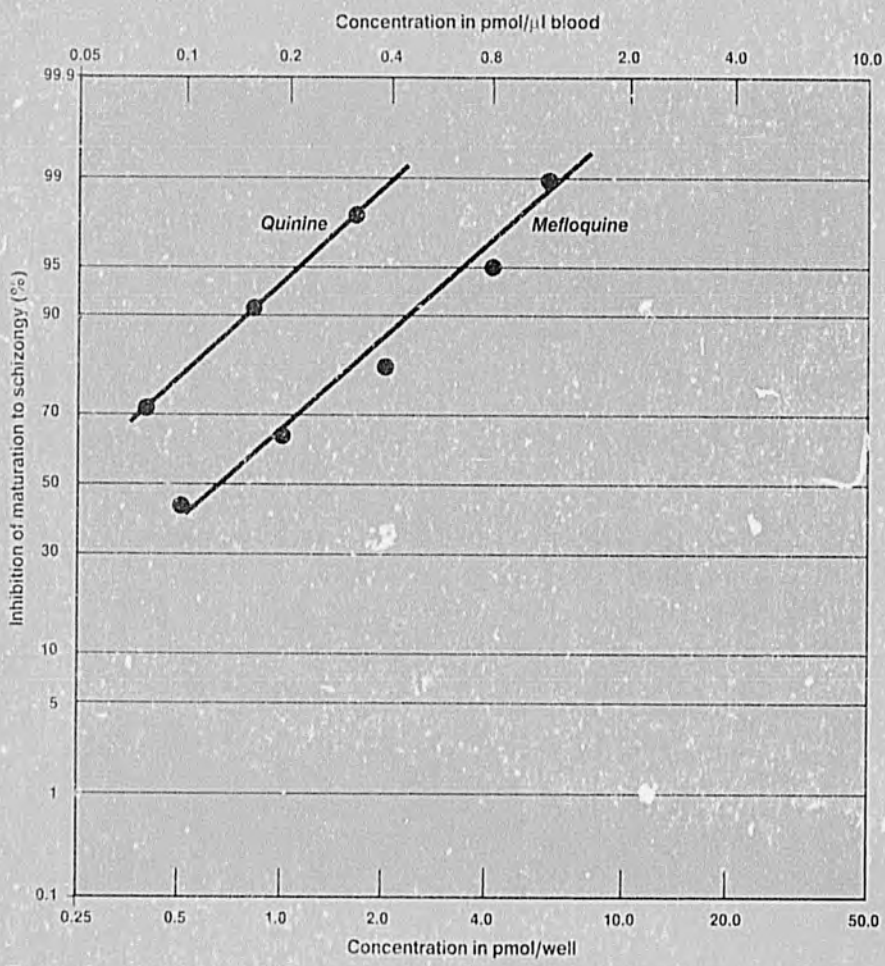


Figure 2. Inhibition of schizogony in Nigerian isolates of *P. falciparum* by quinine and mefloquine using in vitro microtechnique (1986).

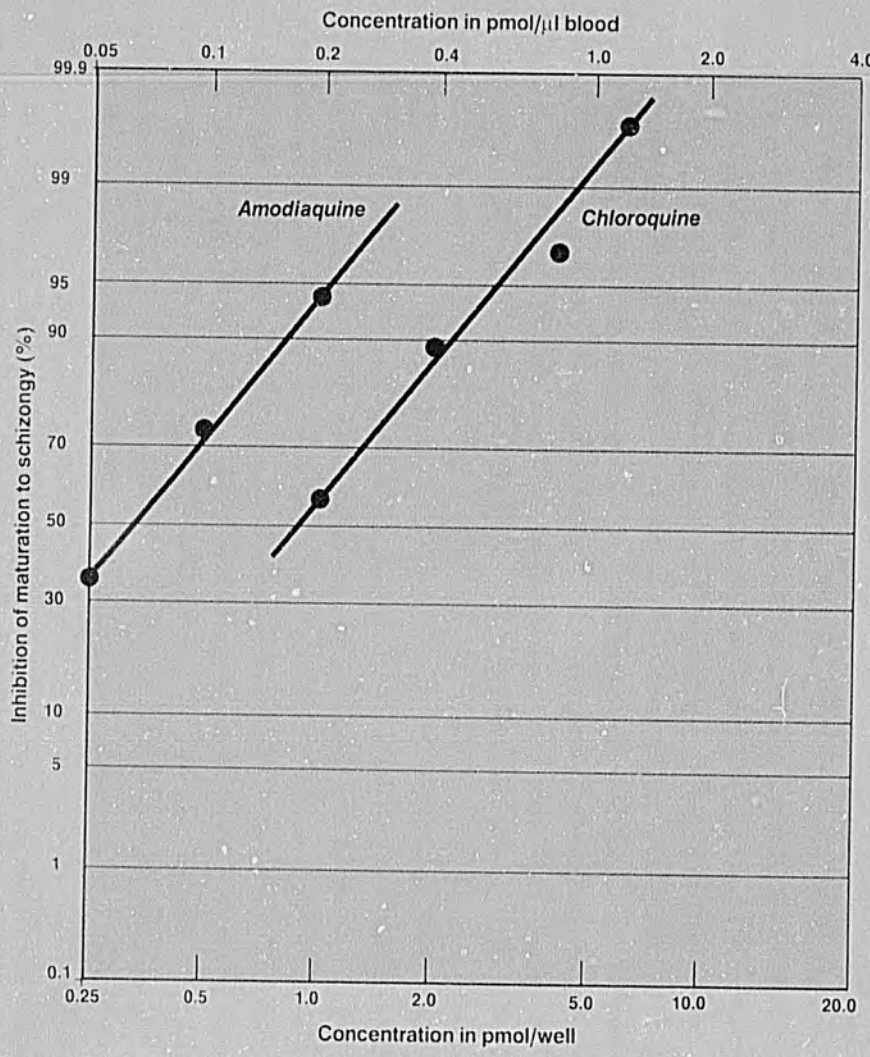


Figure 3. Inhibition of schizogony in Nigerian isolates of *P. falciparum* by chloroquine and amodiaquine using in vitro microtechnique (1986).

*falciparum* isolates from Nigeria are still sensitive to chloroquine although over the years the degree of sensitivity appears to be declining. Thus, in tests performed in 1980,[15] complete inhibition of maturation of isolates was achieved at all chloroquine concentrations above 0.4 µmol/l. In tests performed in 1983,[17] some isolates were not inhibited completely at chloroquine concentration of 0.8 µmol/l and later still, in 1986, one isolate out of 12 grew in a medium containing 1.14 µmol/l chloroquine and was only completely inhibited in concentrations of 1.6 µmol/l and

Table 2 Sensitivity of 1985–1986 isolates of *Plasmodium falciparum* from Oyo State, southwestern Nigeria, to drugs using the in vitro microtechnique.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *Chloroquine** | | | | | |
| Concentration (pmol/μl) | 0 | 0.2 | 0.4 | 0.8 | 1.14 | 1.6 | 3.2 | 6.4 |
| No. of isolates | 26 | 25 | 13 | 6 | 1 | 0 | 0 | 0 |
| % of isolates | 100 | 96.2 | 50 | 23.1 | 3.8 | 0 | 0 | 0 |
| | | | *Amodiaquine** | | | | | |
| Concentration (pmol/μl) | 0 | 0.05 | 0.1 | 0.2 | 0.4 | 0.8 | 1.6 | 3.2 |
| No. of isolates | 11 | 11 | 9 | 4 | 0 | 0 | 0 | 0 |
| % of isolates | 100 | 100 | 81.8 | 36.4 | 0 | 0 | 0 | 0 |
| | | | *Mefloquine** | | | | | |
| Concentration (pmol/μl) | 0 | 0.1 | 0.2 | 0.4 | 0.8 | 1.14 | 1.6 | 3.2 |
| No. of isolates | 27 | 25 | 22 | 20 | 11 | 7 | 0 | 0 |
| % of isolates | 100 | 92.6 | 81.5 | 74.1 | 40.7 | 25.9 | 0 | 0 |
| | | | *Quinine** | | | | | |
| Concentration (pmol/μl) | 0 | 0.08 | 0.16 | 0.32 | 0.64 | 1.28 | 2.56 | 5.12 |
| No. of isolates | 12 | 9 | 3 | 1 | 0 | 0 | 0 | 0 |
| % of isolates | 100 | 75.0 | 25.0 | 8.3 | 0 | 0 | 0 | 0 |

*Data show maturation of schizonts at different concentrations of the drug.

above.[22] The tests with the other drugs show that the local strains of *P. falciparum* are still fully sensitive to amodiaquine and quinine.

Results with mefloquine are difficult to interpret with confidence as it is not yet certain what minimum inhibitory concentration is consistent with sensitivity of the parasites to this drug. It has been suggested that a concentration of 0.8 μmol/l in the microtest should normally completely inhibit maturation of sensitive parasites provided the density of parasitemia is not greater than 90,000/μl. In our recent study, 11 out of 27 isolates matured to schizonts in the presence of 0.8 μmol/l mefloquine, while 7 out of 27 grew in a medium containing 1.14 μmol/l mefloquine (Table 2). These data may indicate some degree of primary resistance of *P. falciparum* to mefloquine in Nigeria. If this is true, it would not augur well for the future of mefloquine as an effective antimalarial drug in the country if it were to be deployed for general use as a single drug.

## CONCLUSION

It can be concluded from the foregoing that malaria in Nigeria is characterized by intense transmission all year round, hyperendemicity, high morbidity and possibly also high mortality rate especially among children under age 5, and a stable prevalence rate in adults past age 40.

To date chloroquine-resistant *P. falciparum* has not been detected in Nigeria. However, the fact that chloroquine resistance has already reached the two countries bordering Nigeria in the east and west (Republics of Cameroon and Benin, respectively) suggests that the appearance of chloroquine resistance in Nigeria is only a question of time. With the appearance of drug resistance, the only affordable way of controlling malaria morbidity and mortality at the present time would be seriously compromised with dire consequences to the health of the people. Alternative safe, effective, acceptable, and affordable means of controlling the scourge of malaria are therefore urgently needed. The malaria vaccine is one such malaria control modality upon which a lot of hope is now pinned. It is hoped that one would soon be available for evaluation and subsequent deployment in malaria endemic countries.

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# Malaria in Sao Tome and Principe[a]

JOAO GUADELUPE VIEGAS DE CEITA

## GEOGRAPHY

Independent since 1975, the Democratic Republic of Sao Tome and Principe is an archipelago covering 1001 $km^2$ with an estimated population of 108,000. The country is situated off the coast of West Africa in the Gulf of Guinea on the equator. Located at a distance of 240 km from Cape Lopez of Gabon, its other neighbors are Equatorial Guinea, Cameroon, the Congo, and Angola.

The islands are of volcanic origin and are very mountainous, with 17 of their peaks reaching an altitude of more than 1000 meters, including Mount Sao Tome, which is 2024 m high. The coastal plains are sequestered by numerous water courses, including man-made ones. They often form swamps, especially on the island of Sao Tome. There is a large unpopulated area with extremely high humidity in the highlands of the southern and central parts of Sao Tome, which covers 546 $km^2$, or 63.5% of the total surface of the island.

## CLIMATE

The climate is characterized by an average annual temperature of 23.6° C, with a maximum of 30.5° C in March and April and a minimum of 19.9° C in July. Humidity is close to saturation in the interior mountain area. The annual rainfall ranges between 1000 mm and 7000 mm, depending on the mountain relief and the direction of the winds. The dry season varies from 1 to 3 months (June to August) according to topography. These climatic features combined with the type of soil, composed of clay and basalt, favor the growth of the dense forest that covers all of the country, with the exception of the northern parts of the island of Sao Tome, where the climate is arid and semiarid (Lains and Silva, 1958).

[a]Translated from French; see French summary at end of paper.

## POPULATION

The origin of the people shows considerable diversity. In the past, Sao Tome served as a trading center for slaves. The population lives in long villages that are distributed along the roads, which facilitates access to even the most distant villages. Houses are made of wood, are raised on foundations, and have corrugated iron roofs. In the towns and agricultural centers, the houses are made of bricks and cement.

Literacy in the general population amounts to about 25%; of these 80% read and speak Portuguese, the official language. Christianity is the religion practiced by the great majority of the population. There is a national radio station and a weekly newspaper. Agriculture, which has remained the only source of foreign exchange for the country, is based on the production of cocoa and copra. The average annual per capita income has been estimated as the U.S. equivalent of $289. (Table 1 shows some demographic indicators for the country.)

## ENDEMICITY

Malaria is endemic in all parts of the country. Despite the control measures taken, infections have remained at a mesoendemic to hyperendemic level. High temperature, humidity, and heavy rainfalls have created conditions favorable for this high transmission.

Malaria is the principal cause of absenteeism from work and school; it has the highest mortality of the indigenous transmissible diseases. The tragic socioeconomic consequences of malaria, especially in children, have led to various malaria control programs that were first started in 1947 under the colonial administration and later continued by the national government.

### Plasmodium Species

The four species of *Plasmodium* that cause human malaria are all found in Sao Tome and Principe: *P. falciparum*, *P. malariae*, *P. ovale*, and *P. vivax*. This fact is surprising considering the geographic location of the country at the west coast of Africa, which has traditionally been free of *P. vivax* infections. Its atypical occurrence in Sao Tome has its roots in the diverse ethnic origin of the residents, some of whom have come from East Africa. This fact indicates that forced emigration of laborers from that part of the continent has continued until the 20th century.

The malariometric studies carried out between 1961 and 1962 have revealed the presence of three malaria species on the island of Sao Tome

Table 1. Selected demographic indicators for Sao Tome and Principe.*

| | |
|---|---|
| Crude birth rate | 40.9/1000 |
| Infant mortality | 68.5/1000 live births |
| Live Crude mortality | 11.9/1000 |
| Annual growth rate | 2.95% |
| Life expectancy at birth | 62 yr |

*Data from *Perfil Sanitario do País, 1983–1984).*

with the following distribution: *P. falciparum*, 76.3%; *P. vivax*, 23.1%; *P. malariae*, 0.6%. In these studies, 1511 blood slides were examined. Of these 481 were positive, corresponding to a parasite index of 31.8% in children between 6 months and 10 years of age. In the 7- to 10-year-old group, the spleen index was 34.5% during this period.

Malariometric measurements carried out from 1978 to 1979 as part of the Preparatory Phase of the Malaria Eradication Program revealed the following results: 1486 children (5 to 14 years) were examined between May and December 1978. Of these, the spleen index varied between 2 and 39.3%, with an average of 15.1%. The corresponding parasite index ranged from 6 and 65.5%, with an average of 16.6%.

In 1979 the average spleen index of 2546 children between 5 and 14 years old was 23.7 % with a parasite index of 18.5 %. The following distribution of malaria species was found in the examinations of 5264 blood slides from children aged 3 to 14 years old: *P. falciparum*, 81.9%; *P. malariae*, 10.3%; *P. ovale*, 3.1%; *P. vivax*, 4.7%. The predominance of *P. falciparum* was strong in both studies. Attention is drawn to the absence of *P. ovale* in the 1961–1962 studies in which 1511 blood slides were examined.

### Anopheles Species

Transmission studies of malaria carried out by the Malaria Eradication Program since the country's independence have all revealed the presence of only one vector: *Anopheles gambiae* s.l. (endophagic and endophylic), before and during the first phase of the intradomiciliary application of DDT. *Anopheles coustani*, which is found throughout the extreme south of Sao Tome, has not been shown to serve as a malaria vector.

## MALARIA CONTROL PROGRAM

### Before Independence (1947–1975)

Because of its great socioeconomic impact, malaria control was always given high priority by the government. Starting in 1947, chloroquine was

first employed for malaria prophylaxis, however, its application was irregular and incomplete. In 1949, residual DDT spraying was added as another control measure, however, the applications were often irregular and spotty. The goal of the program was the reduction of the prevalence of malaria.

During the period of 1971–1973, DDT applications were repeated in cycles of 6 months, combined with fogging of roads and around habitations. DDT was also used as a larvicide. The combined measures did not succeed in the interruption of the transmission cycle as had been foreseen by the Malaria Eradication Program (MEP), which was established in 1968. Therefore, mass chemoprophylaxis with chloroquine was resumed.

### After Independence (1975–Present)

Following the interruption of MEP malaria control activities in 1973, the malaria situation deteriorated. The government decided to remodel the entire program, and with the technical and material assistance of the World Health Organization (WHO), the program was restructured. During the reorganization period chloroquine chemoprophylaxis was continued.

## MALARIA ERADICATION PROGRAM

Four established phases of malaria eradication were included in planning the national program: preparation, attack, consolidation, and maintenance.

The attack phase began on June 24, 1980, after all of the following conditions were met: adequate personnel, material, equipment, and infrastructure; sufficient knowledge of the average surface of the dwellings to be treated (283 $m^2$), the spraying itinerary, and the biology of the vector.

The strategy was based on periodic indoor spraying of all houses with DDT, as 75% wettable powder, at a dose of 2 $g/m^2$ to all interior surfaces, at 6-month intervals. From the first to the sixth spraying cycle the insecticide applications were regular and the coverage of surface complete and effective.

## EVALUATION OF THE ERADICATION PROGRAM

The prevalence of malaria among children between 0 to 14 years old was strongly influenced by the weekly chemoprophylaxis distributed to the households by members of the Malaria Eradication Service.

The initial parasite index of 19.2% decreased gradually over the first three semesters of the program. The results are shown in Table 2. This gradual decline in prevalence corresponds to a parallel decrease of malaria transmission. The observed results were similar to those projected by the WHO Expert Committee on Malaria.

The data based on epidemiologic surveillance between April and December of 1982 are shown in Table 3.

Of 23,461 blood slides examined, there were 201 cases of indigenous malaria, plus 23 imported cases.

Pyrethrin spray-catches have shown that *A. gambiae* s.l. had practically disappeared from the interior of the houses within the first three semesters of operation of the attack phase. Indeed, only seven *A. gambiae* were caught at five different catching points.

There were localities where human-vector contact no longer existed, even outdoors on the veranda, except for Micolo and Neves, in the coastal, arid zones where outdoor human-vector contact has remained of importance. The overall epidemiologic situation remained unchanged during 1982.

Table 2 Results of a weekly chemoprophylaxis program during attack phase (June 1980) of malaria eradication program.

| | Semester 1 | Semester 2 | Semester 3 |
|---|---|---|---|
| PI* | 5.5%† | 1.9%‡ | 0.63%§ |
| PI in general population | 5.4% | 1.5% | 0.59% |
| Population Sample: | | | |
| General | 2411 with 129 cases | 11,061 with 166 cases | 11,974 with 70 cases |
| Children (3–14 yr) | 1550 with 86 cases | 7296 with 136 cases | 8365 with 53 cases |
| Infants (0–11 mos) | 115 with 2 cases | 806 with 3 cases | 392 with 0 cases |

*Parasite index at inception of program was 19.2% (3 to 14-year-old children).
†Or 28.9% of initial PI.
‡Or 9.7% of initial PI.
§Or 3.3% of initial PI.

Table 3. Epidemiologic surveillance between April and December 1982.

| Malaria Species | Number | Indigenous | Imported |
|---|---|---|---|
| *P. falciparum* | 161 | 142 | 19* |
| *P. malariae* | 2 | 0 | 2* |
| *P. ovale* | 32 | 28 | 4† |
| *P. vivax* | 29 | 29 | 0 |
| Mixed Infections | 0 | 0 | 0 |
| Total | 224 | 201 | 23 |

*From Angola, Congo, and Gabon.
†From Angola and Gabon.

## CAUSES FOR THE FAILURE OF THE PROGRAM

When the DDT spraying of houses was regular and complete, there was a rapid decrease of the parasite index from 19.2% to 0.6% in a period of only three semesters. The initial success was the combined result of careful and circumspect planning of the program during the preparatory phase and the meticulous spraying operations and entomologic monitoring during the initial attack phase. However, for sociocultural and economic reasons at all administrative levels, the seventh and eighth cycles were delayed by 2.5 and 9 months, respectively. Moreover, there were other irregularities. The entomologic evaluation teams began to experience serious difficulties.

When the plasmodium index was reduced to only 0.6% in March 1982, surveillance operations by adequately trained field teams should have started immediately. Unfortunately, their recruitment was not possible until several months later. Limited active and passive malaria surveillance was started in April by personnel of the spraying teams and by a few adequately trained sanitation units. The field logistics became increasingly more difficult due to the shortages of supplies, spare parts, fuel, etc.

### Destabilization of Focal Malaria (1983–1984)

The first indications of trouble came in July of 1983 from L'Esprainha, a coastal village (Stratum Cw) with 143 inhabitants where the epidemiologic conditions were highly favorable for malaria transmission. There is also a group of fishermen from the northeast coast of Sao Tome who come to the area each year between June and August, where they take temporary residence in makeshift huts built with palm leaves. A similar type of annual migration can be found along all beaches of the northwest coast.

The parasistological data based on epidemiologic inquiries were as follows: July, 4 cases; August, 20 cases; September, 5 cases; October, 0 cases; November, 5 cases; and December, 11 cases.

During the first two months, most of the cases were found in new arrivals. All cases with *P. falciparum* infection were cured by radical treatment with chloroquine and primaquine.

The entomological data revealed that 102 female and 1 male mosquitoes (*A. gambiae* s.l.) were caught. Pyrethrin collections were made during August in 10 houses, just before the DDT spraying cycle was begun. The collections gave low yields, with only zero to two mosquitoes per catch. Soon there were small outbreaks in Santa Catarina and Neves (Stratum Cw); Praia Guegue-Morro Peixe and Micolo (Stratum A); and D. Augusta and Rebeira Afonso (Stratum Ce). The measures taken to control malaria in these foci included the following:

1. Active detection of febrile cases followed by presumptive treatment with chloroquine and primaquine;
2. Radical treatment of all parasitologically confirmed malaria cases;
3. Mass examination of blood specimens taken from the general population combined with presumptive treatment followed by radical treatment of all confirmed malaria cases. The presumptive treatment was given immediately after the individual blood samples were drawn. Because of material shortages and declining compliance of the population who had been disappointed by unkept promises made in previous campaigns, the foci progressed in number and size.

## THE EPIDEMIC

The consequent malaria epidemic was the result of long and frequent interruptions of the control measures. The expansion of malaria was rapid. Its prevalence increased to levels equal and above those found before the beginning of the eradication campaign, especially in the coastal areas. The potential of a major epidemic with catastrophic consequences was realized. Since 1983 the authorities had been alerted to that possibility. In August 1985 a new malaria control program was proposed as an alternative to the eradication program.

In 1984 there were five deaths from malaria. Until September 1985, the number of fatal cases varied between three to nine per month. Then in October there was a sudden increase in the number of hospitalized malaria cases and deaths (Fig. 1). Before the beginning of the attack phase of the malaria eradication program, malaria morbidity and mortality were, more or less, controlled by weekly chemoprophylaxis given to all children from 0 to 14 years, as well as some high-risk groups of adults. The number of deaths was not very high. As can be seen from Table 4, the monthly number of deaths registered during the epidemic was equal and higher than the annual fatalities reported before insecticide spraying began in June 1980.

As stated before, the malaria epidemic began in October and reached its peak in March of 1986 (Table 4).

## EVOLUTION OF THE EPIDEMIC

The sudden rise in malaria morbidity and mortality, which started in October 1985, continued unabated and the number of deaths seen in the Hospital Dr. Ayres de Menines remained high until May 1986, with the number of deaths varying between 19 to 41 per month. Thereafter, the mortality fell to pre-epidemic levels with six, nine, and seven deaths in

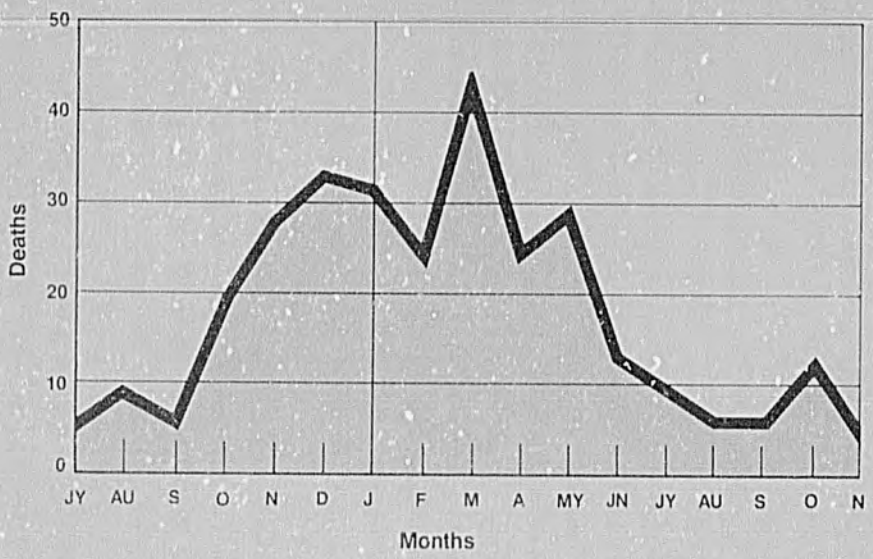


Figure 1. Number of deaths that occurred between July 1985 and November 1986 among patients admitted to the Dr. Ayres de Menezes Hospital.

Table 4. Reported malaria deaths 1972–1986 (October).

| Data Source | Year | No. Deaths |
|---|---|---|
| All health services (1972–1981) | 1972 | 37 |
| | 1973 | 51 |
| | 1974 | 29 |
| | 1975 | 29 |
| | 1976 | 28 |
| | 1977 | no data |
| | 1978 | 34 |
| | 1979 | 30 |
| | 1980 | 24 |
| | 1981 | 0 |
| Hospitals only (1982–1986) | 1982 | 0 |
| | 1983 | 0 |
| | 1984 | 5 |
| | 1985 | 13 |
| | 1986 (Jan–Oct) | 198 |

July, August, and September, respectively. However, in October there was another increase with 12 deaths in 257 hospitalized malaria patients, with a case- fatality rate of 4.7%. During the first 25 days of November there were 235 hospitalized malaria cases with four deaths (1.7%). Hospital admissions of malaria patients from January to October varied between 223 and 491 per month, and deaths between 6 and 41, respectively.

# CRITICAL THOUGHTS ABOUT THE EPIDEMIC AND ITS CONTROL

## Epidemic

The severity of the epidemic which ravaged the population of Sao Tome and Principe recalls historical events of the 16th century when a severe malaria epidemic almost eliminated the Jewish children on the islands. In our view, the extreme gravity of the outbreak has resulted from an unusual constellation of factors.

1. For the first time in history the prevalence of malaria had dropped to very low levels, with practically no transmission for an uninterrupted period of three years in the entire country.
2. The immunity of the population was significantly reduced due to absence of immunity boosting reinfections for three years.
3. Genetic selection of *P. falciparum* could have occurred as a result of the continued, intensive drug pressure of chloroquine given systematically over a decade.
4. During this time, a more virulent variety of *P. falciparum* could have emerged.

## CONTROL

The epidemic was brought under control thanks to the combination of three factors: The first factor was the physiological readjustment of the population to the parasite. The second factor was the administration of systematic chemoprophylaxis to all children under the age of 10 years and to all pregnant women, as well as presumptive treatment of suspected cases by a medical team from the University of Lisbon and the Portuguese Red Cross, with material and financial assistance from the European Economic Community. The third factor involved the occurrence of less than normal rainfall, particularly when compared with the epidemic period when rainfalls were exceptionally heavy.

## PARASITES

As mentioned before, all four plasmodium species are present in Sao Tome. *P. falciparum* has been the predominant species (80–100%) all over the country. Chloroquine sensitivity was originally high. After the residual indoor spraying with DDT, only specimens of *P. falciparum* were caught.

### Appearance of Resistance

During the second semester of 1984, several cases of *P. falciparum* malaria were detected in the vicinity of the international airport. These did not sufficiently respond to the short course of 3-day chloroquine treatment. The existence of chloroquine resistant malaria strains was suspected.

In April 1985 we began a trial with a 7-day course of chloroquine as recommended by WHO. The results of these clinical trials confirmed the presence of drug resistant *P. falciparum*. At present, quinine is also administered in the treatment of malaria.

## VECTORS

### Before DDT Spraying Operations (1980)

The studies by Bakri (1977) have demonstrated that *A. gambiae* s.l. is the only malaria vector in Sao Tome, and that it is endophagic and endophylic and occurs in high densities. Studies carried out on the island of Principe by MEP have given similar results. At altitudes above 300 meters, the vector densities become very low. With regard to insecticide resistance, Bakri found a reduction in sensitivity to DDT in Micolo and Neves with values of 77.4% and 82%, respectively.

In 1980, a French mission of the Laboratories of Toulouse presented these results of their studies: Sporozoite index of 5%; developmental cycle of 8 to 10 days; DDT susceptibility 100% in 3000 mosquitoes tested. The same investigators did not confirm the existence of *A. funestus*, *A. melas*, *A. paludis*, and *A. pharaoensis* on the islands as was reported previously in 1952.

### After the First DDT Applications

After the first DDT applications, the in-house human-vector contact reached practically zero and was also significantly reduced outdoors, except in Micolo and Neves. Saar, who studied the biting rates of *A. gambiae*, thought that this was due to a change in mosquito behavior. More recently, in March and April, 1986, an entomological team of the Institute of Tropical Medicine of Lisbon carried out interesting studies on the bioecology of *A. gambiae*.

The entomological team observed *(a)* that the vector is *A. gambiae* (Giles 1902) *sensu stricto*, Type A; *(b)* anthropophily is strong; vector densities are high; *(c)* biting rates are high; *(d)* endophagy and endophily are pronounced; *(e)* longevity is high; biting rates become practically zero

at altitudes of 300 meters and above; *(f)* there is marked DDT resistance but unimpaired sensitivity to malathion and propoxur.

Sensitivity to DDT and Propoxur on the island of Principe is complete. Malathion was not tested there. The various studies have greatly enhanced our knowledge of the bioecology of the vector *A. gambiae* in Sao Tome and Principe.

## PERSPECTIVES

After having lost an excellent opportunity to eradicate malaria because of sociocultural and economic constraints, the epidemiologic situation has become so complex that one should no longer aim at eradication but at malaria control. Keeping with this change, the new malaria project proposed in 1985 has been implemented. It is based on integrated malaria control at the level of the primary health care services. A training project for community health workers has already become operational.

Professors Cambournac and Linhao of the Institute of Tropical Medicine of Lisbon and technical consultants of the Portuguese Red Cross and the European Economic Community have proposed an integrated control program to be operated with the technical and financial assistance by international organizations. Two WHO experts (Tonn and Rasoloniaina) have made recommendations in support of these proposals. All experts have agreed to the important role to be played by the community health workers.

With the significant advances made in the field of malaria immunology, especially in research on the development of malaria vaccines, we can look forward to a new era that holds great promise. Because of the unique features of Sao Tome and Principe, as an island nation with a small area, easy access to a population living in roadside villages, with an interesting epidemiology of malaria and experienced field staff, we believe that our nation is uniquely qualified to serve as a candidate site for field trials of new malaria vaccines.

## CONCLUSIONS

A national malaria control program must always take full account of the sociocultural, political, and economic realities of the country. In Sao Tome and Principe, malaria has continued to represent a serious public health problem. However, all the control measures taken in the past were met by serious socioeconomic and political constraints.

After the national program of residual DDT spraying there has been a spectacular decline in the incidence and transmission of malaria

sufficiently long to cause a marked reduction of immunity in the population. This loss of immunity was responsible for the subsequent emergence of a severe malaria epidemic with high mortality.

The appearance of chloroquine-resistant strains of *P. falciparum* and of DDT resistance of *A. gambiae* have further aggravated the epidemiologic situation in the country. Chloroquine resistance is seen as the result of selective drug pressure following a decade of systematic, but often irregular chemoprophylaxis. The development of DDT resistance of the vector has had a similar history.

It is thought that physiologic readjustment of the population through natural boosting of antimalarial antibodies could actually reduce the amount of clinically apparent drug resistance in the future.

## RECOMMENDATIONS

For improvement and consolidation of epidemic control, one should commence as soon as possible with spatial applications of malathion in the coastal zones below 300 meters altitude. After consolidation of the situation and after the establishment of adequate diagnostic and treatment facilities, chemoprophylaxis should be limited to pregnant women.

Special attention should be paid to sanitary engineering and other effective environmental measures of vector control.

The development of an effective primary health care system will form the pillars of the fight against malaria by assuring early case detection and presumptive treatment of all suspected malaria patients with fever, as well as of the parasitologically confirmed malaria patients. The success of the program depends on the uninterrupted availability and distribution of all supplies and other essentials required for control. This should be guaranteed from the outset of the program.


## RESUME

Le paludisme en tant que maladie transmissible de morbidite et mortalite elevees, avec un impact redoutable sur tous les efforts de developpement socio-economique, il a merite depuis l'epoque coloniale a nos jours, une attention tres particuliere de la part des autorites.

La chimioprophylaxie et les pulverisations d'insecticides, irregulieres dans l'espace et dans le temps des 1947 n'ont pas reussi a modifier le spectre paludisme.

Son eradication ayant ete decidee par le Gouvernement, on a declenche en juin 1980 une campagne de pulverisations intradomiciliaires avec le DDT, ce qui permis d'amener l'indice plasmodique de depart de 19.2% a 0.63% en trois semestres.

A cause des contraintes socio-culturelles et economiques la Campagne a echoue, et la population fut victime d'une epidemie tres meurtriere. La situation

epidemiologique est autant plus grave vu l'apparition de la resistance de *Plasmodium falciparum* a la chloroquine et d'*Anopheles gambiae* sensu latu au DDT.

La lutte antipaludique integree basee sur les soins de sante primaires est actuellement la meilleure solution. Le recours aux vaccins est un espoir.

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# Malaria in the Sudan

A. A. EL GADDAL

## GEOGRAPHIC DISTRIBUTION

Malaria is the main endemic parasitic desease in the Sudan. The endemicity of the disease ranges from holoendemic in the south to hypoendemic in the north. As seen in Figure 1, the endemicity of the disease follows more or less the natural distribution of the geographic zones.

Malaria is holoendemic in the equatorial and rich savanna belt, hyperendemic in the savanna zone, mesoendemic in the poor savanna and semidesert zone in central Sudan, and hypoendemic along the main Nile in the northern part of the country and along the Red Sea. Accordingly, transmission ranges from perennial in the south to seasonal in the other parts of the country. In the irrigated areas in central Sudan, transmission changed from seasonal to perennial and the endemicity is changing from mesoendemic to hyperendemic.

Sudan shares its boundaries with eight countries and is subjected to population influx from neighboring countries and introduction of various parasite strains. During the last few years, due to the unstable situation in most of the neighboring countries, a great number of refugees have crossed the borders and come to seek shelter and peace in the Sudan. A lot of these people are considered as carriers of different strains of the malaria parasite. This has given rise to a change in the epidemiology of the disease.

## VECTOR SPECIES

Three vector species are identified: *A. gambiae*, which is geographically restricted to the southern part of the country; *A. arabiensis*, which extends from the south and reaches up to the northern limit near the Egyptian border and is considered as the principal vector in the country; and *A. funestus*, which is found in the southern part of the country.

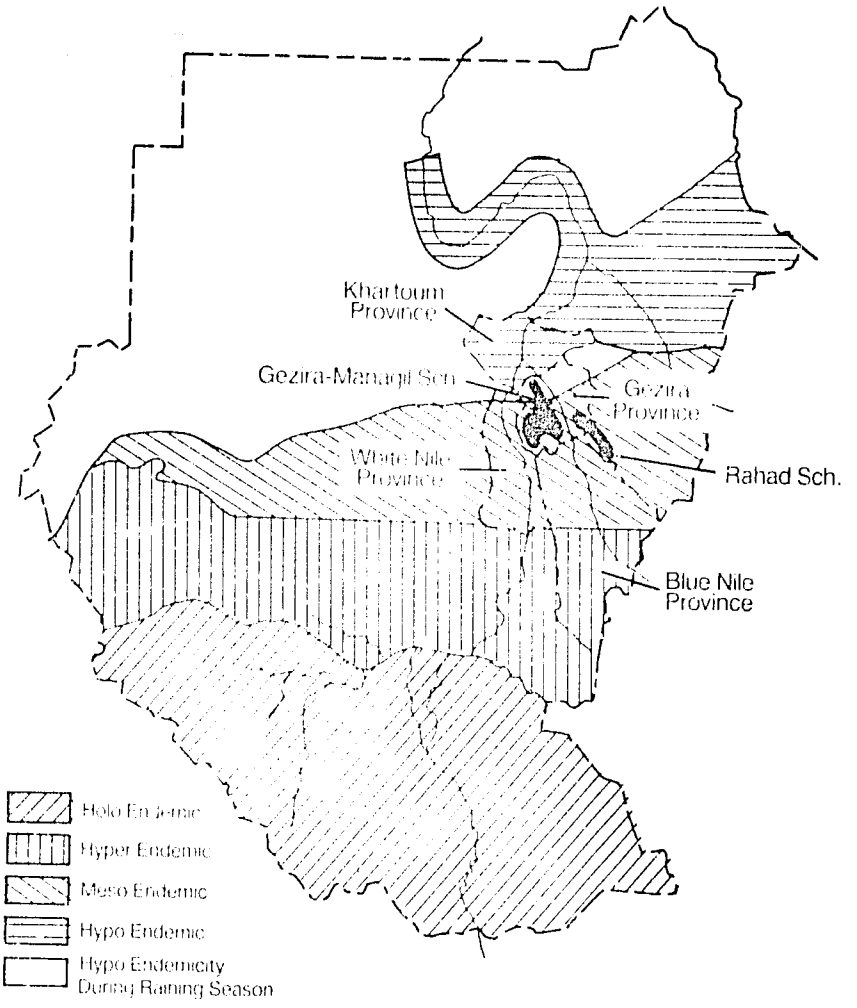


Figure 1. Geographic distribution of malaria in the Sudan by level of endemicity.

## PARASITE SPECIES

The predominant parasite species is *Plasmodium falciparum*, which constitutes about 90% of the human infection. However, *Plasmodium vivax* is widespread in the eastern part of the country and may reach 20% close to the Ethiopian borders. *Plasmodium malariae* is reported mainly in southern Sudan. *Plasmodium ovale* is being reported in a sporadic manner.

# CHARACTERISTICS AND REGIONAL CONTROL OF MALARIA

## Northern Region

Malaria is hypoendemic with seasonal transmission in the winter season and mainly along the river Nile. Malaria is unstable and the population is susceptible to infection. In the absence of control measures, waves of malaria epidemics occur. Chloroquine resistance based on in vivo tests only was first reported in this zone, particularly in the area north of Khartoum, but not confirmed.

Vector control based on residual house spraying is carried out mainly in the major towns and some irrigated projects in this region.

In the northern part of the region, a joint program between the governments of Sudan and Egypt is going on and aims at control of *Anopheles arabiensis* to prevent its introduction into southern Egypt (Gambia Project).

## Central Region

The central region is characterized by having several irrigation projects, among which is the large Gezira irrigation project and several sugar-plantation irrigation projects. Malaria ranges between mesoendemicity to hyperendemicity.

The first malaria eradication pilot project was carried out in the southern part of the region between 1958 and 1963. A major achievement of the project was its collection of valuable epidemiological data about malaria in the area. A malaria training center was established in this area in 1963 and is still functioning. A collaborative research project on malaria, which deals mainly with malaria immmunology and drug sensitivity, is being carried out with Michigan State University with support from the National Institutes of Health (NIH).

## Blue Nile Health Project

The Blue Nile Health Project is an integrated disease control project that deals mainly with the control of water-associated diseases (malaria, schistosomiasis, and diarrhoeal diseases) in irrigation programs of the central region. The strategy of the scheme is based on the application of integrated control measures using chemical control, case detection and treatment, environmental management, biological control, and community participation. The ultimate objective of the project is to reduce reliance on chemical control and emphasize the establishment of long-term control measures.

In the project area malaria prevalence is maintained at a level of about 1%. The major technical problem in the area is the insecticide resistance of the vector to chlorinated hydrocarbons and malathion. Recent sensitivity tests indicated that *P. falciparum* is still sensitive to chloroquine.

An important epidemiological feature in the area is population movement, particularly introduction of migrant labor forces from different parts of the country as well as neighboring countries.

### Eastern Region

In the eastern region, malaria ranges between hypoendemic to mesoendemic. The peak transmission period coincides with the rainy season. Malaria control is concentrated in the irrigated New Halfa Project to protect the nonimmune nubian population.

Recently in vitro tests indicated chloroquine resistance in the eastern borders with Ethiopia, where the influx of refugees is on a very large scale.

### Western Region

In the western region, malaria is mesoendemic with seasonal transmission. Vector control is limited and malaria is dealt with through the primary health care system.

### Southern Region

In the southern region, malaria ranges between hyperendemic to holoendemic. Limited vector control is carried out in major towns. Malaria chemotherapy is made available through the primary health care units.

# Summaries of Recorded Statements

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## TANZANIA

Kilama reported that the National Institute of Medical Research was created in 1980 and has a scientific staff of nine senior national scientists. The institute has been carrying out research on malaria in two major areas: drug resistance of antimalarials and entomological research of malaria vectors. There are cooperative malaria research projects with the Swiss Institute of Tropical Medicine in Ifakara, Central Tanzania; with the London School of Tropical Medicine and Hygiene in epidemiology and entomology; with the University of Rome in vector biology and genetics; and with the Kilimanjaro Christian Medical Center, to name only the major projects.

Chloroquine resistance in the country was first noticed in 1982, but has by now been found all over Tanzania. In addition to chloroquine, drug resistance has been observed to fansidar and mefloquine, in the northeast of the country. There is even a pronounced reduction of susceptibility of *P. falciparum* to the old antimalarial drug quinine. Tanzanian scientists are undertaking studies in combined chemotherapy.

The entomological studies consist of identification of mosquitoes involved in malaria transmission in different ecological settings, sporozoite identification using radio-immune assay and ELISA techniques, as well as malaria control using impregnated bed nets and intradomiciliary spraying of DDT.

In cooperation with the Swiss Medical Research Institute in Ifakara, antisporozoite antibodies and anti-blood-stage antibodies to *Plasmodium falciparum* have been followed in 132 children over a period of 3 years in an area of high endemicity. They examined NANP-40 antibodies and found that there was an increase in the antibody titer with the intensity of transmission and between each age group. Up to about age 5, the child's antibody level is unstable, but rises slowly and becomes less variable after that age. However, approximately 25% of all children have always remained negative in these antibody tests. A negative association was found

between the spleen rate and the antibody titer of the children. The investigators found evidence that malaria transmission is focal.

Finally, studies are underway to test the efficacy of a new larvivorous fish for the control of local malaria vectors.

## CAMEROON

Leke described the work of the new WHO Collaborating Center for Research and Training in Immunology. The center undertakes collaborative graduate and postgraduate programs to train African students under the auspices of WHO. The center has organized workshops for participants of African countries, including training in new laboratory technology supported by manufacturers of new sophisticated equipment like the ultracentrifuge, gamma and beta counters, etc.

Referring to the paper on malaria in Cameroon presented at this meeting by her fellow citizen Dr. Mfonfu, Dr. Leke pointed to the potential of new projects for research and training in the fields of immunology, immunopathology, and immunodiagnosis of malaria and other important parasitic diseases, notably onchocerciasis and three different species of schistosomiasis. Cameroon is bilingual for French and English. Because of the broad ecological spectrum of regions in the country, comprising the rain forest, mountainous areas, the flat lands in the coastal zone, Sudan savanna, dry savanna, rivers, lakes and the sea, diseases can be studied simultaneously in different settings. Dr. Leke invited the participants and organizers to make use of the Center for research and training.

## ZAIRE

Poluku stated that Zaire has a tropical and humid climate with a population of about 30 million people. French is the national language. The infant mortality rate is about 100–120 per 1000 live births. The annual growth rate of the population is estimated as approximately 3%. From 1978 to 1982, there was a malaria pilot control project in Kinshasa, which was evaluated in 1983 and, consequently, divided into vector control under the Department of the Environment, and disease control under the Ministry of Health, which carries out malaria control primarily through its Primary Health Care Service System.

Malaria endemicity is high in Zaire with a parasite formula of 95% *P. falciparum*, 5% *P. malariae*, and 0.5% *P. ovale*.

Malaria has a high morbidity and is estimated to account for 10–15% of child deaths. It is the third most important cause of death in the country. Since 1983 there have been studies on chloroquine resistance of

*P. falciparum*. There is a selective strategy for treatment of children under 5 and for reduction of morbidity in pregnant women. Zaire has a surveillance system to monitor the efficacy of antimalarial drugs. Most treatments are done on a presumptive basis. There is chemoprophylaxis for pregnant women and approximately 70% who attend prenatal clinics are treated.

Research on malaria is carried in cooperation with the three national universities in Kinshasa, Lubumbashi, and Kisangani. There is close collaboration with Centers for Disease Control in the United States and with the Institute of Tropical Medicine in Antwerp, Belgium.

Health education is another important aspect of the national malaria control program. District medical officers and laboratory technicians are trained in surveillance methodology, in techniques for monitoring drug sensitivity, and in approaches to identify risk factors for severe clinical malaria.

## SENEGAL

Diouf described Senegal as being located in West Africa and covering an area of 197,000 km$^2$. Its coastal area is the western most point of Africa. Senegal is situated in the Sahelian zones; its malaria endemicity level varies from hypoendemic in the desertic northern parts near the border with Mauritania, to hyperendemic in the southern Sudan-Sahelian area where the annual rainfall reaches 1800–2000 mm/yr.

About 30 years ago, Senegal was chosen as a pilot zone for the malaria eradication program in Africa. Since that time it has had a special department for malaria and parasitic disease control. The program has reduced the prevalence of malaria in the country through vector control, treatment and, since 24 years, chemoprophylaxis. The present malaria control program aims at the reduction of both the morbidity and mortality of the disease, primarily through the use of drugs. In 1984, a strain of *P. falciparum* was found in the Kaolaou region of central Senegal which was less sensitive to chloroquine. This has been verified by the in vitro test.

Because of the drought that has prevailed in the Sahel over the past decade, Senegal, Mali, and Mauritania decided to build water retention dams, especially on the Senegal River. These changes pose environmental threats for the level of malaria endemicity in the areas concerned. Baseline data have been collected and will be used for comparison to monitor the situation and to take appropriate action to prevent a negative impact on the health situation in the population living near these water impoundments. Senegal is a member of the West African regional organization for the fight against endemic diseases (OCCGE). A unit for monitoring malaria drug resistance has been established in cooperation

with OCCGE. The objectives of this program are described in greater detail in the paper given by Dr. L. Guiguemde at this meeting.

Senegal is prepared to participate in research and field trials of future malaria vaccines.

## ZIMBABWE

Taylor made some comments about the malaria situation in Zimbabwe. The country is bounded by South Africa, Zambia, Malawi, Botswana and Mozambique. Malaria ranges from hyperendemic in the low altitude areas to non-endemic in the highlands. A malaria control program based on vector control has been in effect since 1949. At the present time, this program covers the estimated 4 to 5 million population that live in malaria endemic regions. The cost of the program is about 2 million Zimbabwe dollars (US $1.2 million) per year to operate. It has reduced malaria endemicity from the previous levels of 50–70% to 5% in most areas.

He pointed to the great variations of the epidemiologic patterns and the pronounced seasonality of malaria as presented by the various country representatives who participated in this meeting, and stressed that there is a need for a review of our attitude toward malaria control. One should get away from the idea of a panacea which is going to solve all problems. We have to reconsider this situation, particularly as the general attitude toward new malaria vaccines seems to be that they are not by themselves going to provide the ultimate answer to remove malaria. We are going to have to integrate the new methods into an effective program together with other control measures.

## DISCUSSION

Bruce-Chwatt: It is the prerogative of old age and, in a way, a prerogative of one of the few remaining workers of the colonial period in Africa to make a few general points. I have listened with a great deal of admiration to the presentations of the new generation of the African colleagues. There is one thing I would like them to remember. There is a tremendous amount of valuable information in the old papers and documents of the colonial period. Do not neglect them. They are still valid because the situation in Africa has not changed to any extent, and, if anything, has become worse at the present time because of drug resistance. There is a great deal of basic studies, especially in Zaire and Nigeria and other parts of Africa for the period between 1930 to 1959. Do not neglect this information because it is still valuable today. Those who want to avoid the errors of the present should learn from the past. Thank you for allowing me to make this brief remark.

SECTION III

# EPIDEMIOLOGICAL STUDIES OF MALARIA AND MALARIA IMMUNITY

E.K. N'JELESSANI AND A.A. BUCK
*Chairpersons*

R. MORROW, JR. AND S. DIALLO
*Afternoon Session Chairpersons*

# Section III

N'Jie: Good morning, ladies and gentlemen. I welcome you to this scientific session of deliberations on ongoing epidemiologic studies on malaria immunity and transmission in a number of African countries. I join my colleagues from yesterday in thanking the organizers for making this meeting possible and for inviting us to participate in this conference. I am equally indebted to them for giving me the privilege of chairing this scientific session.

Yesterday we looked at the state-of-the-art in this exciting period of malaria vaccine development. We have looked at the problems that may stand in the way in arriving at an effective vaccine against malaria. Later in the afternoon we were given an overview of the status of malaria in Africa, and we learned more about the diversity and adaptability of the African mosquito fauna involved in malaria transmission, and how we need to start preparing those communities who will be participating in these new vaccine trials. I think this latter point was brought out very clearly by our colleagues from Tanzania and Kenya. We must emphasize that unless our communities truly understand what these studies are all about, our efforts will not really yield the benefits that we anticipate. So, the importance of community participation is something we really must explore.

Later in the evening we considered prospects of new diagnostic tools that could be used in epidemiologic studies of malaria and that could provide vital information that is so necessary before launching any new project in this field of research. It is in that spirit that we will continue this morning, we will be looking at some new epidemiologic findings that will play a significant role in vaccine development.

I am happy to introduce the first speaker, Dr. Louis Molineaux of the World Health Organization in Geneva, who will introduce and elaborate on WHO's guidelines for epidemiologic evaluation of *Plasmodium falciparum* sporozoite malaria vaccines.

# WHO Guidelines for the Epidemiologic Evaluation of *Plasmodium Falciparum Sporozoite Vaccines*[a]

LOUIS MOLINEAUX

The World Health Organization (WHO) guidelines were produced by a working group from the Malaria Action Program (MAP) and the Special Program for Research and Training in Tropical Diseases (TDR). The MAP/TDR group included Drs. Goriup, Martinez, Molineaux, Morrow, Muir, and Spencer, who worked in cooperation with others, inside and outside WHO, in particular the members of three TDR steering committees: scientific working groups on malaria immunology (IMMAL), on field research on malaria (FIELDMAL), and on epidemiology (EPID). The guidelines are not a blueprint which would be premature; they are more general and tentative, which we hope will start a process requiring further work, including inputs from countries where malaria is endemic. A French translation of the guidelines is in preparation. This presentation will of necessity be very selective.

The epidemiologic evaluation of a vaccine must be its use as a tool for public health. It is therefore appropriate to discuss the expected effects and the potential objectives of malaria vaccinations. In residents of endemic areas (as distinct from nonimmune visitors), two broad categories of objectives can be distinguished, namely, the protection of vaccinated individuals and the reduction of transmission. Though not absolute, the distinction is important in the design of vaccine trials.

A discussion of the expected effects of malaria vaccination involves comparison among different types of vaccines (sporozoite, blood stage, etc.), including combinations. Such a comparison should be made in relation to the parasite's life-cycle. This life-cycle (Fig. 1) has three levels at which naturally acquired immunity or vaccination may interfere. It is worth considering the expected effect of stage-specific vaccines on immunity to the other stages and the expected effect of partial immunity. In theory, an increase in immunity against a given stage could lead to a

[a]World Health Organization publication TDR/MAP/SVE/PF/86.5

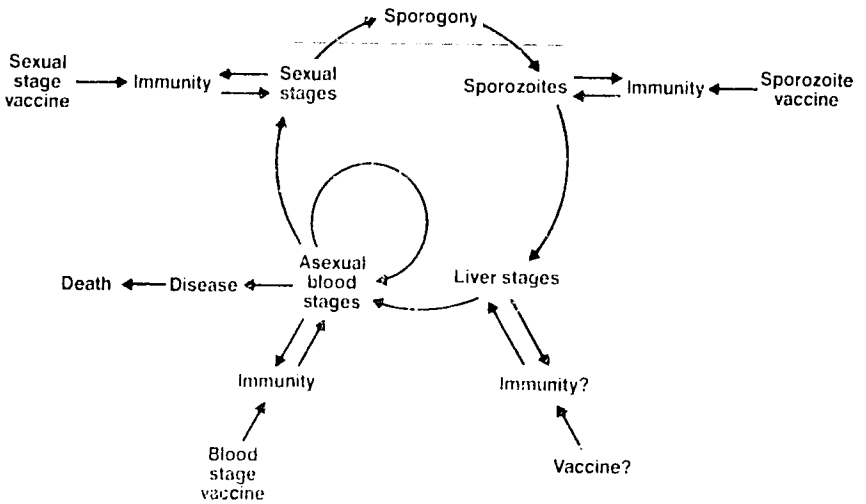


Figure 1. Schematic representation of the malaria parasite's life-cycle, indicating where natural immunity or vaccination are expected to interfere.

decrease in immunity against later stages (through a decrease in antigenic stimulation), and perhaps an increase in immunity against earlier stages (through a decrease in immunosuppression). For example, sporozoite vaccination may prevent the development of immunity against blood stages or allow its decay; if protection is time-limited or partial, the pathogenicity of breakthroughs may be enhanced. The significance of hypothetical partial protection per se against sporozoites is unknown: Would a reduction in the number of "successful" sporozoites tip the balance in favor of the defense mechanisms, or would it mean that only the light (relatively benign?) inoculation would be aborted?

What are the chances of interrupting transmission of malaria and of eradicating it through vaccination? The analogy with smallpox may be misleading. One of the relevant differences concerns the basic reproduction rate ($R_0$) or potential number of secondary cases originating from a primary case. The $R_0$ of smallpox is small (of the order of 4 or 5), whereas the $R_0$ of malaria can be much larger (up to 100 or even much more). There must exist a relationship between $R_0$ and the vaccine coverage required to interrupt transmission. This relationship can be approximated as follows: If $V$ is the proportion effectively protected by vaccination, the interruption of transmission requires

$$R_0 (1-V) < 1 \text{ or}$$
$$V > 1-1/R_0$$

$R_0$ does not have to be very large to make the required effective coverage impossible to achieve. Interruption of transmission will be a realistic objective of malaria vaccination only where $R_0$ is small or can be made so by vector control.

In many situations, particularly in Africa, the objective of malaria vaccination, rather than eradication, is to achieve a new, more favorable, equilibrium with the parasite, which can result in a reduction in morbidity and mortality. Such an equilibrium may ideally include an increased resistance of individuals and a reduction in the inoculation rate that would be sufficient to reduce superinfection but still allow natural boosting of immunity, thus reducing the need for revaccination. (Note the potential conflict in the latter objective.) A blood-stage vaccine may be more likely to achieve a satisfactory new equilibrium than a sporozoite vaccine; the value of sporozoite vaccines for that purpose will depend on the existence and effect of partial protection. Given the use of a blood-stage vaccine, will it be desirable to add a sporozoite and/or gamete vaccine, for the purpose of reducing transmission, given the potential conflict referred to above? The addition of a gamete vaccine may serve the additional purpose of preventing the selection of antigenic mutants refractory to the blood-stage vaccine.

In planning vaccine trials, we must consider the factors, other than the vaccine, that are likely to affect the outcome. Such confounding factors may affect exposure to malaria or the response to vaccination or to malaria. Table 1 is a list of such factors, with special reference to sporozoite vaccines—although many would apply to other types of

Table 1. Factors likely to affect outcome of trial (other than the vaccine).

| |
|---|
| Parasitologic Factors |
| Antigenic diversity and variation |
| Diversity and variation in drug response |
| Human Host Factors |
| Effects of malaria in the host |
| Pre-existing acquired active immunity |
| Passive immunity in infants |
| Suppression of immune response to sporozoites by concurrent blood-stage infection |
| Other malaria species |
| Other Human Host Factors |
| Genetic factors affecting host response to P. falciparum |
| Age and sex |
| Human behavior |
| Other diseases |
| Other vaccines |
| Diet and nutrition |
| Entomologic Factors |
| Control Measures |

vaccine as well. These factors are further discussed in section A4 of the guidelines. They are conveniently grouped into four classes, and they should be taken into account in planning and/or evaluating vaccine trials. The list is also useful for discussions concerning the differences between endemic and nonendemic areas, the limits of extrapolation from the one to the other, and the trials required in either or both kinds of areas.

Section B, the largest section of the guidelines, is dedicated to field trials in malaria-endemic countries and discusses the following:

1. The information required from pre-epidemiologic trials (phases I and II) and the need to repeat the trials in endemic areas;
2. The criteria for selection of trial areas and populations;
3. The design of trials of individual protection, comparing vaccinated and unvaccinated individuals living side by side in the same community, preferably through a double-blind comparison between the vaccine and a placebo, and the possible usefulness of combining the administration of the vaccine with that of an antimalarial treatment;
4. Some of the main differences between a trial of individual protection and a trial of impact on transmission, comparing vaccinated and unvaccinated communities, and having many similarities with trials of the epidemiologic impact of vector control;
5. Methods of evaluation.

Table 2 outlines methods for evaluating malaria vaccine trials. Points to be taken into account include the following: First, the relative importance of the three methods of case detection (passive, active, by survey) will vary with the precise objectives and the logistic possibilities of the trial. Surveys and active case detection will detect cases (of infection with or without symptoms) earlier and more completely than

Table 2 The evaluation of malaria vaccine trials.

| Data Collection Methods | Measurements |
|---|---|
| Human Population: Total population surveys and passive and active case detection | Infection (incidence and density), immune response, disease (incidence and severity), side effects, and behavior (exposure and treatment seeking) |
| Detection and Investigation of Deaths | Malaria mortality |
| Vector Population | Entomologic inoculation rate |

passive case detection, but the latter allows a better estimation of the incidence and severity of perceived illness. Improved peripheral diagnostic and therapeutic facilities are prerequisites of a vaccine trial and are also desirable.

Second, early epidemiologic trials of sporozoite vaccines are likely to concentrate on protection against infection (with immediate treatment of breakthroughs). There will eventually be a desire to evaluate protection against disease (e.g., by a blood stage vaccine or by a partially protective sporozoite vaccine); it cannot be assumed that protection against disease (and death) will be proportional to protection against infection, because protection against infection may be negatively related to the size of the inoculum, whereas severity of disease (and risk of death) may be positively related to it.

Third, while deaths should be investigated, early epidemiologic trials are unlikely to evaluate usefully the prevention of deaths, because sample sizes, calculated in relation to the expected incidence of infection, will be too small, and because "cases" (in either vaccinated or unvaccinated) will be detected and treated early.

Fourth, even in a trial of individual protection there is a place for entomology, because it provides an independent assessment of the challenge (in addition to incidence in the unvaccinated) and because the relationship between inoculation and incidence rates is nonlinear.

Finally, "protection" may be an ambiguous concept. Its simplest definition refers to prevention of the first breakthrough of infection, but other aspects may be important, e.g., severity of infection and frequency of episodes. The definition adopted will affect the calculation of "protection" and of the sample size.

Section C considers relevant preliminary epidemiologic research, as distinct from the collection of baseline data in the strict sense (i.e., data against which to measure the effect of malaria vaccines). Whereas the collection of such baseline data is probably premature, it would be useful to conduct as soon as possible preliminary epidemiologic research on a variety of topics that are relevant to the future epidemiologic evaluation of the vaccines. Indeed, one of the most useful purposes of designing the epidemiologic evaluation trials of the malaria vaccines is to identify as explicitly as possible the relevant preliminary research that could be undertaken immediately. Section C of the guidelines is a brief and tentative list of such topics, which are offered for discussion and elaboration. Table 3 lists the four classes of topics and gives selected examples. Some of the desirable preliminary research is under way, but a more thorough technical review of the subject is desirable in the near future.

Table 3. Preliminary research.

| |
|---|
| Measurement Methods |
| Development |
| Standardization |
| Adaptation |
| Validation |
| Epidemiology of Plasmodium falciparum malaria |
| Transition from passive (maternal) to active immunity |
| Natural patterns of inoculation including distribution of sporozoite loads in vectors |
| Inoculation rate, incidence of infection and disease |
| Size of inoculum and disease |
| Expected Effects of Sporozoite Vaccine |
| Place of chemotherapy |
| Existence and significance of partial protection |
| Effect of antisporozoite antibodies on sporozoites in the vector |
| Simulations |

## DISCUSSION

N'Jessane: Thank you very much, Dr. Molineaux. Since this was a joint endeavor of various parties at WHO, some of whom are represented here in the audience, I wonder whether Dr. Martinez, Secretary of IMMAL, or Dr. Goriup, Secretary of FIELDMAL, or Dr. Morrow, Secretary of EPID, or Dr. Najera, Director of MAP, would like to add something to the presentation we just have heard from Dr. Molineaux.

Since that is not the case, it is my pleasure to open the floor to comments and questions.

Chulay: I agree completely that the only way we can evaluate efficacy is by placebo-controlled clinical trials. The question I have for people who work in communities where field studies will be done is this: Will the average rural population where malaria is endemic accept the concept of controlled trials and participate in a study where half of the people receive a placebo?

Doumbo: Good fortune has been present here at this meeting with experts in malaria control in the audience. I have been asking myself many questions since yesterday. I work at the primary health care level; therefore, we are the middlemen between the decision makers and those working at the grassroot level. We convey your message. I am very worried about what I am going to say when I go back to my country.

A community study was proposed to us several years ago. We had to make enormous efforts at the community level, first of all to clean up the environment. When we try to clean up excavations around the villages by covering them with soil, we immediately create other areas where

mosquito larvae can breed. Another effort concerned chemotherapy. We made great efforts to promote the campaign over many years. Then, in 1985, a new message was conveyed telling us that chloroquine should not be applied systematically as a chemoprophylaxis, but only in clinical cases.

Now we are speaking about malaria vaccines, and many questions are being asked by the scientific community. Can you, the experts, give me a clear message to take back with me concerning the new vaccines at this time?

MOLINEAUX: I think that the problem we have posed is a very broad one. I really do not have the answers to all of the different aspects you have highlighted. There is, indeed, a large difference between smallpox and malaria. We should not hope for a malaria vaccine that would be as effective as the smallpox vaccine, and we should not think it undesirable for a new vaccine to render only partial protection against malaria infection. This is especially relevant for adults in Africa who have acquired immunity levels that make malaria tolerable to them, in contrast to infants and young children in whom malaria is a major cause of mortality. If it were possible for a human being to acquire through vaccination a level of immunity similar to that obtained after 5 to 7 years of natural exposure and without having to pay the price of high mortality, then the new vaccine would be a significant accomplishment.

Your first question about the credibility is a very important one. One can not continue to make promises to the people about effective new tools for malaria control when several previous attempts have failed. Under these circumstances it is true that the compliance will decline through loss of credibility. There is, however, one important aspect which I have only mentioned in passing. To evaluate a malaria vaccine good case detection methods are essential. Yes, it is good to detect malaria cases even if we do not have a vaccine. I say this because of my own clinical experience in Africa. The people want to see some kind of treatment for their children who are ill with malaria. Readily available treatment will have to be included in the evaluation process of a new vaccine. Unfortunately, I can not answer all of the questions you have raised.

N'DIAYE: Thank you, Dr. Molineaux. With your permission, I propose that we take up some of these questions in our workshop discussions and move now to the next papers. Thank you once more, Dr. Molineaux, for your wonderful presentation. May I now invite Dr. N'jie and Dr. Greenwood from the Gambia.

N'JIE: Thank you, Mr. Chairman. Please, allow me to join the earlier presenters to thank the organizers for giving me the privilege of attending this meeting even though at times I have wondered what I am doing here.

However, as the conference has evolved, the rationale for getting such a curious mix is becoming more and more apparent to me. My presentation, Mr. Chairman, will be brief and general to allow time for some of the important research findings that we have made in the Gambia to be shared with the members of this conference.

To put the malaria research that is going on within the Gambia government into perspective, we need to look at the evolution of the primary health care strategy in the Gambia, in an effort to focus our activities onto those areas where we thought we could have a quick impact on the problems that faced us. In the late 1970s, we tried to evolve a primary health care action plan that was based on the available information within our national health system and on the epidemiologic profile. It was not surprising that on the basis of these relatively crude data available to us, acute respiratory infections, malnutrition, diarrhea, the immunizable diseases and malaria were issues of priority. Despite the omnipresence of appropriate technology, we had to look at these problems within the context of our national structure through which we had to deliver some of these programs. The national health program, therefore, was restructured and specific control programs were developed for key areas we had identified.

It soon became obvious that the almost euphoric expectation of a rapid decline in our rather disgraceful levels of infant and child mortality would not materialize because we could not simultaneously sustain an optimal mix in these important program areas. It is, therefore, in this context that we view the exciting new research possibilities on malaria. We are concerned as to how these possibilities will benefit and function within the realities of our national health system; i.e., not within the ideal framework of adequate resources, adequate numbers of trained manpower, and the actual response of the communities to these technological breakthroughs. It was in this context that early on in our primary health care implementation, we approached the Medical Research Council Laboratories in the Gambia — with whom we enjoy rather unique relationships — and asked them to include items of research into their study that we in the public health sector identify for their expert attention. The report which will be given soon by Dr. Greenwood indicates some of the concerns relating to malaria that we have had.

Our National Malaria Control Program has been based on expert groups, mainly from WHO. Primary health care, including early diagnosis and treatment, is given by community health workers and is linked to the referral system through the different levels of the health service structure. Some efforts are directed at restricting human-mosquito contact through the use of bed nets, more recently impregnated bed nets. As I said before, the results have not been as one would have expected. Although some impact has been obvious, the significant drop in overall mortality has not

been there, because this year we appear to be saving the children from malaria only to let them die of diarrhea. If we concentrate our efforts on diarrhea next year, they will die of malaria again. If an optimum package of activities can be put together to ensure the ordinary conditions under which we operate, one can make use of these scientific breakthroughs. It is, therefore, Mr. Chairman, my intention not to dwell too long on this, but invite Dr. Greenwood to give details of our own findings in the Gambia. Thank you.

NIEUSSYS: May I ask Dr. Greenwood to, please, come forward for his presentation.

# Epidemiology of Malaria in the Gambia

BRIAN M. GREENWOOD

The Gambia is a small West African country situated on the north and south banks of the river from which it takes its name. The population, approximately 800,000, is still predominantly rural although there is a population shift to the periurban areas near to the capital, Banjul. Cereals and groundnuts are the main agricultural crops. The climate is characteristic of countries of the Sahel and sub-Sahel with a long dry season, which extends from November through June, and a shorter rainy season. Rainfall in recent years has been approximately 600 mm/yr. The Gambia is a poor country with an average per capita income of only $250, but, nevertheless, it has a good health care system and an excellent record of childhood immunization.[1]

The epidemiology of malaria in the Gambia has been studied by staff of the Medical Research Council (MRC) for over 30 years. The classical studies undertaken by Sir Ian McGregor and his colleagues in the villages of Keneba and Manduar defined clearly the pattern of malaria as it occurred in a rural area of West Africa in the early 1950s.[2,3] Their findings are summarized in Table 1.

## CHANGING EPIDEMIOLOGY: 1950s–1980s

The epidemiology of malaria in the Gambia is now different from the pattern observed in Keneba in the 1950s. Surveys undertaken in Keneba at the height of the severe Sahelian drought (1972–1973) showed a marked reduction in spleen and malaria parasite rates.[4] In recent years it has not been possible to study the epidemiology of malaria in Keneba because the pattern of this and other infections has been changed radically by the presence in the village of a resident physician and midwife.[5] However, studies in other parts of the country suggest that amelioration of the 1970s drought situation has not been accompanied by a return in the level of malarial endemicity to the level seen in the 1950s.

There are probably two main reasons for this change. A study of annual rainfall figures for Banjul shows that, although rainfall has been better in the 1980s than in the 1970s, there has been a slow but steady

**Table 1.** Epidemiology of malaria in the village of Keneba, the Gambia in the 1950s.*

- High infant mortality rates (approximately) 200/1000 and 100/1000 respectively
- Marked seasonal variation in childhood mortality with most deaths occurring during the rainy season.
- High parasite rates reaching nearly 100% in children aged 2–5 yr, with a rapid decline in parasite prevalence in older children
- High prevalence of splenomegaly with a similar age distribution to that of parasitemia
- Low spleen and parasite rates in adults
- Plasmodium falciparum and P. malariae the dominant parasites
- Anopheles gambiae s.s. and A. melas the dominant vectors

* Data from McGregor 1964 and McGregor et al. 1966.

decline in rainfall over the past 100 years.[6] Increased utilization of antimalarials is a second factor that is likely to have contributed to a decline in the level of malaria infection in the Gambia during the past few decades. Chloroquine consumption is currently estimated to be about five tablets per person per year. It is likely that chloroquine consumption in the 1950s was much less than this.

## MORTALITY AND MORBIDITY FROM MALARIA: 1982–1983

### The Farafenni Project

Because of uncertainty about the current importance of malaria as a cause of mortality and morbidity in the Gambia, a new study was started in 1982 in an area near to the town of Farafenni on the north bank of the river. The study measured mortality and morbidity from malaria in this rural community prior to the introduction of a primary health care program. This study area comprises 41 villages and hamlets with a total population of approximately 13,000. Villages within 10 km of the town, which, at the time of the first surveys, had a government dispenser and one private physician, were excluded. Details of the study area and the study population are given elsewhere.[7]

### Mortality

Attempts were made to record all deaths in children under the age of 7 years throughout the study area during a 1-year period in 1982–1983 using a system of village reporters and MRC field staff. Cause of death was established as accurately as possible through a postmortem interview with the family of each deceased child, conducted by a physician, and by

Table 2. Cause of death among 184 children under the age of seven in a rural area of the Gambia in 1982–1983.*

| Diagnosis | No. Deaths | % Deaths |
|---|---|---|
| Neonatal | 42 | 23 |
| Acute respiratory | 27 | 15 |
| Malaria | 25 | 14 |
| Meningitis | 17 | 9 |
| Malnutrition/Chronic Diarrhea | 16 | 9 |
| Acute gastroenteritis | 14 | 8 |
| Measles | 6 | 3 |
| Septicemia | 6 | 3 |
| Chronic respiratory | 5 | 3 |
| Miscellaneous | 7 | 4 |
| Unknown | 19 | 10 |
| Total | 184 | 101 |

*Data from Greenwood, Greenwood et al., in press.

examination of any health card records that were available.[8] The overall infant mortality rate was 142/1000 and the child mortality rate (deaths in children aged 1–4 years) was 43/1000/yr. Malaria was thought to be the second most frequent cause of death in children who survived the first month of life (Table 2).

Further study of the deaths that were thought to have been due to malaria showed that they nearly all occurred during a short 3-month period at the end of the rainy season (Fig. 1).[9] Malaria was a relatively more important cause of death in children aged 1–4 years than in infants (Fig. 2) and accounted for 25% of deaths in children in this age group. The malaria-specific mortality rate in children in the latter age group (11/1000/yr) is very similar to the level found in Lagos in 1950 during one of the few studies in West Africa in which measurements of this kind have been attempted.[10] Of the 25 children thought to have had malaria, 23 died at home and only 6 had received any conventional treatment. Terminal illnesses were usually short, with an average duration of only 2.8 days.

## Morbidity

Morbidity from malaria was assessed in a 1:5 cohort of approximately 600 children under the age of 7 years using three methods: clinic attendance, fever surveys, and clinical surveys.

*Clinic Attendance.* Health cards, held by a high proportion of Gambian children, were examined for records of attendance at a clinic or dispensary with an illness diagnosed (usually without microscopy) as malaria. In children under the age of 5 years, the average number of such attendances was about 0.3/yr.

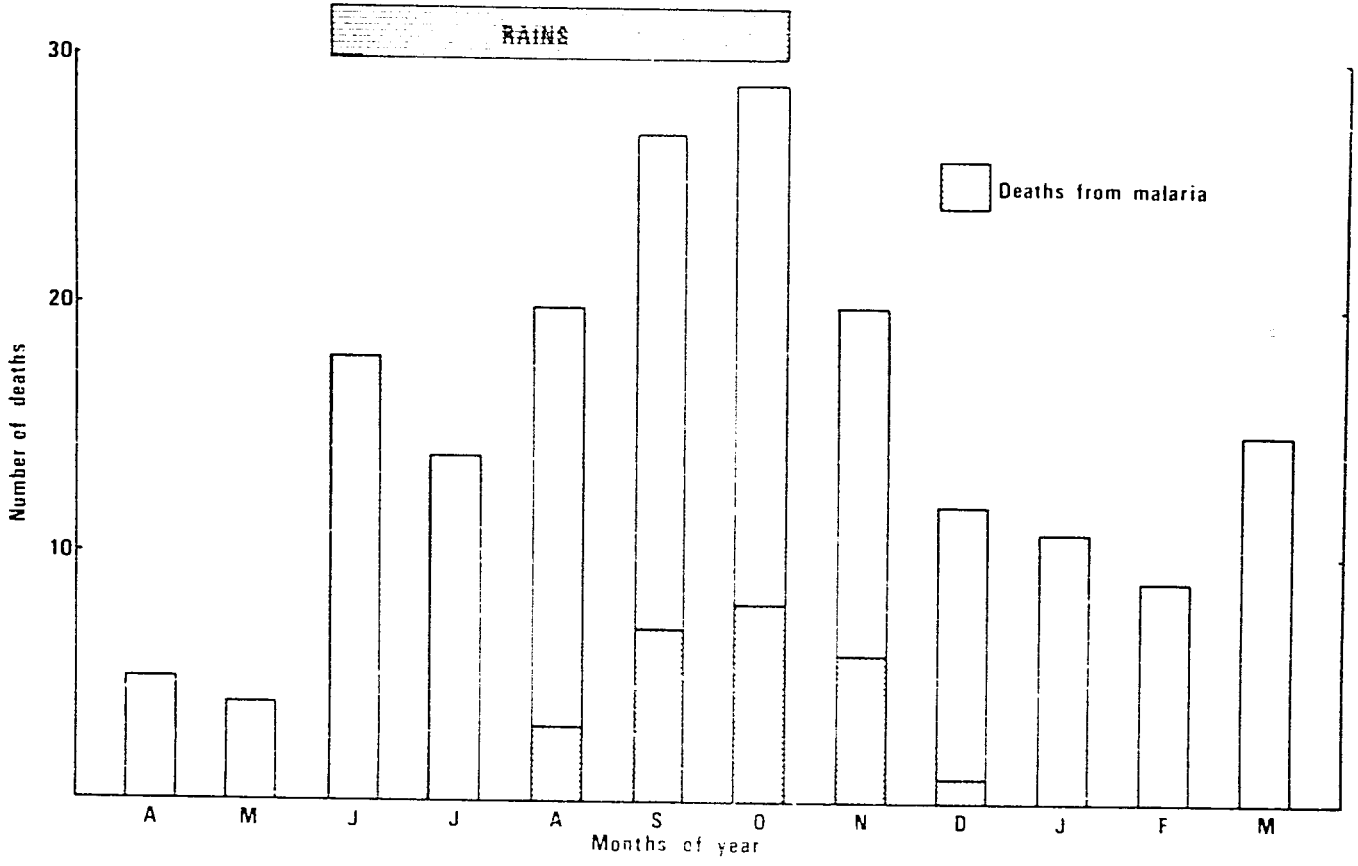


Figure 1. Deaths by month of year in 3398 Gambian children aged less than 7 years old (midyear population). (From Greenwood, Bradley, et al., in press.)

Figure 2. Deaths by age in 3398 Gambian children aged less than 7 years old (midyear population). (From Greenwood, Bradley, et al., in press.)

*Fever Surveys.* Because only a proportion of children with febrile illnesses were brought to a clinic for treatment, a more active surveillance system was introduced. Each child was visited once a month by a field assistant, temperature was recorded and a blood film made if an axillary temperature of 37.5°C or higher was found.[9] With the use of this technique, about 0.2 episodes of fever accompanied by malaria parasitemia were detected per child per year. Episodes of fever and malaria parasitemia were noted most frequently at the end of the rainy season (Fig. 3). In a subsidiary study in which observations were made weekly about 0.8 episodes of fever and malaria parasitemia were recorded during one rainy season. These studies suggest that, in the Farafenni area, children under the age of 7 years experience at least one clinical attack of malaria per year. About half of all episodes of fever were accompanied by parasitemia. Calculation of etiologic fractions suggested that malaria was responsible for about 40% of episodes of fever.

*Clinical Surveys.* Conventional malaria surveys established an overall level of parasitemia of about 40% in children aged 1–6 years which varied little by season. Spleen rates increased from 10% in infants to 50% in children aged 6–7 years. The packed cell volume (PCV) showed a sharp fall in children aged 1–3 years.

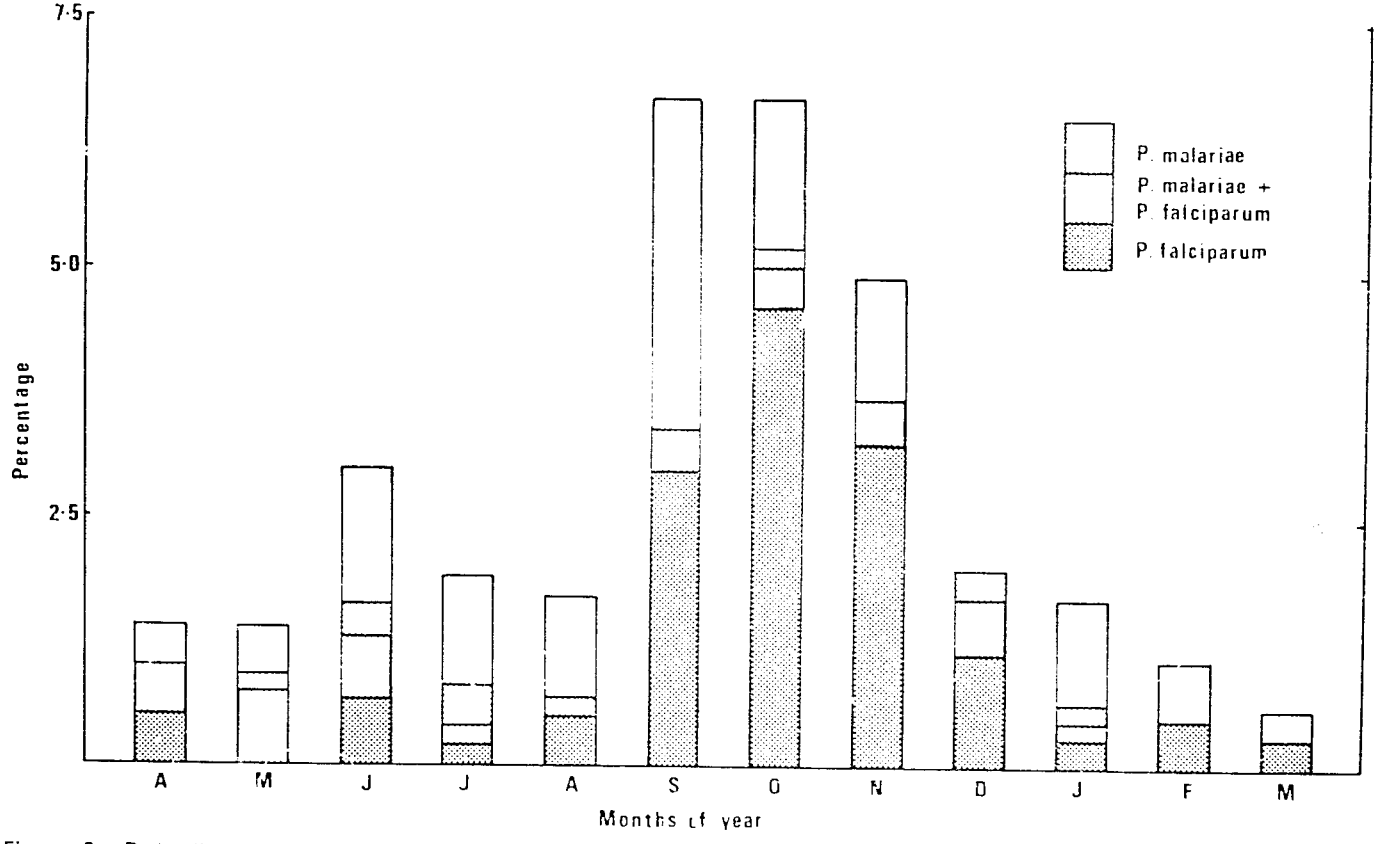


Figure 3. Detection of fever in an average of 657 Gambian children less than 7 years old examined monthly for 1 year and the presence of associated parasitemia. (From Greenwood, Bradley, et al., in press.)

## ENTOMOLOGY

Surveys undertaken by Bryan and associates in 1980 and 1981 showed that the main vectors in the Farafenni area were *Anopheles gambiae* s.l. and *A. melas*.[11] A few *A. arabiensis* were caught in the town. More detailed entomological studies were started in Farafenni villages in 1986 (Lindsay, unpublished data). These have shown that mosquitoes of the *A. gambiae* complex can be found in huts only during a period of a few months at the end of the rainy season. Ninety percent of biting takes place during the period 11 PM to 6 AM. The average inoculation rate is about 50 infected bites per person per year.

Sporozoite antibodies, detected by radio-immunoassay, were found rarely in Farafenni village children under the age of 10; by which time children have probably experienced about 500 infected bites (Marsh et al., unpublished data). After the age of 10 years the prevalence of sporozoite antibodies increases rapidly. Children with sporozoite antibodies are subsequently less likely to have a clinical attack of malaria than children without sporozoite antibodies, but this relationship is not a strong one. The prevalence of sporozoite antibodies in young children is not increased by chemoprophylaxis.

## SEASONALITY OF CLINICAL MALARIA

Although there are only modest seasonal variations in malaria parasite and spleen rates among children in the Farafenni study area, the main clinical impact of malaria is restricted to a relatively short period at the end of the rainy season. This seasonality facilitates malaria control in this community through chemotherapy, chemoprophylaxis, or vector control.

## LOCAL VARIATIONS IN THE PATTERN OF MALARIA

Surveys in villages throughout the Farafenni area have shown marked variations in the pattern of malaria between villages situated in apparently similar geographical situations. Furthermore, clustering of cases of clinical malaria and of splenomegaly is frequently found within a village (Snow et al., unpublished data). It is probable that both environmental and genetic differences contribute to these local variations in malaria epidemiology. Differences in mosquito numbers have been observed between adjacent villages, and the prevalence of splenomegaly is influenced by ethnic group. Among adults large spleens are found more frequently among Fulas than among other ethnic groups; this finding was noted previously in Nigeria.[12]

## SPECIAL EPIDEMIOLOGICAL SITUATIONS

It is probable that the epidemiology of malaria found at Farafenni is reasonably characteristic of the situation that prevails in most parts of rural Gambia and in similar areas in adjacent countries. However, there are at least two areas in the country where different epidemiological situations prevail.

### Urban and Periurban Areas

In periurban areas near the capital of Banjul, the prevalence of splenomegaly and parasitemia is much less than that found in Farafenni villages, and yet severe clinical attacks of malaria occur frequently in this community. A survey undertaken at the end of the 1986 rainy season in Bakau's new town showed spleen and parasite rates of less than 10% in children aged 0–10 years (Aihie et al., unpublished data). Perhaps, in urban areas, a higher proportion of infections are accompanied by clinical illness than in rural areas where children have a greater degree immunity.

### Irrigated Rice Swamps

A large swamp rice project has recently been started at Jakhaly-Pacharr in which irrigation is achieved with water pumped from the river Gambia. In this area large numbers of *A. gambiae* s.l. have been found during the dry season, but it has not yet been established whether these mosquitoes can transmit malaria at this time of the year when it is very hot and dry.

## MALARIA CONTROL

Following the collection of baseline data in the Farafenni area, a number of intervention studies have been started including trials of treatment of fever by village health workers, chemoprophylaxis, and bed nets. It is hoped that the results of these studies will provide some indications of how malaria can best be controlled in the Gambia until an effective malaria vaccine becomes available.

### ACKNOWLEDGMENT

Support of the Gambia government's Medical and Health Department for the Farafenni project is gratefully acknowledged.

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# Yekepa Project in Liberia

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The malaria situation in Africa is now more serious than ever, mainly because of the development and spread of drug resistance. However, in the past few years a great deal has been learned about malaria parasites and the immune responses they evoke in the host. These advances and the new developments in molecular biology are exciting and may inspire optimism. A close collaboration between immunologists and molecular biologists in sophisticated Western laboratories and laboratories and research units in the endemic areas is and will be increasingly essential. The importance and benefits of such close links between the basic laboratory research and the applied clinical epidemiological research have been apparent in the Yekepa Project with the collaboration between the Yekepa Research Unit and the malaria unit of the Immunology Department in Stockholm. The objectives are to further pursue the project along these lines towards future vaccination trials.

This review focuses on different immunological parameters in relation to the development of natural protective immunity, especially in the holoendemic area.

## YEKEPA MALARIA RESEARCH UNIT

The Yekepa Malaria Research Unit was established in 1977 in Liberia. It represents a subunit of the Liberian Institute of Biomedical Research (LIBR). The unit was formed as a collaboration between the Liberian Institute and the Roslagstull Hospital of the Karolinska Institute. At present, Dr. A. P. Hanson is the director of the LIBR and Dr. A. Björkman is the principal investigator of the project. Two physicians and one entomologist are presently attached locally to the project. During the last years Stockholm University has increasingly participated in this project with the close collaboration of Professor Perlmann's malaria unit.

## THE STUDY AREA

Yekepa is situated in Nimba County, northern Liberia, close to the borders of Guinea and the Ivory Coast (Fig. 1). Yekepa town was built in the 1950s and the 1960s as the base for the iron ore mining company. The town now has about 15,000 inhabitants; a nearby suburb, Camp 4, has another 10,000 inhabitants. Within a radius of about 15 miles there are 10 villages with between 500 and 2,000 inhabitants each (Fig. 2). The main occupation is mountain rice farming.

The altitude at the foot of the Mount Nimba ridge is 500 m. The climate is tropical with a dry season from November to April and a rainy season from May to October. The relative humidity ranges from 55% in the dry season at the time of the desiccating Harmattan wind from the Sahara, to 95% in the wet season. The temperature is generally between 21 °C and 32 °C. The annual rainfall is about 1700 mm. The natural vegetation is rain forest. The villages are like isolated islands within this deep forest.

There is a main hospital in Yekepa town with rather modern Western-type facilities. The people from the suburb have access to a missionary clinic, but they also have partial access to the facilities of the Yekepa Hospital. Besides the services rendered by a mobile "under 5" clinic that operates in close collaboration with the Malaria Research Unit, there is only local traditional medical care in the villages.

## MALARIA EPIDEMIOLOGY

At the beginning of the research project, the study area was divided into four areas according to different ongoing antimalarial activities (Table

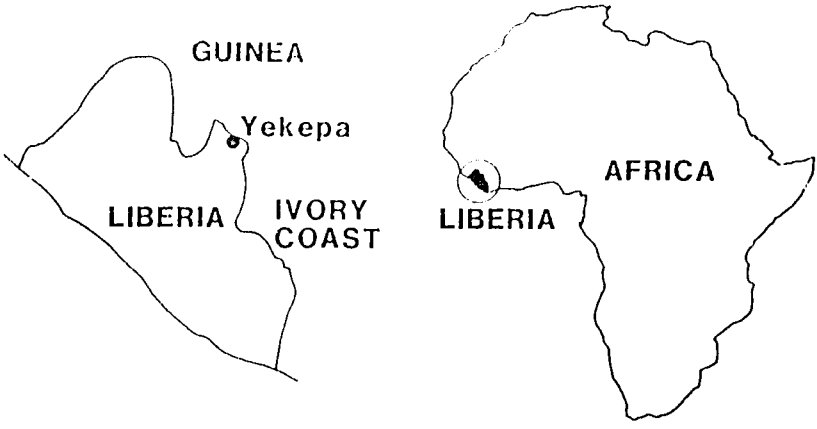


Figure 1. Localization of Yekepa.

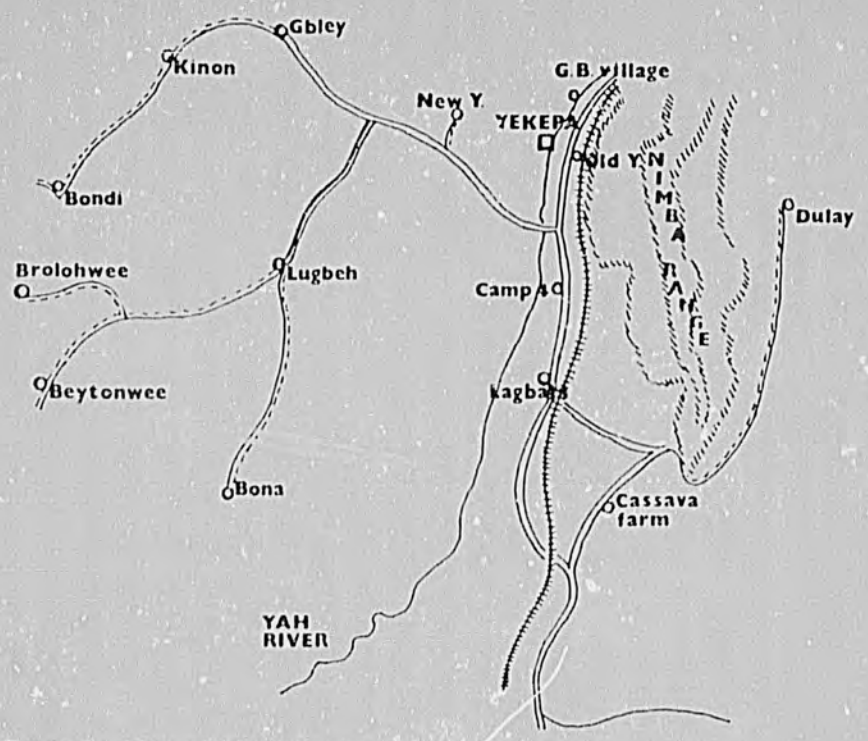


Figure 2. Map of Yekepa region.

1).[1,2] Different levels of endemicity malaria were found from hypoendemicity to holoendemicity (Table 2). In the outer holoendemic areas, malaria transmission was stable and perennial.

In 1977, malaria control with drugs was introduced in some of the holoendemic villages and the effects of different models of drug administration on clinical parameters of morbidity and immunity were studied in different age groups.[1,3,4] The susceptibility of *Plasmodium falciparum* to different drugs was also continuously related to the different modes of drug use.[1,3–9] Finally, one special field of research within the project has been the epidemiology of β-thalassemia trait, which was found to be highly prevalent in the Yekepa region.[10,11]

The studies on clinical epidemiology and drug resistance have provided valuable baseline information that will be useful for future vaccination trials.

## CLINICAL PROTECTIVE IMMUNITY TO MALARIA

The assessment of clinical protective immunity is based on two indirect or reverse parameters: morbidity and parasitemia.

Table 1. Antimalarial activities in the Yekepa region of Liberia.

| Locality | Vector Control | Availability of Chemotherapy* |
|---|---|---|
| Yekepa town | indoor spraying | + + + |
| Close area | water pond spraying | + + |
| Middle area | none | + |
| Far area | none | (+) |

*Chloroquine

Table 2. Epidemiologic indices in Liberian children (age,2–9 yr) from four adjacent areas with malaria control activities.

| Locality | Inoculation rate* | No. children test (n) | Parasite rate† (%) | Spleen rate (%) | Seropositivity rate (%) | Total IgG (g/L) |
|---|---|---|---|---|---|---|
| Yekepa | | 243 | 13 | 11 | 74 | 19 |
| Close area | 0.01 | 232 | 22 | 40 | 85 | 21 |
| Middle area | 0.06 | 195 | 68 | 95 | 99 | 30 |
| Far area | 0.17 | 378 | 92 | 99 | 99 | 33 |

* Rate, which was estimated in the early dry season, equals inoculations per individual per night.

†Prevalence of asexual parasites.

‡*Plasmodium falciparum* seropositivity measured by indirect immunofluorescence.

From age 2, the geometrical mean density of asexual parasites in the general population decreases with increasing age (Fig. 3). The spleen size reflects the variations of these parasite densities. This successive decline of parasite load with age is steady in the holoendemic area (the "Far Area"), whereas for Yekepa there is only little decrease of mean parasite density between the ages of 2 and 9. The "close" and "middle" areas show in-between patterns.[1]

The estimation of morbidity is more complex. The incidence of the combination of fever and high parasitemia (10,000 parasites per microliter of blood) was compared in the Far Area and Yekepa (Table 3). In the Far Area, fever and high parasitemia were largely confined to the first years of life. The children had fever with high parasitemia about 10% of the time (i.e., a total of 4 months during the first 4 years of life).[1] In Yekepa, fever and high parasitemia occurred in all ages and the age distribution of the clinical cases closely followed the age distribution of the population to 15 years of age. In Table 4, a general model is suggested for clinical expression of illness, mean parasitemia, and the corresponding level of protective immunity.

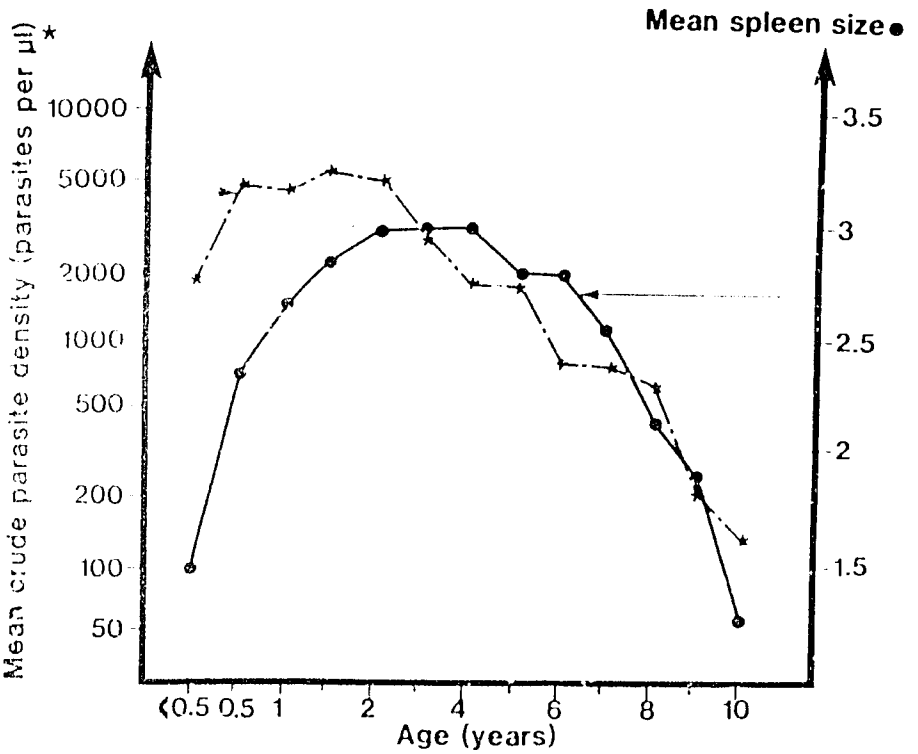


Figure 3. Geometrical mean parasite density (•-) and mean spleen size by age group in Liberian children (N 769), 1 10 years old, living in villages with holoendemic malaria in the Yekepa region (Far Area).

## IMMUNITY TO THE ASEXUAL BLOOD STAGE

Different antigens from the antigenically complex asexual blood stages have been assessed for their possible involvement in protective immunity. One *P. falciparum* antigen of molecular weight of 155,000 initially designed as Pf155, has been identified.[12] This antigen is deposited in the erythrocyte membrane during merozoite invasion. Pf155 was shown to be the same antigen as RESA (ring-infected erythrocyte surface antigen),[13] a *P. falciparum* antigen described in Australia.[14]

Different immunological parameters may be related to age and possibly to development of clinical protective immunity. Hence, sera from individuals living in the holoendemic area outside Yekepa have been examined in several investigations. The total IgG concentrations showed a steady increase up to 5 years of age.[1] A somewhat different pattern was obtained for the seroreactivity against crude *P. falciparum* schizont

Table 3. Age distribution of Liberian patients with recorded fever episode and concomitant high *Plasmodium falciparum* parasitemia ($10^{-1}$ parasites/$\mu$l) in Yekepa town with hypoendemic malaria and in villages with holoendemic malaria (Far Area).

| Age (yr) | Age distribution in Liberian population (%)* | Age distribution of patient (%) | |
|---|---|---|---|
| | | Yekepa Town | Far Area |
| 1 | 12 | 7 | 19 |
| 1–2 | 22 | 20 | 51 |
| 3–4 | 18 | 16 | 16 |
| 5–9 | 32 | 33 | 5 |
| 10–15 | 25 | 24 | 0 |

*Data from Jacobsen: *Public Health in Yekepa*. Internal report, 1971

Table 4. Standard relations between age, clinical illness, median parasitemia, and spleen size and suggested expression for corresponding protective immunity in a population living in a holoendemic area of Liberia (far area).

| Age (yr) | Clinical Expression of Illness | Median Parasitemia* | Median Spleen Size† | Suggested Expression for Immunity Level |
|---|---|---|---|---|
| 6/12–12/12 | high fatality risk | 4000 | no data | nonimmune |
| 1–2 | some fatality risk | 4000 | 3 | hypoimmune |
| 3–4 | severe symptoms | 2000 | 3 | semi-immune |
| 5–9 | light symptoms | 600 | 2 | semi-immune |
| 10–15 | mostly asymptomatic | 50 | 1 | hyperimmune |
| 15– | mostly asymptomatic | 20 | 1 | hyperimmune |

*Parasites per microliter

†According to Hackett's index

antigen as [illegible]ured by immunofluorescence technique.[14,15] In this instance, a plateau level was apparently reached by 2 to 3 years of age. In contrast, in a group of children 1–15 years old, the prevalence of seropositivity against Pf155 and its mean titer increased significantly and continuously with age.[15] However, substantial interindividual differences that remain unexplained were found within each age group. In the oldest age group there was a wide variation of the titers, although all had substantial clinical immunity.

To further study the individual differences of Pf155 seroreactivity, we investigated the parasitemia and seroreactivity of 28 adult men. The same individuals were examined on four different occasions over a period of 1.5 years. All men were asymptomatic at each survey, and the parasitemias were mostly low grade and showed only minor individual fluctuations despite constant reinfections. This confirms that the 28 men were all clinically hyperimmune to malaria infection. Interestingly, the antibody titers against Pf155 varied among the individuals but were rather constant

within the same subject on the different occasions. There appeared to be high, medium, and low responders.

The Pf155/RESA molecule has been sequenced and found to contain two regions of tandemly repeated sequences of amino acids. One sequence in the C terminus of the molecule consists mainly of an octapeptide subunit tandemly repeated four to five times. This is followed by about 30 to 40 repeats of the first half of this sequence, the tetrapeptide subunit (EENV).[14] Towards the N-terminus there is a second repeat region consisting of six imperfect subunits of 11 amino acids.[16]

To further investigate the degree of specificity of the individual patterns of seroreactivity, we investigated the antibodies to different repeated sequences of amino acids in the Pf155/RESA molecule. In a first study, different sera were examined for their reactivity against the octapeptide and the dimer tetrapeptide 2 × 4.[17] The sera were from 36 adult men and women who lived in the holoendemic area and who had anti-Pf155 titers over 1000. In 32 of 36 sera, specific antibodies were found binding to ELISA plates coated with the synthetic peptides. Many sera contained antibodies reacting with both peptides but 21 sera contained antibodies reacting mainly or exclusively with the dimer tetrapeptide while 11 sera contained antibodies reacting mainly with the octapeptide. These results have focused further interest on possible genetic reasons for the differences in seroreactivity against different defined peptides.

The different components of sera from individuals with different levels of immunity have been further studied with regard to their inhibitory effect on *P. falciparum* in vitro growth. Crude IgG from one hyperimmune donor (Kinon) strongly inhibited reinvasion in vitro of human erythrocytes by the parasite.[18] Interestingly, when different sera were tested, those with high anti-Pf155 titer usually showed better inhibition by crude IgG. Enrichment of anti-Pf155 by affinity purification on infected RBC strongly increased the inhibition. The purified anti-Pf155 was 40 times more efficient than the original IgG. After further purification, both antioctapeptide antibodies and antitetrapeptide antibodies were found to inhibit merozoite reinvasion, but the latter were more efficient.[19,20]

Protected T-cell responses in vitro in patients with acute *P. falciparum* malaria, but not in those who are clinically immune, are suppressed when the cells are exposed to crude parasite antigens.[21] To study the effect on regulation of defined *P. falciparum* antigens, we have set up analogous T/B-Cooperation Systems and lymphokine assays permitting assessment of both T-cell stimulation and T-cell dependent secretion of antimalarial antibodies in vitro. Lymphocytes were stimulated either with crude *P. falciparum* antigen or with a preparation highly enriched in Pf155. T-cells from Pf155 seropositive donors

responded normally to Pf155 and only weakly or not at all to the crude antigen. Responding T-cells induced analogous B-cells to secrete anti-*P. falciparum* antibodies including antibodies to Pf155 when the lymphocytes were derived from donors with elevated levels of serum antibodies to this antigen. Thus these data show that the intact Pf155 contains epitopes for the induction of T-helper cells.[22] To define these epitopes, we have as our major objectives today: (*a*)the mapping of Pf155 for T-cell reactive epitopes; (*b*)defining the oligopeptides that are most efficient in inducing T-cell response; and (*c*)comparing these cellular results with data on seroreactivity, clinical protective immunity, and previous exposure to malaria.[20]

## IMMUNITY TO THE SPOROZOITE STAGE

Studies on antisporozoite protection and/or seroreactivities to circumsporozoite antigen have now been included in the project together with in-depth entomologic studies. The first study was concerned with antibodies to *P. falciparum* sporozoites.[22] Sera were collected from infants, children, and adults in the holoendemic area outside Yekepa. The antibodies were detected by ELISA using the recombinant circumsporozoite protein $R_{32}tet_{32}$ and by the inhibition of sporozoite invasion (ISI) of hepatoma cells.[23] Both assays showed significant correlation and demonstrated that antisporozoite antibodies increase with age and protective immunity. ISI was more sensitive than ELISA. Hence, in all adults there was complete ISI (90% inhibition).

### ACKNOWLEDGMENT

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## DISCUSSION

N'JELESSANI: Thank you very much for that thought-provoking presentation. I am sure there will be a lot of questions and comments when we come to the discussions which will be in the next session. We will now adjourn for a 15-minute coffee break . . .

BUCK: Our next presentation this morning is about Zambia. Our chairman, Dr. N'jelessani, was the director of the Ndola Center at the time these studies have been carried out. Dr. Fred Wurapa who is now in charge of parasitic disease control at the Regional Office for Africa of the World Health Organization was at that time the senior investigator of the epidemiologic research program at the center. He was the team leader of

the studies that will be discussed here today. The presentation will be made by the new director of the Research Center for Tropical Diseases, Dr. D.M. Mukunyandela.

# Association of Malaria and Schistosomiasis in Northern Zambia[a]

M. MUKUNYANDELA

Malaria continues to be a major problem in Zambia today. It accounts for up to 20% of all hospital admissions and 30–50% of the hospital outpatients clinical workload, making it the largest single cause of morbidity in the country.

Since the late 1970s the Tropical Diseases Research Center (TDRC) in Ndola, and more recently the Malaria Research Laboratories in Lusaka, have carried out epidemiologic and pharmacologic studies in malaria.

Recent studies suggest a changing epidemiological pattern in relation to prevalence, endemicity, and use of antimalarial drugs in which multiple resistance of *Plasmodium falciparum* has been shown to be on the increase.

This study was part of the multidisciplinary longitudinal epidemiological study in Kampumbu (Fig. 1). The study sample of 1341 individuals living in eight villages was selected from a study population of 10,000. The population pyramid of the sample population is shown in Figure 2.

A thick blood smear for malaria parasitology was obtained and 100 oil-immersion fields were analyzed for 917 persons. Stool samples were collected from 722 individuals and examined for *S. mansoni*. Results for both malaria and *S. mansoni* were obtained on 690 individuals.

Thick blood films were prepared and examined using standard methods. Stool samples were examined by the formol-ether concentration method in the field and also by the same method used on preserved samples (MIF) at the base laboratory in Ndola. The maximum number of *S. mansoni* recorded was coded for statistical analysis.

## MALARIA

The prevalence rates for malaria parasitemia (any species) are shown by age and sex in Table 1, and by species according to age in Figure 3. The

[a]Based on a study by F.K. Wurapa et al. WHO TDRC Technical Report. Geneva. World Health Organization, 1985, chap 6, pp 43–84.

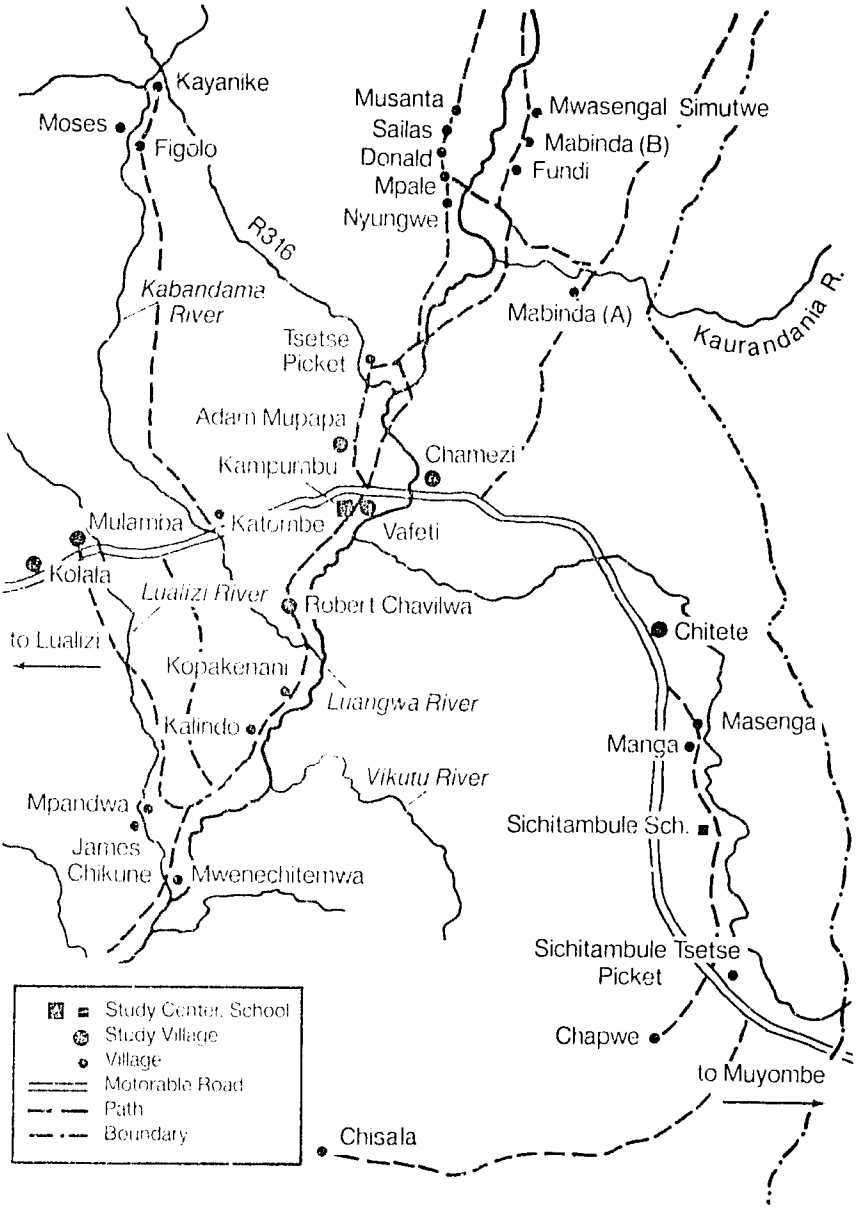


Figure 1. Villages in the study area of Kampumbu, Zambia.

prevalence was greater in those under 15 years of age than in adults. The prevalence of each of the three species reached its peak in children aged 1–4 years and declined with increasing age.

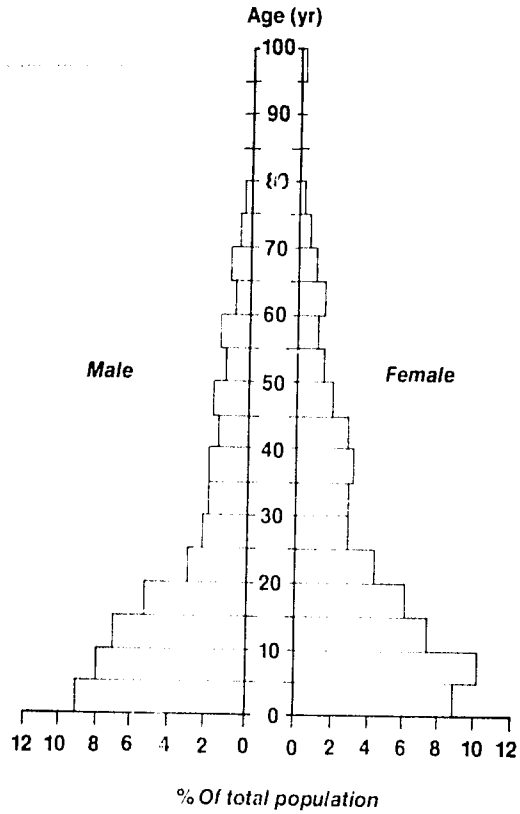


Figure 2. Population pyramid of the study populations of Kampumbu.

Since three malaria species were found in the population, i.e., *P. falciparum*, *Plasmodium malariae*, and *Plasmodium ovale*, it was possible to examine the frequency of multiple infections in individuals (Fig. 3). As the male and female prevalence rates in each age group were similar for each of the three species of malaria parasites, the two sexes were combined for subsequent analysis. Table 2 shows the prevalence of infection with *P. malariae* and *P. ovale* among those infected and not infected with *P. falciparum*. Of 124 children under 5 years of age infected with *P. falciparum*, 60 (48%) were also infected with *P. malariae*. Of the 52 children not infected with *P. falciparum*, none was infected with *P. malariae*. The number of *P. ovale* infections was lower than that of *P. malariae*, but individuals infected with *P. falciparum* were more likely to be infected with *P. ovale* than those not infected with *P. falciparum*.

Sixty-four percent (322/503) of those with *P. falciparum* had all 100 fields positive, whereas only 9% (16/180) of those infected with *P.*

Table 1. Prevalence of malaria parasitemia (any species) in Kampumbu by age and sex.

| | Male | | | Female | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| Age | No. examined | No pos. | % | No. examined | No pos. | % | No. examined | No pos. | % |
| 0 1 | 21 | 8 | 31.8 | 23 | 17 | 73.8 | 44 | 25 | 56.8 |
| 1 4 | 62 | 50 | 80.6 | 70 | 51 | 72.9 | 132 | 101 | 76.5 |
| 5 9 | 75 | 50 | 66.7 | 108 | 85 | 78.7 | 183 | 135 | 73.8 |
| 10 14 | 67 | 56 | 83.6 | 61 | 36 | 59.0 | 128 | 92 | 71.9 |
| 15 19 | 37 | 16 | 43.2 | 43 | 20 | 46.5 | 80 | 36 | 45.0 |
| 20 39 | 68 | 29 | 42.6 | 125 | 59 | 47.2 | 193 | 88 | 45.6 |
| 40 59 | 46 | 17 | 37.0 | 67 | 24 | 35.8 | 113 | 41 | 36.3 |
| 60+ | 22 | 5 | 22.7 | 22 | 8 | 36.4 | 44 | 13 | 29.5 |
| Total | 398 | 231 | 58.0 | 519 | 300 | 57.8 | 917 | 531 | 57.9 |

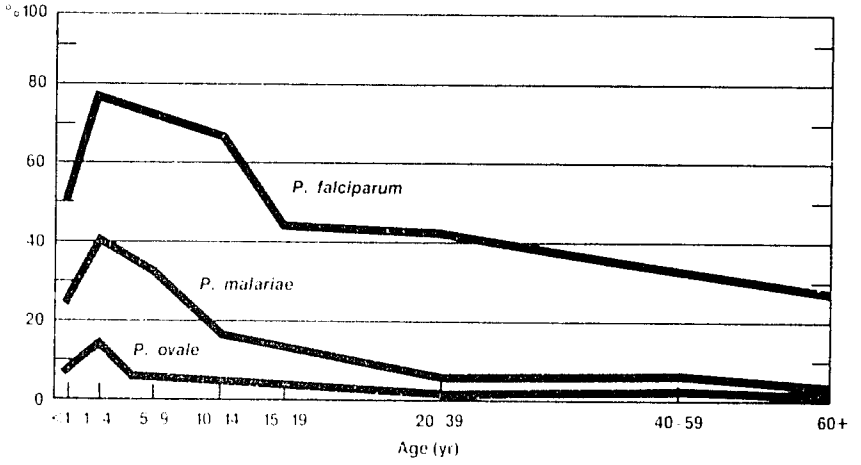


Figure 3. Prevalence of malaria parasitemia in Kampumbu by species and age

*malariae* and 15% (6/41) of those infected with *P. ovale* had 100 fields positive for these parasites. *P. falciparum* was therefore the most intense as well as the most prevalent infection found. There was a trend of decreasing intensity with increasing age for *P. falciparum*. No such trend was apparent for infections with *P. malariae* and *P. ovale*. An analysis of multiple malaria species infections and splenomegaly also revealed that individuals with multiple species infections showed significantly higher prevalence of marked splenomegaly (Hackett's classification of 3 and above) compared with those infected with only one species or with those who were not infected at all.

Table 2. Kampumbu. Prevalence of infection with *P. malariae* and *P. ovale* among those infected and not infected with *P. falciparum*.

| | Pf negative | | | | | Pf positive | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Age | Total | Pm+ | % | Po+ | % | Total | Pm+ | % | Po+ | % |
| 0–1 | 21 | 0 | 0.0 | 2 | 2.9 | 23 | 7 | 30.4 | 1 | 4.3 |
| 1–4 | 31 | 0 | 0.0 | 0 | 0.0 | 101 | 53 | 52.5* | 17 | 16.8* |
| 5–9 | 51 | 3 | 5.9 | 0 | 0.0 | 132 | 55 | 41.7* | 14 | 10.6* |
| 10–14 | 38 | 2 | 5.3 | 0 | 0.0 | 93 | 22 | 42.4* | 4 | 4.4 |
| 15–19 | 45 | 1 | 2.2 | 0 | 0.0 | 35 | 11 | 31.4* | 1 | 2.9 |
| 20–39 | 109 | 4 | 3.7 | 0 | 0.0 | 84 | 12 | 14.3 | 0 | 0.0 |
| 40–59 | 75 | 3 | 4.0 | 0 | 0.0 | 38 | 6 | 15.8 | 2 | 5.3 |
| 60+ | 31 | 0 | 0.0 | 0 | 0.0 | 13 | 1 | 7.7 | 0 | 0.0 |
| Total | 401 | 13 | 3.2 | 2 | 0.5 | 516 | 167 | 32.4* | 39 | 7.6* |

*P < 0.001 Comparing Pf+ with Pf−.
*P < 0.05 Comparing Pf+ with Pf−.
Pf = *P. falciparum*
Pm = *P. malariae*
Po = *P. ovale*

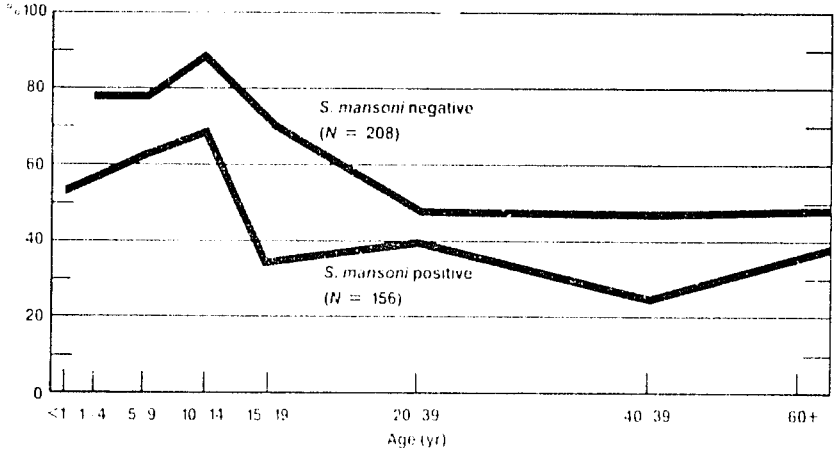


Figure 4. Prevalence of *P. falciparum* infection in Kampumbu by age and infection status with *S. mansoni*.

## ASSOCIATION BETWEEN MALARIA AND *S. MANSONI* INFECTIONS

Figure 4 shows the prevalence of *P. falciparum* parasitemia in those infected and not infected with *S. mansoni*. There are striking differences in the parasitemia rates between the two groups, that are statistically significant at ages 5–9, 10–14, and 40–59 years. The overall difference between the two curves is highly significant: chi$^2$ (Mantel-Haenszel) =

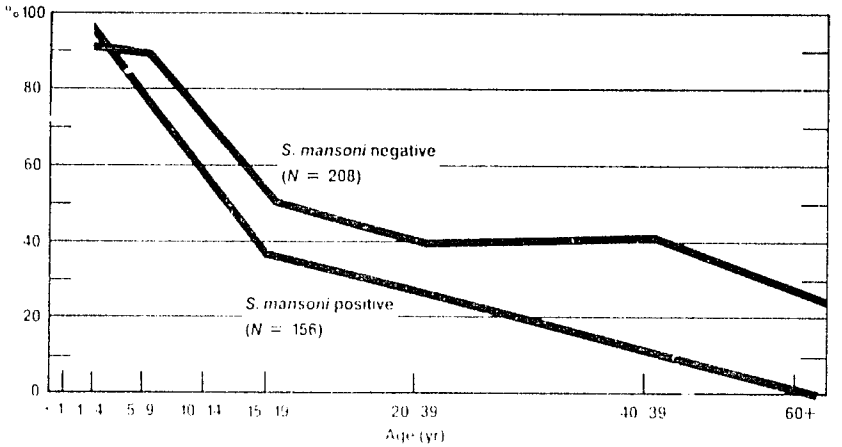


Figure 5. Percent of *P. falciparum* infected individuals in Kampumbu, with 50 or more blood fields positive, by age and infection status with *S. mansoni*.

21.97; P < 0.001. Furthermore, the evidence suggests that those with malaria parasitemia have less intense infections if they are concurrently infected with *S. mansoni* (Fig. 5). The difference in the percentage of persons having 50 or more positive fields for *P. falciparum*, between those with and those without *S. mansoni* infection, is statistically significant: chi$^2$ (1 DF; Mantel Haenszel) = 7.57; P = 0.01.

There was considerable variation in prevalence of both malaria and schistosomiasis amongst the villages: crude malaria prevalence rates ranged from 47% to 74% and *S. mansoni* rates from 22% to 63%. After direct age standardization an inverse correlation was seen between the *P. falciparum* and *S. mansoni* prevalence by village (Spearman Rank Test, $r_s$ = 0.798).

## COMMENT

The greater likelihood of individuals with *P. falciparum* parasitemia being infected with another malaria species had previously been reported from the Garki Project (Molineaux and Gramaccia, 1980), but the relative risk found in the Kampumbu study was much larger. This could possibly be explained by a differential exposure, but the additional finding that individuals with multiple malaria infections had significantly greater splenomegaly than those with single infections suggests that an immune host factor may be of importance.

The finding of an inverse relationship between *S. mansoni* infection and malaria parasitemia has not been previously reported, and studies are

continuing to confirm it. The finding that *P. falciparum* infection in the presence of *S. mansoni* is associated with lower malaria parasite densities than in the absence of schistosomiasis suggests that this is a true relationship possibly involving a host factor. The inverse correlation in the prevalence of the two infections among the different villages is consistent with differential exposure but also with the possibility that *S. mansoni* infection may suppress malaria parasitemia. *S. mansoni* infection may enhance the reticuloendothelial system and cause a more rapid clearance of infected red blood cells. Further studies to confirm, clarify, and extend these findings are underway.

# General Discussion

BECK: I now open the floor for the discussions of the papers. I recognize Professor Bruce-Chwatt.

BRUCE-CHWATT: I would like to make a few points about previous presentations and would like to start with the Gambia. Dr. Greenwood mentioned direct and indirect mortality. Perhaps he could have stressed that indirect mortality is due not only to malaria infection itself, but also to the fact that malaria infection of the placenta of many Nigerian women leads to birth weight of newborns that is below the normal level. This means that a large proportion of the infants are already born with a handicap. Neonatal mortality is very much increased by this handicap. Because of the tremendous amount of background information on malaria I feel the Gambia is one of the countries where clinical and field trials of new malaria vaccines could be done with a great degree of reliability.

A point about Liberia. I was surprised that Dr. Björkmann referred to the infants of Liberia as "nonimmune". Infants have a large degree of passively transferred transplacental immunity until the age of about 6 to 9 months. This is an extraordinary situation because it shows that nature has produced a biological shield which lasts for only 6 months. Thereafter, between the age of 1 to 4 years there is the highest mortality due to malaria and other diseases.

The report from Zambia is very interesting and rather worrying because of the observation of quinine resistance. But here I would like to call on somebody from WHO to find out whether in vitro tests for resistance to quinine have already been established as a standard procedure.

Finally, I was very much interested in Dr. Molineaux's presentation of the problems of testing immunity that has resulted from malaria vaccines. Altogether I think that we are in a very peculiar situation that can be summarized at the present time with the well known quotation: "If we had some fish, we could have fish and chips, if we had some chips".

BECK: Thank you very much, Professor Bruce-Chwatt. I think we should have some more comments before we start responding to the interesting points raised by Professor Bruce-Chwatt. Professor Kilama, please.

KILAMA: Thank you Mr. Chairman. I have just two comments. The first concerns the report from the Gambia where one is given the impression

that this country has been going through a period of "drying up". Yet when one looks at the percentage of mosquitos that are found of the three carrier types that Professor Coluzzi described yesterday, there is this cluster of *Anopheles arabiensis*. I would like to request that the Gambians explain to us how this is coming about and whether they think that in the near future this species might actually become more predominant than it is now.

My second comment concerns the presentation from Liberia. I got a bit worried when I saw that they had an area they call the "Far Area" where people are left untreated. I would like to know whether there is any ethical consideration to have left this area unattended, perhaps for the sake of the study. I request some explanation.

Lastly, a comment on the observations that in Ethiopia and in the Sudan: *Plasmodium vivax* has become the next common parasite after *P. falciparum*. Is there an explanation?

DIALLO: In Senegal splenomegaly has dropped following widespread use of chloroquine, but parasite prevalence has remained high. What age groups in the Gambia were considered for the collection of data on splenomegaly? Again, in urban areas which age groups were studied in the report of splenomegaly? In endemic areas fever may be caused by other agents than malaria, yet malaria parasites are always present in the blood. What were the criteria for defining a malaria case?

IMBOUA-BOGUI: My question is similar to that of Dr. Diallo. In the Côte d'Ivoire we continue to define a clinical case of malaria. Yesterday Dr. Breman asked about the local definition of malaria, but the question has not been answered. I also reject the statement made by Dr. Björkmann that newborns are nonimmune. They are partially immune because they have received passively transferred antibodies from their mothers.

N'JELESSANI: Thank for your comments. There is time for one more question.

MOLINEAUX: I would like to make two brief comments, one on drug resistance and the second on the species problem. It was very interesting to hear what has happened to drug resistance in Ethiopia and the Sudan. If I look at it from Ethiopia it seems to be coming from the Sudan. But if I look at it from the Sudan, it seems to be coming from Ethiopia into my country. Many things make border areas different. In these areas the parasite population may be much larger making it more likely for drug resistance to develop. However, this does not necessarily mean from invasion of resistant strains.

My second comment concerns the species problem of plasmodia. One day I was sitting next to Dr. Louis Miller in a meeting, which gave me a great chance to ask him a few questions. After all he proved why *P.*

*vivax* is not in certain population groups. I asked him why he thought *P. ovale* seems to have occupied the territory that *P. vivax* can not occupy. His hypothesis is that *P. vivax* suppresses *P. ovale*. In other words, it is a competitive exclusion. There would be chances in Ethiopia of exploring this phenomenon on an individual basis and to investigate the relationship of these two species of plasmodium.

N'JELESSANI: Thank you very much, Dr. Molineaux. May I now ask Dr. Greenwood to respond to some of the comments.

GREENWOOD: Dr. Bruce-Chwatt mentioned the indirect effect of malaria. Rather to our surprise, in our recent studies it has been quite difficult to show that malaria plays any part in either maternal or neonatal mortality. Dr. Kilama asked about *A. arabiensis* but this is difficult for me to answer. There is one thing peculiar about the weather changes. Although the total rainfall has decreased it has affected particularly the month of August but not the entire period of the rainy season. The period of the rainy season has not become shorter, only the heavy rain in August has decreased. Therefore, the effect on mosquito behavior has not been as pronounced.

With regard to splenomegaly we are not sure what is the proportion of acute episodes of illness with low levels of parasitemia that cause splenomegaly. It is our impression that only chemoprophylaxis can reduce the spleen rate in the population. This is an area for further research.

It is also difficult to determine what percent of febrile episodes are malaria. Previous work has suggested that parasite densities of greater than or equal to 5000/mm$^3$ are related to increased body temperature. It looks now that this level may be lower. It is important to look at other factors that play a role in the mechanism of fever. That is one of the questions we are studying in the Gambia now.

N'JELESSANI: Thank you very much. Would Dr. Björkmann like to comment on the issues raised by Drs. Kilama and Bruce-Chwatt?

BJÖRKMAN: I am sorry that I have not expressed myself clearly. I wanted to say that nonimmunity of infants begins at 6 months of age. In reply to Professor Kilama's comments I should like to mention that we have treated the population in the "Far Area" of our project. What I wanted to say in my presentation was that the area in question had no health infrastructure at the beginning of our study.

N'JELESSANI: Would Dr. Mukunyandela or Dr. Salako care to comment on the question of quinine resistance?

MUKUNYANDELLA: Quinine in vitro tests were carried out in Zambia in 1985 and 1986 using tests that were acceptable to WHO.

SALAKO: A precoded plate for measuring quinine resistance is now available through WHO.

N'JELESSANI: Next on my list are questions to Ethiopia and the Sudan.

TEKLEHAIMANOT: With regard to the question of malaria outbreaks in famine areas there appeared to be more outbreaks following the drought of 1983–1984 but a complete analysis of all data has not yet be done. In some areas *P. vivax* has become more frequent and may even be more prevalent than *P. falciparum*. I have not seen *P. ovale*, but it is there in very small foci. It looks very unlikely that there is sufficient drug pressure to induce the emergence of chloroquine resistance. Chemoprophylaxis is not used but presumptive treatment of fever cases is practiced in all areas without sufficient diagnostic facilities.

EL GADDAL: The prevalence of *P. vivax* is 20% in the areas of the Sudan bordering Ethiopia due to refugees entering the region.

N'JELESSANI: Thank you. I think this is the right moment for a break. I would like to thank you, ladies and gentlemen for your contributions this morning, particularly the speakers and those who have provided useful comments during our discussions. I am sure that we shall have more opportunities to continue our discussions during the workshops tomorrow. We meet again at 2 o'clock this afternoon.

## AFTERNOON SESSION

DIALLO: We are going to open this afternoon's session. The meeting is called to order. Let us continue our overview of the malaria situation in Africa. As the previous chairmen have done , I would like to begin this session by thanking the organizers for having invited me. I would even go further and say as the dean of the delegates from French-speaking Africa, since I am the oldest among them, I would like to speak on their behalf and thank the organizers for their kind invitation. We hope that next time there will be more countries represented in meetings such as this one. Having said that I would like to offer the floor now to my colleagues from Burkina Faso, Drs. Lamizana and Sabatinelli first and Dr.Lamizana and Dr. Esposito thereafter.

# Malaria in Ouagadougou, Burkina Faso[a]

G. SABATINELLI AND L. LAMIZANA

After the serious setbacks of the global malaria eradication campaign in the tropics (WHO 1969), the World Health Organization (WHO) has encouraged alternative strategies of malaria control at the primary health care level. These stategies are based on community participation and are supported by applied field research projects aimed at improvements of malaria control under the local conditions (WHO 1983). Vector control is part of these new strategies. In the past, vector control was based on antilarval measures combined with the application of residual insecticides (Hamon et al. 1959). These measures have been ineffective in interrupting the transmission of malaria in large areas of Africa, especially in rural zones of the savanna. By contrast, in urban areas the combination of antilarval measures, chemical and biological vector control, and environmental improvements through sanitary engineering can have a considerable impact on the level of malaria transmission. In Ougadougou, about 70,000 new cases of malaria are reported to the Ministry of Health annually.

Considering the large-scale migration of the African population from rural areas to the large cities that has taken place recently, about 20% of the African people are now residing in an urban milieu. For this reason, the development of new malaria control strategies geared to urban malaria has become an urgent task. With this perspective, the Malaria Control Program for the city of Ouagadougou was conceived as a cooperative endeavor between the Department of Cooperation and Development of the Ministry of Foreign Affairs of Italy and the Ministry of Health of Burkina Faso. A longitudinal study of the epidemiology of malaria, combining entomological and parasitological parameters, was started in 1984 as the first phase of the Malaria Control Program in Ougadougou.

The study has three major objectives:

1. To identify the distribution of larval breeding sites for the malaria vectors throughout the city;

[a]Translated from French.

2. To determine the level of malaria transmission in the urban zone in the different seasons of the year;
3. To measure the prevalence of infection in different parts of the city during the high transmission season.

## STUDY ZONE

### Geography

Ouagadougou, the capital of Burkina Faso and administrative seat of the Province of Kadiogo, is situated at 12°22′ north latitude and 1°21′ west longitude. The city occupies a central position of the Mossi plateau. The predominant type of vegetation in the periurban area is that of wooded savanna. A protected forest of about 150 hectares in size, called the "Bois de Boulogne" lies adjacent to the northeastern border of the town.

### Climate

The climate is of the "North Sudan" type, with maximum temperatures around 40° C during the dry and hot season (April), and a minimum of about 15° C in the dry and cool season (January). The average amount of rainfall between 1961–1970 was 886 mm/yr. The total rainfall in 1984 was deficient.

### Urban Settlement

For the last 20 years the city has developed around a center of traditional quarters and dwellings. The adminstrative plan divides the city into 30 sectors, including 56 of the old "quartiers". Around the central zone with the commercial and administrative centers are new residential areas with well-defined plots, a viable infrastructure, including provision of electric power and piped water supply. This new type of settlement represents less than 40% of the total surface area of the city, which measures 18,000 hectares. The remainder is made up of relatively simple habitations established by spontaneous settlement of new residents of modest economic means. Buildings are usually of the traditional type, i.e., made of *banco*, locally made sun-dried bricks of clay. In the zoned settlement area, one finds houses with several rooms built with brick and cement on well-defined lots. A traditional residence is defined by its "concession", which is either a brick wall or pallisade surrounding one or more simple dwellings, according to the importance of the family.

A particular feature of Ouagadougou is the presence of three water-retention dams in the northern part of the city that extend from west to

east. They are linked together by a system of sluices. Of the three water impoundments, only the third forms a reservoir that is well developed with its water made available to the public for consumption. There are four canals; two of them are finished and well maintained. They serve as the major storm and waste water drains, run through the city, and empty their water into retention area No. 2, which forms a large reservoir at the edge of the Bois de Boulogne. Ouagadougou has 450,000 inhabitants, of whom one-third are living in properly zoned areas with access to public utilities. The resident population of Ouagadougou grows by about 10% every year.

The ethnic group of the Mossi forms the largest segment of the population (40%), but almost all of the other ethnic groups of the country are represented fairly eveniy in all of the city sectors, with the exception of sectors 10 and 23, where relatively large groups of Peul herdsmen have settled together with their livestock.

## METHODS

To reach our objectives of measuring the level of transmission and to determining the prevalence of malaria in the city, we created a surveillance system in five ecologically different sectors that serve as representative sample areas of Ouagadougou (Fig.1). The following sectors were selected:

Sector 14 (Saint Camille), located in the southeastern part of the city. It is a zoned area traversed by the nonimproved canal of Zogona.

Sector 24 (Nongremasem), situated to the north of water impoundment No.3. It is a traditional area of settlement with many small creeks in the rainy season.

Sector 11 (Kologh-Naba), a zoned district located to the south of water impoundment No. 2.

Sector 9 (Gounghin Nord), a zoned district located in the northwest.

Sector 1 (Saint Leon), the centrally located, zoned area of the city.

## RESULTS

During 1984, we identified more than 200 important breeding sites of *Anopheles gambiae* s.l. in the city. In the urban setting, the larval breeding sites are very heterogenous. One can distinguish between temporary and semipermanent places. The former are created by small puddles of stagnant, muddy water exposed to the sun. These are found in large numbers during the rainy season all over the city. These puddles are

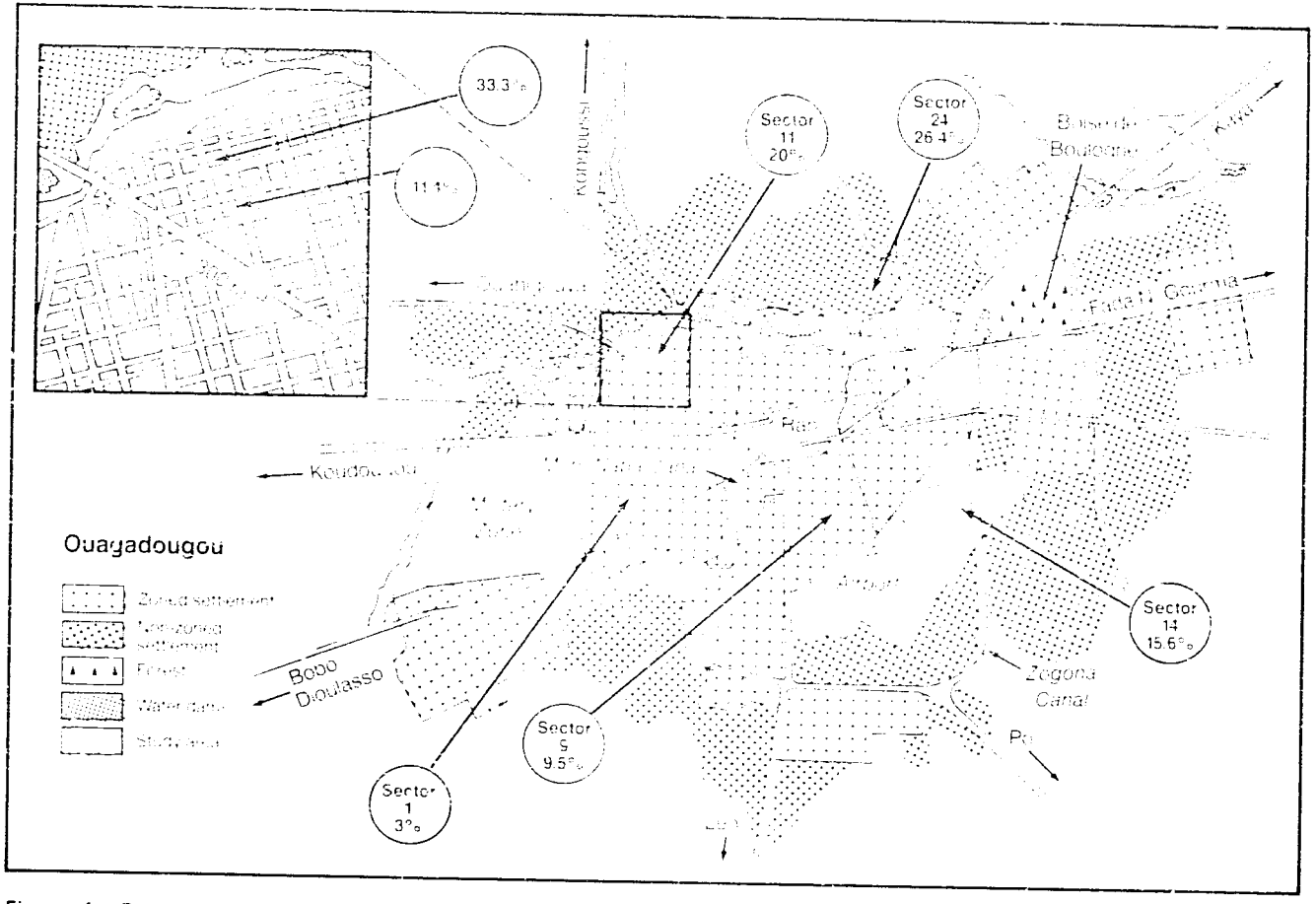


Figure 1. Prevalence of malaria in different sectors of Ouagadougou in children, ages 0–5 years, during August 1984.

predilection sites for *A. gambiae* breeding, and are practically the only places where larval development takes place in the zoned areas of Ougadougou. In the traditional settlement areas, these sites are even more numerous, where they can be found in many important locations. Such is the case of the unfinished canal of Zogona, which crosses the eastern parts of Ouagadougou from south to north for about 4 km. After each rain, the uneven canal bed has an array of small pools of stagnant water that become progressively smaller through evaporation in the dry season.

Water impoundment No. 2, which is also unfinished, represents an important semipermanent breeding place for *A. gambiae*, with considerable seasonal variations in its dimension. During the rainy season it rapidly fills with water, which forms pools along the banks in close vicinity to the first houses. During the dry season a major part of the lake dries out, leaving numerous puddles at the bottom in which larval breeding continues unabated.

Another important area for mosquito breeding is found around the northern edge of reservoir No. 3, where the water from the northern part of the city collects.

In areas where clay is dug out for the production of banco, special problems have been created at the periphery of the city, where new quartiers are springing up in rapid succession. The massive immigration of rural people into the capital has greatly increased the demand for banco bricks. In areas where clay is dug out almost uninterruptedly throughout the year, many new man- made breeding sites for malaria vectors are created.

Wells, once considered as important places for *Anopheles* breeding (Le Gac et al. 1945), are of relatively little importance for malaria transmission in the city according to our findings. Of 300 wells examined only 10 were found to contain *Anopheles* larvae.

At times storm drains and roadside gutters may also play a significant role in the development of *A. gambiae* s.l., but only when their water is not polluted too much.

## TRANSMISSION

To evaluate the density of the vectors resting indoors in the different sectors of the study, we performed pyrethrin spraying collections in the houses. These collections were made from March to December between 8 AM and 10 AM in five selected houses of each of the sample sectors.

The total yield of these pyrethrin spray collections was 2756 females of *A. gambiae* s.l. and 46 of *A. funestus*. A cyotaxonomic study carried out in August of the same year (Petrarca et al. 1985) revealed that the *A. gambiae*

complex was composed of a mixture of *A. arabiensis* (46.5%) and *A. gambiae* s.str. Of the latter, 77.5% belonged to the "mopti" form, 19.4% to the savanna form, and the remainder, 3.1%, were hybrids of the two.

Table 1 shows the average number of the collected females of *A. gambiae* s.l. per room. The collections in the city were made twice per month during the rainy season. These collections are indicated as follows: July 1, July 2, August 1, August 2, September 1, and September 2. In sectors 9 and 1 we encountered very low vector densities during the study period with a maximum of 4–5 females per room during the September catches. In sectors 11 and 24 there was an appreciable vector density already between March and April, which increased to a maximum of 52 in July (sector 24), followed by a decline with low values of 6.6 and 1.2 per room, respectively, which were reached in December. In sector 14 the vector density remained low until May (1.6), when it began to rise to a maximum of 22.8 females per room in September, followed by a rapid decline to December.

For studies of the sporozoites we have dissected more than 2000 females of *A. gambiae* and 46 of *A. funestus*. In sectors 9 and 11 the sporozoite index always remained zero throughout the period of the study. In sectors 11 and 24 the sporozoite index became positive at the begining of the rains (1.5% and 1.3% respectively), but no mosquitoes were found infected in sector 14 until August to October (4.2% sector 14, 11.5% sector 11, 5.1% sector 24). With the onset of the dry season the indices fell to zero, with the exception of sector 24, where this level was not reached before December.

By multiplying the sporozoite index by the observed number of infected *A. gambiae* bites, one can calculate the annual inoculation rate ($h_e$) as follows: sector 1, $h_e = 0.32$, or one infective mosquito bite in 3 years; sector 9, $h_e = 0.95$, or about one infective bite per year; sectors 11, 14, and 24, 9 to 12 infective bites per year.

## PREVALENCE

The study involved 1267 children aged 0–5. Because of their limited radius of movement, they represent a good sentinel population for the determination of the prevalence of malaria in the sectors selected for the study. The longitudinal entomological investigations were carried out in the same sectors. The examination of the children took place from August 28 to September 13, which is the period of maximal transmission. Blood was taken from each child for preparation of thick and thin smears, which were examined for malaria parasitemia.

The overall result of these examinations yielded a plasmodium index of 14.8% in the urban area. In the semirural border area of the city the

**Table 1.** Mean indoor resting density of *Anopheles gambiae* s.l. in houses of selected sectors of Ouagadougou, March-December 1984*

| Sector | Mar | Apr | May | June | Jul 1 | Jul 2 | Aug 1 | Aug 2 | Sep 1 | Sep 2 | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | — | — | — | 2.4 | 0.7 | 0.4 | 1.0 | 0.6 | 0.6 | 0.6 | 0.0 | 1.0 | 0.0 |
| 9 | 0.0 | 0.5 | 0.2 | 1.8 | 1.2 | 1.4 | 3.4 | 2.2 | 0.4 | 5.4 | 0.4 | 0.0 | 0.0 |
| 11 | 3.0 | 17.2 | 14.0 | 38.2 | 11.4 | 30.2 | 16.0 | 21.4 | 7.4 | 22.2 | 5.0 | 0.8 | 1.2 |
| 14 | 0.0 | 0.2 | 1.6 | 14.4 | 5.6 | 13.4 | 14.4 | 10.2 | 17.8 | 22.8 | 5.6 | 6.2 | 0.2 |
| 24 | 3.3 | 5.4 | 9.6 | 16.4 | 52.0 | 24.8 | 16.8 | 37.2 | 21.6 | 24.0 | 7.8 | 22.6 | 6.6 |

**Table 2.** Plasmodium index by age and species in different sectors of Ouagadougou, August 1984

| | 0–11 Mos | | 11–23 Mos | | 2–5 Yr | | 0–5 Year | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sector | N | $IP_{tot}$% | N | $IP_{tot}$% | N | $IP_{tot}$% | N | $IP_{tot}$% | Pf% | IGf% | Pm% | Po% |
| 1 | 25 | 0.0 | 32 | 6.2 | 171 | 2.9 | 228 | 3.0 | 3.0 | 0.8 | 0.4 | 0.0 |
| 9 | 44 | 2.3 | 49 | 6.1 | 212 | 11.8 | 305 | 9.5 | 9.1 | 0.9 | 0.6 | 0.3 |
| 11 | 36 | 8.3 | 36 | 16.6 | 203 | 22.1 | 275 | 20.0 | 20.0 | 3.6 | 1.4 | 0.3 |
| 14 | 15 | 6.7 | 46 | 4.3 | 157 | 19.7 | 218 | 15.6 | 15.6 | 2.7 | 0.0 | 0.0 |
| 24 | 36 | 13.8 | 40 | 17.5 | 162 | 31.5 | 238 | 26.4 | 26.4 | 7.9 | 0.0 | 0.0 |

N = number examined
$IP_{tot}$ = total parasite index
Pf = *Plasmodium falciparum*
$IP_{tot}$ 0–5 years: X2 = 46.88
IGf = Gametocyte index for *P. falciparum*
Pm = *Plasmodium malariae*
Po = *Plasmodium ovale*

index reached the high level of 62% (Sabatinelli et al. 1985). The parasite formula was *P. falciparum* 14.7%, *P. malariae* 0.5%, *P. ovale* 0.2%.

The gametocyte index was 3.2% (40 positive cases). *P. falciparum* was present in 99.5% of all infections. *P. malariae*, responsible for some cases, was almost always observed as a double infection in combination with *P. falciparum*.

As shown in Table 2, in the age group of children under the age of 5, the plasmodium index increased significantly with age. The study revealed important differences in prevalence between the individual sectors of the city. We even found significant differences in the plasmodium rates over only short distances within the sector. We studied in greater detail the frequency of parasitemia for subgroups of children residing in sector 11 by the distance of their residences from the dam of the water retention area No. 2, which serves as an important breeding site for *A. gambiae*. There is a statistically significant difference in the prevalence of malaria parasitemia by the distance away from the dam: 33% of the children living at a distance of 100–300 m were found infected as compared to only 11.4% for those whose residence was between 400–1050 m away from the water impoundment (Fig. 1).

## CONCLUSIONS

The entomological data of this study have identified *A. gambiae* and *A. arabiensis* as the only malaria vectors in Ougadougou. The results of the entomological study have also shown that there are large differences in the infection rates among the various sectors of the city, in addition to the seasonal variations common to all of them. The intersectorial differences reflect variations in transmission levels. These are the results of differences in the nature and size of the vector breeding places in each sector. We found perfect geographical concordance between sectors with high malaria transmission and existence of permanent and semipermanent vector breeding sites.

On the basis of these results, we can now stratify the malaria situation in Ouagadougou on the basis of the epidemiologic evidence into three major transmission zones as follows:

1. Zone of low transmssion ($h_e$ = 0.32–.95) and prevalence ($IP_{tot}$ = 3–9.5%), which includes the zoned sectors of the city center, far away from the large water impoundments, where the availability of piped water supplies and properly maintained storm and waste water systems limits the number and duration of vector breeding sites.
2. Zone of intermediate transmission ($h_e$ = 7.1) and prevalence ($IP_{tot}$ = 15.6 %), formed by sectors without zoning plans and traversed

byopen, poorly maintained canals with numerous temporary breeding sites favoring high anopheline densities and malaria transmission for at least 3 months (July to September).

3. Zone of intensive transmission ($h_e$ = 9.13–12.17) for at least 6 months of the year and with high prevalence ($IP_{tot}$ = 20.6–26.5%), formed by sectors with and without zoning plans, located in proximity to major permanent and semipermanent breeding places. In some of the areas, anopheles breeding is maintained during the dry season by water collecting at sites where clay is excavated for brickmaking.

As for the observed differences in malaria prevalence over short distances within the same sector of the city, we assume that the limited flight range of the vector, as indicated by the studies in sector 11 (Sabatinelli et al. 1985b), could have been one of the factors that has contributed to the focality of malaria transmission in the urban setting.

## RESUME

Dans le cadre du Projet de lutte contre le Paludisme financé par le Gouvernement d'Italie, les études entomologiques et protozoologiques menées à Ouagadougou, ont montré que la transmission du paludisme à Ouadadougou est de type saisonnier et que les seuls vecteurs sont: *Anopheles gambiae* (forme "Mopti" et "Savane") et *A. arabiensis*.

Des différences remarquables dans les niveaux entomologiques de transmission et dans les indices plasmodiques ont été observées dans différents secteurs de la ville, en relation avec la localisation et la nature des gîtes larvaires.

Les différences des indices entomologiques et protozoologiques à l'intérieur d'un même secteur à des distances de 300 mètres ont mis en évidence le rôle de la faible dispersion de *A. gambiae* s.l. comme l'un des facteurs caractèristiques de la transmission du paludisme en zone urbaine.

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# Sporozoites and Their Antibodies: A Field Approach

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A bilateral, cooperative project for malaria control was started in 1983 in Burkina Faso, West Africa, under the auspices of the local government and of the Italian Ministry for Foreign Affairs. The program included a longitudinal survey, the main goal of which was to evaluate the antisporozoite immune response in the field and to perform extensive monitoring of malaria vectors. A detailed analysis of the obtained results will be reported elsewhere (Esposito et al., submitted manuscript; Lombardi et al., in press), but we would like to avail ourselves of the opportunity provided by this conference to give an overview of the results and new questions that have arisen from this field study.

## OBJECTIVES AND METHODS

It is well known that adults are more resistant than children to malaria infection, but it is difficult to evaluate the respective roles of antibodies to sporozoites and to the blood forms of *Plasmodium falciparum* in this increased resistance. Acquisition of natural resistance to malaria with age is accompanied by the development of antibodies to the blood stages of the parasite.[1]

A greater insight into the development of immunity can be obtained by measuring the antisporozoite antibodies under field conditions. The specificity of induction of these antibodies has recently been questioned on the basis of in vitro reactions of antisporozoite antibodies with both sporozoite and blood-stage antigens.[2] It is apparent that, if antibodies to sporozoites were mainly the result of an immune response to a cross-reactive epitope present on blood stages, it would be impossible to measure the degree of protection against malaria conferred specifically by the antisporozoite antibodies. We tried to approach this problem directly in the field.

Throughout the experiments, the sporozoite antigen employed was (NANP)3, the immunodominant epitope of *P. falciparum* circumsporozoite antigen.[3] The induction of antibodies to (NANP)3 in the field was

studied in two groups of children, from 6 to 30 months old, living in the same village, on the opposite sides of rice fields.

## RESULTS

### Transmission

The intensity of malaria transmission in the area was analyzed, and we found inoculation rates above the value of one infective bite per human per night for a fairly long period, reaching values as high as seven infective bites per human per night.

It should be emphasized that the entomological survey providing these results was performed in close correspondence with the protozoologic and immunologic surveys, that is to say in the same places and at the same time. The first baseline survey was carried out before the beginning of intense transmission, the second near to the peak, and the third at the decline of transmission, after the end of the rainy season. Immediately after the first survey, in June, one of two groups of children was treated with therapeutic doses of the antimalarial drug chloroquine to clear their blood from parasites. From that moment on, these children, although exposed to sporozoite inoculations, were protected from exposure to *Plasmodium* blood-stage antigens by weekly chemoprophylaxis with chloroquine. Children belonging to the other group were closely followed and treated when they showed malaria symptoms. Thus they were exposed to both, sporozoite and blood-stage antigens. The trends in prevalence of parasitemia, prevalence of antibodies to (NANP)3, and in the mean anti-(NANP)3 antibody levels were monitored in the two groups through the three surveys.

### Prevalence

The baseline parasitemia prevalences in the two groups were almost identical and averaged 40%. At the successive surveys, as the transmission season proceeded, parasitemia prevalence reached 86% and 89% in the group not receiving chemoprophylaxis, but went down to 12% and later to 0% in children under chloroquine prophylaxis. In contrast, no significant difference was found in the prevalence of antibodies to (NANP)3. These antibodies kept increasing in both groups, even after the beginning of chemoprophylaxis. It is remarkable that more than 90% of the examined children had produced anti-(NANP)3 antibodies at the end of the transmission season.

The presence in the circulation of blood stages of the malaria parasite did not seem to induce or depress the production of antibodies to

sporozoite antigens. These findings point to the sporozoites as the major trigger of anti-(NANP)3 antibody production. We cannot exclude an effect of parasitemia in sustaining a certain level of anti-(NANP)3 antibodies, possibly due to a weakly immunogenic, cross-reactive, blood-stage epitope.

### Anti-Blood-Stage Antibodies

The trend of the anti-blood-stage antibodies was also studied through the same three surveys, and it was shown to follow the expected pattern. The anti-blood-stage antibodies increased in the nonprotected group and decreased in the children under chemoprophylaxis. They appeared therefore completely unrelated to the antibodies to (NANP)3.

### Role of Antibodies to (NANP)3

Once established that antibodies to (NANP)3 were mainly elicited by sporozoites inoculated by mosquitoes, a further investigation was carried out to determine the possible protective role played by these antibodies. This was done by evaluating the parasitemia prevalence in relation to the anti-(NANP)3 antibody levels in different age groups.

The most significant results refer to individuals living in the village of Kuiti, where the intensity of transmission reached the exceedingly high values mentioned above. Individuals belonging to each age group were subgrouped according to whether they showed anti-(NANP)3 antibody levels above or below the median value of their group. The parasitemia prevalences were then determined separately for each subgroup of individuals.

At the beginning of the rainy season in June, under low-transmission conditions, the parasitemia prevalences were lower in individuals in whom the antibody levels were higher than the median value. The difference in parasitemia prevalence was striking in the group ranging from 20 to 35 years. In this group none of the individuals with high levels of antibodies to (NANP)3 was parasitized, whereas 40% of the individuals with low levels of antibodies were parasitized. This result pointed to a possible protective role for these antibodies.

The pattern changed at the second survey, corresponding to the peak of malaria transmission, when entomological inoculation rates as high as seven infective bites per human per night were measured in the relevant village. Accordingly, in all age groups the parasitemia prevalences were higher than those found in the preceding survey. The parasitemia prevalences did not show any relation to the antibody level, whatever it was. Thus, no obvious protective effect was observed.

When transmission started to decline, after the end of the rainy season in November, the protective effect again became apparent in the 20- to 35-year-old group. In the individuals with the higher antibody levels we found a parasitemia prevalence of 10% that was significantly lower than the 56% found in the others.

No correlation was ever found between parasitemia prevalence and anti-blood-stage antibody levels, measured by immunofluorescence assay (IFA), in each age group.

The above results on parasitemia prevalences in relation to antibodies to (NANP)3 indicated two possibilities: (*a*) A significant protection from malaria infection could be afforded only by the high anti-(NANP)3 antibody levels found in the sera of adults living in a hyperendemic area. (*b*) Anti-(NANP)3 antibodies might offer protection against malaria infection, provided that the sporozoite loads are not overwhelming. Thus, it appears reasonable to assume that individuals possessing high levels of antisporozoite antibodies might enjoy complete protection from malaria infection, provided that the human-vector contacts are reduced. New experimental data from ongoing studies are in support of this assumption.

Cotton curtains impregnated with pyrethrum-related insecticides were installed before the rainy season of 1986 in two villages with different intensities of malaria transmission. The data of this large-scale experiment, involving all 4000 inhabitants of these two villages, are still being analyzed, but it is already apparent that a huge reduction in the sporozoite inoculation rate was obtained in both villages. With regard to the levels of parasitemia, we anticipate that in accordance with our previous assumptions, the adults of Kuiti village were significantly less parasitized in June of 1986 (2% parasitemia positives) as compared with the prevalence during the same period in 1985, when 13% parasitemia positives were observed. These data are based on examinations of the same individuals. Although these results do not demonstrate unequivocally a protective role of the antisporozoite antibodies, they indicate that in some age groups and certain transmission conditions, individuals with higher levels of anti-(NANP)3 antibodies are less frequently parasitized than those with lower antibody levels.

## EPIDEMIOLOGIC CONSIDERATIONS

Another set of results, obtained during these studies, indicates the interesting possibility of using the anti-(NANP)3 antibodies as an epidemiologic tool. Usually the assessment of malaria transmission intensity in a region requires extensive, expensive, and complicated entomological surveys. The aim of these surveys is essentially to establish the entomological inoculation rate (EIR). We found a relationship between the EIR

and the anti-(NANP)3 antibodies. This relationship was investigated in groups of children living in areas with different intensities of malaria transmission, and it was found that the prevalence and the level of anti-(NANP)3 antibodies in each area were related to the number of infective bites received by a resident of that area, that is to say to the EIR. Thus, these parameters may represent reliable probes of the human-infected vector contact and appear to correlate to the intensity of malaria transmission in a defined area. Further studies are needed to obtain the details of this relationship. It is imperative that these future investigations combine entomological and immunological measurements in the field concomitantly.

## ENTOMOLOGIC CONSIDERATIONS

Studies like those undertaken in this project require intense and accurate entomological surveillance. In the course of this activity, some peculiarities emerged at the level of the malaria vectorial system. These peculiarities concern a hypothetical refractoriness in mosquitoes belonging to the main Afro-tropical malaria vector *Anopheles gambiae*.

Starting from the beginning of the rainy season, in June, the villages of Kuiti and Zagtouli were periodically visited until November 1985. In these two villages a research was planned to study the localization of sporozoites in the vectors. From each specimen, salivary glands were dissected from the thorax and examined with phase-contrast illumination to detect *Plasmodium* sporozoites. After microscopic observation, the salivary glands from individual mosquitoes were transferred into a microtiter plate for later processing. The thorax was also collected and placed in an individual microtube for storage until processing. Monoclonal antibodies to the circumsporozoite (CS) antigen of *P. falciparum*, kindly provided by Prof. Ruth S. Nussenzweig, were employed in the Zavala's assay[4] to assess the presence of CS antigen in the thorax and/or in the salivary glands obtained from the same mosquito. The overall proportion of salivary glands positive for CS antigen was 35 over 587 examined. Of the 552 mosquitoes which were found to be negative for *P. falciparum* CS antigen in salivary glands, 18 contained the CS antigen in the thorax. It was astonishing to discover that 17 of these came from the same village of Kuiti and only one from Zagtouli. Moreover, these 17 mosquitoes represented 43.5% of all the CS antigen positive specimens of Kuiti. We tried to investigate this unexpected phenomenon, i.e., the fact that in the Kuiti mosquito sample the CS antigen was frequently found only outside the salivary glands. To rule out the possibility that our findings reflected merely the time lag required for sporozoites to pass from the thorax to the glands, three samples of freshly fed *Anopheles*

females from Kuiti village were processed after a holding period sufficient to allow the completion of sporogony. It was found that, after 14 days since the capture, still as many as 10 of the 22 specimens positive for CS antigen remained uninfected in their salivary glands. This proportion of 10 over 22, or 45.4%, corresponds closely to the figure of 43.5% that was observed in the wild-caught mosquitoes of Kuiti.

## SUMMARY

The results of these studies can be summarized in the following way: in two villages with different intensities of malaria transmission, the vector populations became infected at the same level. However, in one of the villages, 45% of the infected mosquitoes did not become infective, because the sporozoites remained in the thorax leaving the salivary glands uninfected. The frequency of this phenomenon was similar in samples collected in the field throughout the transmission season and in experimental studies carried out in the insectary. Various mechanisms could be proposed to explain this phenomenon of discrepancy between the sporozoite rates in thorax as compared with salivary glands. These include the possibility that immune or non-immune components of the mosquito may interfere with the assembly of the CS antigen on the sporozoite surface, may promote the disruption of normally assembled sporozoites, or may prevent their penetration into the salivary glands. Factors able to provoke these kinds of effects on sporozoites (e.g., antibodies to CS antigen) could even be ingested by the mosquito with the bloodmeal taken on the human host. The inability to complete the morphogenesis and differentiation of sporozoites might also depend on the genetic characteristics of a particular *Plasmodium* strain. If it emerges that some peculiar mosquito trait is at the base of the observed phenomenon, the chance of relating it with a particular genotype will open up new perspectives. We are currently planning some laboratory and field studies to clarify this point.

It did not escape our attention that unexpected results, like those we got, could question the absolute reliability of immunoassays for the determination of sporozoite indices and strengthen the opinion that, before these assays are generally accepted for use, they must be extensively tested under different field situations. Nevertheless, we are convinced of the potential value of these newly developed methods for studies of the epidemiology of malaria and of the naturally acquired protective immunity. The constellation of results presented here indicates that these methods, in addition to displaying unquestionable technical advantages, may also contribute to the better knowledge of the mechanisms of malaria transmission.

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# Malaria Research in Kenya

M. MUGAMBI

The Kenya Medical Research institute (KEMRI) was established as a corporate body by an act of Parliament in 1979. The functions of the institute as stated in the act are:

1. To carry out research in the field of biomedical sciences;
2. To cooperate with other organizations and institutions of higher learning in training and matters of relevant researches;
3. To liaise with other research bodies within and outside Kenya carrying out similar research;
4. To disseminate research findings;
5. To do all such things as appear to be necessary, desirable or expedient to carry out its functions.

In its operations the institute is guided by the general policies as articulated in the national science and technology policy. Specific research objectives are determined by the management board and implemented through its research departments. The institute is administratively accountable to the Ministry of Health. The results generated in our research are expected therefore to be of practical application to the country at large and more specifically to the Ministry of Health in its efforts to promote better health. To ensure relevance in research, we select disease priorities in close consultation with the Ministry of Health and other relevant authorities. KEMRI works very closely with the Division of Vector-borne Diseases (DVBD) which is a department of the Division of Disease Control of the Ministry.

According to available health returns the three most prevalent diseases accounting for most of the morbidity and mortality in Kenya are malaria, ARI, and diarrhea. The institute therefore has designed programs that give prominence to these diseases.

KEMRI has an excellent infrastructure with adequate well-equipped laboratories. Currently it has a manpower strength of 695, of which 270 are scientific or technical staff. The following groups are responsible for the planning and implementation of research programs: Situated in Nairobi are the Center for Microbiology Research (CMR), Clinical

Research Center (CRC), Respiratory Diseases Research Center (RDRC), Medical Research Center (MRC), Biomedical Sciences Research Center (BSRC), and the Traditional Medicines and Drugs Research Center (TMDRC), Virus Research Center; and situated in western Kenya in the vicinity of Lake Victoria are the Alupe Leprosy and Skin Diseases Research Center (ALSDRC), and the Vector Biology and Control Research Center (VBCRC). Research coordination is ensured through several scientific and programs committees. These same committees determine the general direction of research and minimize duplication. Final approval of research is the responsibility of the management board.

The prevalence of malaria in Kenya is not uniform but varies with altitude and other climatic differences. The disease is holoendemic in the western and coastal belts of Kenya, where infection rates may be as high as 90% in young, school-age children. Despite the public health importance of malaria, Kenya has not had well-articulated policies in either research or control of malaria. Through our work in KEMRI we are attempting to rectify this deficiency.

KEMRI initiated its malaria program in 1980 with the help of the World Health Organization (WHO) and the Centers for Disease Control (CDC). In the last two years our initial efforts in malaria research have further been strengthened by the participation of Walter Reed Army Institute of Research (WRAIR) and the Wellcome Trust. Initially our research concentrated on drug sensitivity studies to *Plasmodium falciparum* infection in the two holoendemic areas. Results so far obtained have been of great value to the Ministry of Health. The malaria program has now expanded to include some of the following research:

1. A community-based malaria control project in Saradidi, Kenya, which sought to reduce morbidity and mortality through community distribution of chloroquine;
2. Response of *P. falciparum* to other antimalarials, singly or in combination, including pyrimethamine, amodiaquine, fansidar, chloroquine and erythromycin, halofantrine, chloroquine, and dapsone;
3. In vitro studies of *P. falciparum* response to dihydrofolate reductase inhibitors and sulfonamides;
4. Bioassay for blood and serum levels of antimalarials by radioisotopic methods and pharmacokinetic studies of several antimalarial compounds;
5. Monitoring of malaria vectors (*A. gambiae* and *A. funestus*) including chromosome separation, bloodmeal preparations and sporozoite inoculation rates;
6. Adaptation of malaria vectors to changing environments;

7. Attempts to colonize *A. funestus*;
8. Biological control of malaria vectors using indigenous fish (*Nothobranchius spp* and *Tenopoma spp*).

Preparation for phase IIb vaccine trials have recently started and, progress to date will be reported in a subsequent paper by one of our collaborators.

This short resume aims at highlighting the importance of malaria in Kenya and the need to intensify applied research. We are so far satisfied with the collaboration between KEMRI and the two U.S. Institutions. We have adequate facilities for good laboratory research and well-defined field study areas for operations research. More collaboration with countries in the east African region would ensure more productive research through the exchange of information and manpower. The Agency for International Development (AID) could be quite instrumental in enabling the establishment of appropriate research networks.

# Transmission of Malaria in Western Kenya

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We have initiated studies of the transmission of *Plasmodium falciparum* malaria in Kenya in anticipation of a field trial of a *P. falciparum* sporozoite vaccine. This first trial will be conducted using nonimmune American volunteers who will be immunized with placebo or vaccine and then travel to Kenya, where they will be exposed to natural infection.

The current study was designed to define transmission by studying the incidence of malaria, rather than the prevalence, in order to precisely define seasonal changes in transmission. Only young children, under 6 years of age, have been studied because they are the least immune cohort of the local population. Thus, we expect that the incidence of malaria observed in these children will approximate best the expected incidence in the nonimmune volunteers of a vaccine field trial.

Entomological and meteorological data were correlated with corresponding changes in the incidence of malaria. These correlations should allow us to predict future periods of peak transmission, and thus allow us to decide the appropriate time and duration of a field trial of a vaccine.

## MATERIAL AND METHODS

The Saradidi community in the Siaya District, Nyanza Province, located in western Kenya, about 50 km west of Kisumu near the northern shore of Lake Victoria, was selected as the site for our studies. This area has been extensively studied by investigators from the Kenya Medical Research Institute, the U.S. Centers for Disease Control, and the University of Nairobi. These attributes make it a good potential site for future studies of *P. falciparum* vaccines: (*a*) the prevalence of *P. falciparum* malaria in school children is high, ranging from 80% to 90%, while the prevalence of other malarias is low; 3% for *P. ovale* and about 10% for *P. malariae* (A. D. Brandling-Bennett, personal communication); (*b*) the area is easily accessible and possesses a well-organized community health system, the Saradidi Rural Health Project.

We have studied the transmission of malaria in this area since January, 1986. Every four weeks, cohorts of 43 to 50 (mean = 47) children, 6 months to 6 years of age, were enrolled in the study. They were examined and their blood was drawn for preparation of thick and thin malaria smears and for antisporozoite antibody assays.

All children were treated with sulfadoxine/pyrimethamine (Fansidar, F. Hoffman-La Roche & Co., LTD., Basel, Switzerland). Thereafter, they were seen daily for 12 weeks to promptly detect new illnesses and to insure that they were not given antimalarial drugs, except those given within the study.

Whenever a child became ill, he was evaluated the same day at the Saradidi Rural Health Project. Each time a child was seen for an illness, malaria smears were repeated. If recurrent malaria infections were documented, they were treated promptly. Other illnesses were also treated, but with drugs that are known not to have an effect on malaria.

Additionally, routine malaria smears were performed on all children 1, 2, 4, 6, 8, 10 and 12 weeks after treatment.

All malaria smears were stained with Giemsa, and 200 oil-immersion fields were read independently by two observers. Children who developed recurrent parasitemia on or before day 28, or week 4, were considered to have had a recrudescence; these children were treated and dropped from the study. The remaining children, who had remained negative through week 4, were then checked biweekly for the development of a new parasitemia. When these children developed a new parasitemia, they were treated and dropped from the study. All children who did not develop recurrent infections were followed for 12 weeks after their enrollment.

The incidence of new malaria infections was calculated for biweekly periods, after week 4, and was expressed as the proportion of children who developed parasitemia during a 2-week period among those who were negative at the beginning of the period.

Concurrent studies were performed on the *Anopheles* vectors of malaria in this community. Once a week, six pairs of men collected mosquitoes from one another for half-hour intervals, followed by one-half hour of rest, from 6 pm until 6 am in six houses. All mosquitoes collected were examined in the laboratory the next morning. The *Anopheles* were identified and dissected for parity. Only three species of *Anopheles*, *A. gambiae*, sensu strictu, *A. arabiensis*, and *A. funestus*, were found. Parous *Anopheles* were examined for the presence of sporozoites in their salivary glands. The entomological inoculation rate was then calculated by multiplying the average number of *Anopheles* collected per man per night times the proportion with sporozoites in their salivary glands.

Meteorological data were also collected: maximum and minimum temperatures, relative humidity, and rainfall were recorded at two sites in the Saradidi community.

## RESULTS AND DISCUSSION

The incidence of *P. falciparum* infections, expressed as the proportion of children who developed infections in each fortnight, was about 0.1 in January, rose to a plateau of 0.6–0.7 from late April until August, and then fell to about 0.1 in September.

*Anopheles* biting rates averaged less than one *Anopheles* per man per night from all night human-bait collections from January through March. The biting rate then rose rapidly to a peak of about 15 mosquitoes per man per night in late May, and was greater than three mosquitoes per man per night from April to August. Thereafter, the biting rate fell to levels of less than one by September. Sporozoite rates were about 0.2 in January, fell to a low of 0.01 in April, began to increase again in May, and then rose gradually to a peak of 0.3 in late August, and then declined again. The entomological inoculation rate was about 0.2 in January, fell to a low of 0.005 in March, and rose to a peak of about 1.4 in June. The inoculation rate remained above 0.2 from April through August and then fell to 0.01 in September.

Little rain fell from December 1985 to February 1986. Intermittent rains occurred in February and March. The heaviest seasonal rains, which peaked at 9–10 mm/day, occurred in April. Rainfall was above 1.5 mm/day from mid-February to early June. Then the seasonal rains tapered off and little rain fell from July to September.

The seasonal patterns of the incidence of *P. falciparum* malaria and the entomological inoculation rate were similar, with the exception that the incidence plateaued at about 0.6 to 0.7 (60–70% incidence in each 2 weeks) from late April to end of August, while the entomological inoculation rate continued to a peak in June, and then declined. The difference between the incidence and inoculation rate at high entomological inoculation rates was due to the fact that the entomological inoculation rate is the number of infective bites received per man per night, and thus could exceed one, while the proportion of children infected per fortnight could not exceed one, since we could not detect multiple simultaneous *P. falciparum* infections.

We also compared the entomological inoculation rate and the incidence of *P. falciparum* per day, since the entomological inoculation rate was expressed as the number of infective bites per day. To make this comparison, we recalculated our incidence data using the maximum likelihood estimate of attack rates published by Norman Bailey (1982). In

February and March, and again in September, entomological inoculation rates were 0.01 to 0.06 and the attack rates were about 0.01. The similarity between the inoculation rates and the attack rates during these periods of low transmission suggests that one infective bite is sufficient to cause a *P. falciparum* infection in these young children. However, in May, June, and July the inoculation rates ranged from 0.23 to 1.75 while the observed attack rates were only 0.05–0.09. We speculate that the children probably did suffer multiple simultaneous infections during these periods of high transmission. Thus, the attack rates observed are probably lower than the true incidence of *P. falciparum* infections during these periods.

The attack rates of both *P. malariae* and *P. ovale* were 0.001–0.009, very much lower than those of *P. falciparum* during periods of peak transmission. The attack rates of *P. malariae* and *P. ovale* appeared to increase gradually from July to September, and in September approached the attack rate of *P. falciparum*. However, these data on the attack rates of *P. malariae* and *P. ovale* should be interpreted with caution. We treated all children with recurrent *P. falciparum* infections promptly and this treatment may have prevented the development of patent *P. malariae* and *P. ovale* parasitemias. Also, we treated the children with sulfadoxine/pyrimethamine when they were enrolled in the study. Since this treatment has no effect on exoerythrocytic stages of malaria, we could not be sure that the recurrent *P. ovale* parasitemias were not relapses, as opposed to new infections.

## CONCLUSION

In conclusion, we have carefully studied the transmission of malaria in western Kenya, and have found that the incidence of *P. falciparum* malaria was highest from late April through August. This increase in incidence was preceded by increased rainfall, which peaked in April, increased numbers of *Anopheles*, which increased in early April, and higher entomological inoculation rates, which increased in mid-April.

We shall use these data to decide the timing and duration of exposure for volunteers in a field trial of a *P. falciparum* sporozoite vaccine. The volunteers will be given three doses of the vaccine or placebo at monthly intervals, with the last dose given about one month before their exposure in the field. If a similar pattern of transmission is seen at the time of a field trial as was seen this year, vaccination should be started at the beginning of the rains, in February. The volunteers would be ready for field exposure in May, shortly after the beginning of the high transmission season.

To adequately test the protective efficacy of the vaccine, the volunteers should be exposed for a period sufficient for about 70% of the

unprotected, placebo recipients to acquire *P. falciparum* malaria. Using the incidence of *P. falciparum* in children observed this year during the period of peak transmission (May, June, and July) to predict risk, we estimate that it would require about 2½ weeks for 70% of the unprotected volunteers to acquire malaria. However, the average entomological inoculation rate for May, June, and July was 0.79. At this level of risk, it would take only one night of exposure for over 70% of the unprotected volunteers to acquire malaria. If the volunteers are exposed during the period of peak transmission of *P. falciparum* malaria, our incidence data suggests that there will be substantially less risk of acquiring either *P. malariae* or *P. ovale*. Therefore, we expect little difficulty in detecting a protective response induced by a vaccine specific for *P. falciparum*. However, because of the design of our current transmission study, we cannot be sure that our data on the attack rates of *P. malariae* and *P. ovale* are accurate. Therefore, in collaboration with investigators from the Centers for Disease Control, we are now retesting all of the *Anopheles* that were found to have sporozoites in their salivary glands with ELISAs that can specifically detect *P. falciparum*, *P. malariae* and *P. ovale* sporozoites. With this additional data, we shall be able to assess the relative risk of infection with these three malaria species in Saradidi.

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# The Malaria Program at KEMRI

A.D. BRANDLING-BENNETT

In describing the Malaria Program at the Kenya Medical Research Institute (KEMRI), I should like to emphasize that the studies I will discuss have been performed by several investigators of various nationalities. A major concern at the start of the program in 1980 was the occurrence of chloroquine-resistant *Plasmodium falciparum*, which had been reported in tourists to the coasts of Kenya and Tanzania in 1978.[1] These were the first reports of chloroquine resistance on the African continent, coming some 20 years after the discovery of chloroquine-resistant *P. falciparum* malaria in Thailand and South America. Together with staff of the Division of Vector-borne Diseases of the Kenyan Ministry of Health, in vivo and in vitro tests were undertaken along the coast and near Lake Victoria. The principal study sites have been Malindi, which is on the northern part of the coast, and communities west of Kisumu, which is in Nyanza Province on the shore of Lake Victoria.[2] These areas were chosen because malaria transmission is holoendemic in both locations.

In vivo resistance was not detected in Kenyan residents until 1982, when 1 of 42 infants treated in Kisumu failed to clear his infection after receiving chloroquine 25 mg/kg.[3] Other cases of resistance were found on the coast and in western Kenya in 1983. In rural areas of Nyanza Province, the proportion of cases resistant in vivo has increased since 1982,[4] though rather gradually; currently about 5% of infections fail to clear, that is, have an RII pattern and 13% recur by day 7, that is, have an RI pattern. In vitro testing indicates that about one-third of parasites are resistant to chloroquine. Along the coast, 20–30% of children fail to clear their parasitemia after treatment, while a small proportion will have recurrent infections. Actually, the level of RI resistance is underestimated at day 7 since 30% of children will have recurrent parasitemias if followed for 28 days. In vitro tests indicate that two-thirds of parasites are resistant to chloroquine. As a result of our studies, we estimate that about one-half of infections on the coast and one-fourth of infections in the west are resistant to chloroquine. In collaboration with the African Medical and Research Foundation, we also tested the chloroquine sensitivity of

parasites obtained from various remote locations in Kenya, and chloroquine-resistant parasites were identified in all areas. Therefore, we conclude that chloroquine-resistant *P. falciparum* is present in all malarious areas of Kenya, though at varying levels.

Because chloroquine has been the drug of choice for the treatment of malaria throughout Africa, we studied the clinical response to chloroquine in 100 children who were febrile and parasitemic at the time they were seen in the outpatient clinic at Malindi Hospital on the coast. All except two of the children had rapid resolution of fever and other symptoms a mean of 2 days after starting chloroquine. The continued use of chloroquine in areas of low to moderate resistance seems justified because of its clinical efficacy, safety, and low cost. Nonetheless, we have been interested in the effectiveness of other drugs and drug combinations. Studies in 1983 and 1985 have shown that in vivo resistance to amodiaquine is significantly lower than resistance to chloroquine.[5,6] In in vitro tests, 90% or more of Kenyan parasites have been sensitive to amodiaquine.[7] The combination pyrimethamine/sulfadoxine (Fansidar) cures 85–95% of infections, and resistance to this drug appears to be uncommon.[8] The experimental drug halofantrine also appears to be highly effective in limited trials conducted to date. In addition, we are interested in chemoprophylactic agents, which are recommended for certain individuals in Kenya, including pregnant women and nonimmune persons visiting areas of high transmission. Our studies have shown that weekly chlorproguanil (Lapudrine) is not effective,[9] but preliminary data suggest that proguanil (Paludrine) offers approximately 80% protection when taken in an adult dose of 200 mg/day.

Because drugs have been the mainstay of efforts to control morbidity and mortality from malaria in Kenya, as in much of tropical Africa, a study of community-based chemotherapy and chemoprophylaxis was initiated in Saradidi in 1980. The Saradidi Rural Health Project involves about 50,000 persons residing west of Kisumu on the northern shore of Lake Victoria. For the study, the community was divided into three areas, designated *A*, *B*, and *C*. In areas A and B, chloroquine was provided by selected and trained village health helpers to all persons seeking treatment for malaria. In area A, chloroquine chemoprophylaxis was also offered to pregnant women. Until 1985, area C served as a control. Analyses of the large amount of data collected to evaluate the project were performed primarily by Dr. Dan Kaseje and Dr. Harrison Spencer, whose findings were published in 1987 in a supplement to the *Annals of Tropical Medicine and Parasitology*. I will attempt only a very brief summary of some of their findings.

Malaria is holoendemic in Saradidi. Prevalence rates commonly exceed 80% in preschool and school-aged children, and seropositivity

rates rise rapidly with age to 100%.[10] Changes in mortality rates were monitored before and during the community-based distribution of chloroquine. Mortality rates did decline in children aged 1–4 years in areas A and B, where drug was available, but these rates were not significantly different from those in the control area.[11] Infant mortality rates in the community were about 70/1000 live births. It appears that mortality rates were high in the period before intervention in areas A and B, especially among children 1 to 4 years of age, because of a measles epidemic. The benefit of chemoprophylaxis in pregnancy was demonstrated by lower rates of malaria and higher hemoglobin levels in women who used prophylaxis.[12]

The Saradidi Project has accomplished a great deal. The community has organized itself, has identified health priorities, and has begun to intervene. Prenatal, child health, and family planning services have become available to a very underserved population. Within the project, considerable information is now available about demographic characteristics, mortality, morbidity, and health-related knowledge and behavior in the community. With the continued understanding and participation of the people, Saradidi is an ideal location in which to undertake further studies of malaria epidemiology and control. For that reason, KEMRI, in collaboration with CDC and the Walter Reed Army Institute of Research, has initiated new studies of malaria transmission in and near Saradidi, which we hope will lead to the vaccine trials with which this conference is primarily concerned.

During the past 18 months, four locations have been identified in which sporozoite inoculation rates and the acquisition of antisporozoite antibodies will be measured over a 3-year period. Two of these communities are within the Saradidi Rural Health Project and lie along the shore of Lake Victoria on relatively flat land that floods during the rainy seasons. A third area is situated 20 km north of the lake on rolling hills. These three locations have malaria prevalences of approximately 80% in children 10 years and under. The fourth site, 10 km north of Kisumu, is on precipitous terrain on the Nandi Escarpment, where the malaria prevalence in children is about 50%. Resting anophelines are collected for 1 week each month in each location and are being tested for sporozoites of *P. falciparum*, *P. malariae*, and *P. ovale* by ELISA.

In a preliminary sample of approximately 2600 mosquitoes collected in November and December 1985, 8% were found to have sporozoite infections when tested by ELISA.[13] Following the start of systematic collections in April 1986, peak numbers of mosquitoes were collected in May and June, 6 to 8 weeks after the onset of the heavy rains. At that time, mosquitoes in the *A. gambiae* complex were the principal anophelines present. During the dry season, *A. funestus* was found in equal or

greater numbers. We plan to continue the collections and analyses of mosquitoes for 3 years, in order to define seasonal transmission patterns in the four locations.

Data from a pilot survey conducted in September 1985 indicate that antisporozoite antibodies are commonly found in children under 10 years of age in areas of high transmission, while they are less frequent in areas of lower transmission. For example, antibodies were found in 50% or more of children aged 3 years or more in location A, but were absent in the sera of children under age 5 living in the area of low transmission.[14] The prevalence of *P. falciparum* malaria appeared to be unrelated to the prevalence of antisporozoite antibodies, indicating that we did not observe a protective effect of these antibodies. We are enrolling cohorts of newborns in locations A, B, and C and have enrolled a cohort of children 5 to 9 years of age in location D. We are also conducting cross-sectional surveys for parasitemia and antisporozoite antibodies in each community. These serological measures will be correlated with the sporozoite inoculation rates determined entomologically.

A major purpose of our work in Kenya is to train Kenyan professional and technical staff in field and laboratory methods of malaria research. As a result, we anticipate that KEMRI will be an ideal focus for future research with malaria vaccines that are ready for field testing. In addition, communities like Saradidi, with well-organized and knowledgeable people and with considerable data about malaria epidemiology, may be highly suitable locations in which to conduct vaccine field trials.

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## DISCUSSION

MORROW: Thank you Dr. Oster [and Dr. Brandling-Bennett] for your very fine presentations. Because of constraints in time and auditorium space we have to reschedule the discussions and the talk by Dr. de Ceita and two additional papers on the malaria situation in Cameroon and Tanzania. I am told that these country reports will be included in a special section of the proceedings together with the other situation reports presented here. All day tomorrow there will be separate meetings of the

eight different workshops. Their summaries and recommendations will be presented for comments to all participants during the last plenary session of this conference on Thursday. I wish you all a pleasant evening. The afternoon session of today is adjourned.

SECTION IV

# WORKSHOP REPORTS

C.G. CAMPBELL AND M. COLUZZI
*Chairpersons*

# Workshop Objectives

The objective of this conference is to consider in depth the preprequisites for field trials with the *Plasmodium falciparum* sporozoite vaccine and to investigate potential sites that will be best suited to carry out these trials as well as concomitant research relevant to the epidemiological evaluation of *P. falciparum* sporozoite vaccine trials.

The conference will serve several purposes intimately concerned with the above-stated objective, viz:

1. It will bring together African scientists and other responsible people who have major interests in malaria as a public health problem.
2. It will provide a forum for discussion of issues relating to malaria vaccine testing.
3. It will provide an opportunity to develop an overview of current research program in Africa that are relevant to vaccine trials, and to identify areas where further research is needed to evaluate the results of these trials.
4. Participants will consider the parameters necessary for objectively assessing vaccine trials and will address the role of a malaria vaccine in the context of primary health care.
5. It will provide the opportunity for discussing the sociological perceptions of malaria within several communities and such prerequisites as may be needed to make the vaccine acceptable to those communities.
6. Attempts will be made to identify the manpower requirements and training facilities necessary to monitor the epidemiologic parameters of malaria, both in the context of trials, and for long-term assessment of effects should a product be introduced.

The following are questions that will be considered by the various workshops: Workshop 1, "Malaria, and Maternal and Child Health", will explore the natural history of protective immunity in pregnant women and infants in malaria endemic areas. At what age will vaccination elicit optimum protection in infants? How will malaria vaccines interact with other immunizations normally given to young children? Can malaria vaccination be part of the EPI program? How do malaria vaccines affect children with various patterns of exposure to transmission and also with various genetic traits, e.g., sickle cell trait? Given that the immune response alters in the progression of pregnancy, what would be the role of the vaccine(s) in immunizing mothers, especially primigravida?

Workshop 2, "Sociocultural Considerations for Vaccine Trials," will consider concepts of malaria as a disease: What other disease syndromes are confused with malaria? How do communities view it as a problem? Does this differ in various societies? What herbal remedies are applied to treat malaria or malaria-like diseases? Are there taboos that may affect the acceptance of a vaccine for malaria? What would be the motivation of various societies to participate in vaccine field trials, and in the implementation of vaccination for malaria control?

Workshop 3, "Vaccine Development," will discuss current thoughts in this area, critical considerations of the present vaccines under trial, and methods for enhancement of the protective response.

Workshop 4, "Epidemiology" will attempt to identify those factors that are most important in the selection of sites for vaccine field trials? How will these be measured and evaluated? Will antigenic diversity of *P. falciparum* affect the vaccine(s) and is this in any way related to drug resistance in the parasite? Will a standard vaccine be equally effective in various geographical regions? How will the following factors affect the immunization process, and how will they be determined; e.g., genetic factors, age, levels of nutrition or malnutrition, immunodeficiency disease, intercurrent or concurrent parasitic infections? Do multiple malaria infections constitute a problem and how can this be evaluated? Will P. malariae become a greater problem if *P. falciparum* is reduced by immunization? What parameters will yield information about the efficacy of immunization?

Workshop 5, "Vector Biology" considers the use of modern technologies in the identification of vector species, and species complex members. Is it necessary to determine vectorial capacity of potential vectors? Are there ecological factors that may alter the relative importance of a species as a vector, and can this be anticipated? What is the importance of sporozoite inoculation rate in the development of high-morbidity malaria? What techniques should be part of the malaria entomologist's repertoire? What is the entomologist's role in the measurement of impact of vaccine trials? Standardization of procedures? How?

Workshop 6, " Malaria Control" will discuss how vaccination trials can interface with ongoing malaria control programs? What will be the important measurements that will reflect the presence of protection or breakthrough? Is it necessary to achieve sterile immunity, or will partial protection still reduce the morbidity of malaria as a disease? How will ongoing treatment availability, either through control programs, or by over-the-counter availability impact the measurement of protection? Will drug-induced immunosuppression be important? How will it be determined? How do health delivery personnel see malaria immunization, as

part of a vertical malaria control program, or the more horizontal EPI program? What is the definition of malaria as a disease? Is it necessary to conduct studies on the pathology of the disease, particularly in relation to various levels of perceived disease? What are practical constraints in carrying out field trials with readily available treatment available? Assign priorities as to the safety measures to be investigated prior to the field testing of a vaccine.

Workshop 7, "Manpower and Training," discusses the localities for central training institutions, curricula, ideatification of instructors, funding requirements, etc.

# CHAIRPERSON'S INTRODUCTION

CAMPBELL: Good morning, ladies and gentlemen, I am sorry to have to rush you, but we are on an exceedingly tight schedule this morning and afternoon. My name is Kent Campbell, and I am going to moderate this session together with Professor Coluzzi. We would like to begin the series of presentations from the working groups that met yesterday. The objective of our workshops was to come together and think about particular aspects of the overall problem of how to use vaccines when they come along and to look at this from a variety of aspects. This morning we will have seven presentations. We will attempt to adhere strictly to a 15-minute limit to allow for ample discussion time. There will be some overlap of necessity between the presentations and, I think, this will be beneficial as we go through. We would like to give you ample time for interchanges so that everybody can benefit from the ideas that, up to this point, have been shared only in small groups in the hall or over meals. We shall begin with the first presentation from the maternal and child health group. Dr Steketee, the rapporteur of that group will present.

# Workshop 1: Malaria and Maternal and Child Health

L. BRUCE-CHWATT
*Moderator*

R. STEKETEE
*Rapporteur*

Children under the age of 5 and pregnant women represent the groups at greatest risk for the adverse effects of malaria in hyperendemic and holoendemic areas of sub-Saharan Africa. Consequently, these two groups represent the primary target population for malaria intervention strategies, including malaria vaccines as they become available. Malaria vaccines are likely to be complementary to other available interventions and their ultimate role will only be determined after vaccine efficacy and potential for delivery is fully understood. In advance of such knowledge, additional information is necessary in the following areas: (*a*) immune response to malaria infection and alterations/deficiencies in the response during childhood and pregnancy; (*b*) efficacy of presently available interventions both in the controlled setting and in the field setting where constraints such as drug resistance, poor patient acceptance/compliance, and difficulties of delivery exist.

Recent advances in the development of malaria vaccines have provided encouragement that a new tool might soon join the presently available interventions. In sub-Saharan Africa, where *Plasmodium falciparum* is transmitted in hyperendemic and holoendemic settings, children under the age of 5 and pregnant women are the target populations most likely to benefit from antimalarial interventions. This report considers aspects of knowledge of the natural history of malaria in these populations where additional information is required before phase III vaccine trials could be considered in these groups.

An effective malaria vaccine will complement other presently available interventions and will need to be incorporated into the primary health care–MCH (maternal and child health) system before it could be effectively delivered. Thus, while a malaria vaccine offers promise, malaria intervention strategies cannot wait for vaccine and country ministries of

health must continue to develop present malaria control measures and appropriate infrastructure to support these strategies. It is in this context that malaria control in pregnancy and children under the age of 5 is considered.

## PREGNANT WOMEN

### Natural History of Malaria Infection

The adverse effects of *P. falciparum* infection during pregnancy, which have been well documented among women living in malaria endemic areas, include maternal anemia and low-birth-weight infants (Table 1). Low birth weight (< 2500 g) is a strong risk factor for neonatal and infant mortality.

The role of malaria in the risk of premature delivery, abortion, and stillbirth is less well understood. Women in their first pregnancy are at increased risk for developing potent malaria infection and higher parasite densities and for delivery of low-birth-weight infants. Women in subsequent pregnancies appear to be at lower risk. Malaria-infected pregnant women in endemic areas remain largely asymptomatic (Table 2).

The explanation for why primigravidas appear to be a particular risk for malaria has remained elusive. However, recent unpublished information suggests that gravid status and not age is the relevant factor, and the first exposure to malaria during pregnancy may render subsequent pregnancies relatively more protected. The possible immunologic mechanisms that might explain this observation have not been delineated.

### Presently Available Interventions

Interventions presently available during pregnancy include two major approaches: decreasing exposure to infected mosquito bites and antimalarial

Table 1 Natural history of malaria infection in women and children from hyperendemic and holoendemic areas.

| |
|---|
| Pregnant Women |
| Primigravida (first malaria-exposed pregnancy) |
| Second and third trimester |
| Humoral, immunologic, and physiologic alterations |
| Anemia |
| Impaired placental function |
| Newborns |
| Congenital infection (rare) |
| Low birth weight leading to risk of infant mortality |
| Prematurity and intrauterine growth rate (IUGR) |
| Stillbirth and abortion |
| Infants |
| Relative protection of those under 6 months |
| Relative susceptibility of those 6 months to 2–4 years |

Table 2. Outcome measures show that a high price is paid for acquiring immunity.

| Outcome | Pregnant Women | Child |
|---|---|---|
| Malaria infection | x | x |
| Malaria morbidity | | x |
| Malaria mortality | | |
| Direct | | x |
| Indirect | x | x |

For the purpose of control (vaccine trials) a standardized case definition for each of these should be developed.

Surveillance should be established as a basic requirement of monitoring malaria.

drug use for treatment or chemoprophylaxis. Present interventions have focused on chemotherapy and chemoprophylaxis; however, results of carefully controlled trials of chemoprophylaxis are still limited. Recent investigations have shown that problems of drug resistance, poor patient acceptance compliance, and difficulties in maintaining an effective delivery system limit the potential impact of the chemoprophylaxis interventions.

## Potential for Immune Modulation (Vaccine) Interventions

There is only limited information on the immune alterations of pregnancy that specifically affect response to the malaria parasite. Although our understanding of the effects of vaccines are largely in the realm of humoral responses, new evidence suggests that cellular immune alterations may be more important during pregnancy. The immune response to a malaria vaccine may be different in pregnant women than in adult male volunteers. Therefore, additional information is required to understand the immune response to malaria infection during pregnancy.

## Recommendations

Accordingly, the following recommendations are made:

1. Additional information is required on presently available interventions to understand the benefit that might be obtained in ideal artificially controlled setting and the real-life field setting. This information will provide a basis for understanding the role that a vaccine might have in malaria control during pregnancy.
2. Further information is required concerning humoral and cellular immune response to malaria infection during pregnancy. Differences in immune response between the first malaria-exposed pregnancy and subsequent pregnancies should be evaluated. This information will

provide a basis for determining the potential for immune modulation during pregnancy.

3. Phase III trials should be conducted in pregnant women.
4. The possible effects of a vaccine used in pregnant women include: the prevention of infection; the control of parasite density; the reduction in low-birth-weight babies; and the enhancement or maintenance of maternal antibody transfer to the newborn.
5. Once a vaccine is available, further evaluation of stage- and species-specific vaccine should be carried out and operational issues such as optimal timing of delivery and the potential for incorporation into the antenatal care system should be evaluated.

## CHILDREN UNDER THE AGE OF FIVE YEARS

### Natural History of Malaria Infection

Congenital transmission of malaria is a rare event in settings of endemic malaria. The newborn appears to have relative protection from clinical disease for approximately the first 6 months of life. The child then reaches a period of relative susceptibility from age 6 months to 2–4 years (depending on local endemicity). The impact of malaria during infancy and childhood has been measured by infection rates (prevalence and incidence), morbidity rates and mortality rates (direct and indirect mortality). As these are outcomes of malaria exposure and disease and potential outcome measures of intervention trials, further consideration needs to be given to definitions of infection, morbidity and mortality due to malaria.

### Presently Available Interventions

Available intervention include efforts to decrease exposure to infected mosquito bites and prompt treatment of clinical illness. Problems of drug resistance, cost, and logistic difficulties of maintaining a drug delivery system limit the potential impact of drug treatment. Cost and lack of infrastructure limit the potential of interventions that attempt to decrease exposure.

### Potential for Vaccine Intervention

Baseline information is needed on both humoral and cellular immune response to malaria infection in infants and children to begin to understand the potential efficacy of vaccine in these age groups. Ideally,

protection elicited by vaccination would be most beneficial during the susceptible period of 6 months to 2–4 years. The immune response to vaccine in young children is likely to be different than in adult male volunteers, thus, studies of postvaccine antibody response in this young age group should precede vaccine phase III trials.

## Recommendations

Accordingly, the following recommendations are made:

1. Further information is required concerning humoral and cellular immune response to malaria infection and malaria antigen vaccines.
2. Phase III should be conducted in children under 5 years and should be carried out in a manner that closely approximates the way in which the vaccine might ultimately be used.
3. Outcome measures of vaccine trials will include infection, morbidity, and mortality rates. These need to be further defined, and definitions standardized for comparability across studies.
4. Phase III trials should be conducted separate from operational issues; however, evaluation of timing of delivery and potential incorporation into the extended program of immunization (EPI) should be done shortly thereafter.
5. Vaccine trials should be conducted in different epidemiologic settings and should take into account certain host factors (Hgb, HLA markers, etc.) and additional infections (AIDS) that might affect immune response.
6. Indications for vaccine use (Table 3) might include:
   a. When it is incorporated into EPI or antenatal clinic settings;

Table 3. Interventions.

- Decreasing Exposure
  - Vector control
  - Sanitation/environmental
  - Personal protection
- Chemotherapy/Prophylaxis
  - Ideal application
  - Field application
    - ex: Chemoprophylaxis in pregnancy
      - Drug efficacy
      - Drug availability
      - Patient availability
      - Patient acceptance/compliance
      - Delivery logistics/infrastructure
- Vaccine

b. In a setting of drug failure;
c. In a setting where vaccine is safer or more effective than available drugs;
d. In isolated communities where other interventions are not available.

Thus vaccine may have a role, but not an early prominent role, in malaria control strategies.

## DISCUSSION

CAMPBELL: Are there questions or comments? I know that there are a number of individuals in the room who have had considerable experience in the area of malaria and maternal and child health. Dr. Nussenzweig.

NUSSENZWEIG: I have two questions. The first is the general notion that during the first pregnancy malaria is more severe than in later pregnancies. On the other hand, you indicated that the morbidity is relatively low. So, my question is what do you actually see in primigravidae? Is it the child with low birth weight, more parasitemia, fever, what do you see? My second question concerns your statement that the cellular response during pregnancy might be different. What is the basis for that? What do we know?

SICKETEE: To respond to your first question, I suppose in primigravidae there is both a higher prevalence of malaria infections and a higher parasite density that is subsequently expressed as a higher parasite density in the placenta. It is felt that this is the mechanism for mediating the low birth rate. There is also evidence for some impaired placental function. Subsequent pregnancies have much lower rates of parasitemia and parasite densities. As I mentioned, it appears from several recent studies that they have even lower parasite densities than nonpregnant women of the same age. There may be some conferred protection from having had malaria during the first pregnancy.

What is the basis for speculating that cellular immunity is more important than humoral immunity and that it is altered during pregnancy? Well, in studies of humoral immunity during pregnancy there is no evidence of a difference between the first and subsequent pregnancies. There are slight differences between pregnant and nonpregnant women. It is hard to attribute the effect of malaria in primigravidae to some alteration in the humoral immune response. At this point, we have no tools capable of measuring differences in humoral responses. On the other hand we do not have practical tests that can be applied to measure the degree of cellular immunity. Experimentally these tools exist. There have

been experiments with *Plasmodium berghei* in mice which have shown that what happens in the mouse seems to be similar to what we observe in pregnant women of malarious areas. If one exposes the first pregnancy in a mouse to malaria and keeps the mouse alive, subsequent pregnancies will be relatively protected from challenge infections. I am afraid that we really do not have a great deal of information at this point to support my view. I am merely speculating that cellular immunity might play an important role in pregnancy.

NUSSENZWEIG: If I may be allowed a quick follow up question. I am aware of that situation. But the answer I would like to have, if possible, is about the role of cell-mediated immunity during pregnancy in other infectious diseases.

STEKETEE: The problem is that we do not have an infectious disease model. Most of the infections we see in pregnancy are the viral diseases and those, indeed, have been felt to be a cell mediated immunity problem. Mycobacterial infections are also considered as important during pregnancy. We do not have a model of an infectious disease to which a woman will be exposed during pregnancy, subsequently cured, and then re-exposed in a subsequent pregnancy.

CAMPBELL: Perhaps we could take Professor Bruce-Chwatt's challenge and make certain that we do not miss something that was already discovered a number of years ago. Dr. Greenwood, do you have any comments from the heritage of work that has been conducted on malaria and pregnancy in the Gambia

GREENWOOD: The answer is very simple. I cannot really see what is happening at all. It does not seem to make a great deal of sense immunologically. As the rapporteur said, we know that these various changes do happen in pregnancy. It is very difficult to identify any immunologic change that happens in the first pregnancy that does not happen also in subsequent ones. As many of you know, Sir McGregor suggested that one may deal with some sort of local immunity in the uterus or in the genital tract and that this is stimulated in subsequent pregnancies. I think that is certainly a provocative hypothesis. Unfortunately, it is a rather difficult one to investigate in any way that one could think of as being ethical. But that hypothesis is, as far as I am aware, the only sensible suggestion that is available to explain the difference. If one looks at delayed type response to *Candida albicans* or PPD I am sure one would be able to show differences between pregnant and nonpregnant women, but as far as I know nobody has shown a difference between the first and subsequent pregnancies. There really remains a big mystery.

STEKETEE: Dr. Campbell, you have referred to this presentation of rapporteur under my name. I would like to stress that this is a presentation of the entire group. It does not necessarily reflect my own views. Nevertheless, it represents the average thinking of the group with a coefficient of great uncertainty fitted into it.

CAMPBELL: Thank you Dr. Steketee for this clarification. Let us now move to the next presentation, the summary and recommendations of the Working Group of Social and Cultural Considerations. Dr. Kaseje will function in the dual role of chairman and rapporteur for his group and will present the sense of that meeting.

# Workshop 2: Sociocultural Considerations for Vaccine Trials

DANIEL C.O. KASEJE
*Moderator and Rapporteur*

The group considered their task to be limited to phase III vaccine trials and other antimalarial interventions. It was noted that there are many sociocultural factors that will affect the implementation of trials and should be understood and be taken into account in the planning and implementation of vaccine trials.

## COMMUNITY PERCEPTIONS OF MALARIA AS A DISEASE

Although most communities in endemic areas associate fever with malaria, their perceptions of malaria as a disease and their response to it vary from place to place. Therefore, it is important that local perceptions and behaviors regarding malaria be thoroughly studies and understood before undertaking any interventions or trials. The knowledge of perceptions may provide linkages or ruling points for health education on malaria, vaccine trials, and vaccine use in the future.

## RESPONSES TO PERCEIVED DISEASE AT THE LOCAL LEVEL

The involvement of family members and other local community decision-making systems appear to be important in disease management. This implies that involvement of individuals in a trial will have to take this fact into account.

Several tiers of diagnosis and treatment sources for malaria include traditional healers, formal health care facilities, and commercial sources. These sources of care will need to be identified and the information used in planning the trials.

## MOTIVATION FOR PARTICIPATION IN TRIAL VACCINES

The group underscored the importance of maximum possible community involvement at all stages of planning, implementing, and evaluating a trial. This implies a meaningful community dialogue that must be initiated and maintained by people skilled in methods of community motivation and involvement. Some of the key elements in this include:

1. Involvement of administrative and other well-thought-of community leaders;
2. Involvement of the health sector and other sectoral service providers;
3. Clear simple information and explanations regarding the process of the trial and the expected results in terms of benefits and possible side effects.

If the community is involved, it may also make suggestions that may be crucial to the success of the trial. These recommendations may include services that should be provided along with vaccine. These services should be within the existing health care system and should be available to the wider community.

## POSSIBLE CONSTRAINTS

These main constraints were identified: Collection of specimens that may meet local opposition based on taboos, etc; injections to children or pregnant mothers that may sometimes be unacceptable; and any previous negative experiences that the community may have had.

## PRIMARY HEALTH CARE PRINCIPLES

The following primary health care principles were considered important: Community participation; involvement of community leaders; and involvement of health- and other related extension workers.

## ETHICAL CONSIDERATIONS

The group considered the following ethical requirements: Informed consent be provided within local and national context; no harm be done to participants; treatment be provided to the participants according to well-defined criteria; concomitant services or benefits not be denied to nonparticipants; a placebo be included to insure that the results of the study are scientifically valid.

## RECOMMENDATIONS

The group made the following recommendations:

1. Since community perceptions of and responses to malaria vary from place to place, preliminary studies before vaccine trials should include an assessment of these perceptions and responses.
2. Various levels of diagnosis and treatment services that are already available in the target community should be investigated during preliminary studies.
3. Planning and implementation of trials should involve all levels of the national health, administrative, and political system.
4. Services and benefits should be provided to the participating community in addition to clear and simple explanation of benefits and possible side effects.
5. Study design should recognize local beliefs and practices that may influence specimen taking. This may limit the number and or frequency of specimen collection.
6. Study and trial team should include a person skilled in communication with villagers.
7. Complex ethical issues will require a special group to consider the control group problem, the treatment issues, and the ethical review procedure. This group should come up with guidelines for ethical review or approval at the local, national, and international levels.

## DISCUSSION

CAMPBELL: Certainly, a number of issue were raised here. Are there questions, comments, or perhaps other opinions from participants in the group?

BRUCE-CHWATT: I was one of the participants in this group, and I am grateful to Dr. Kaseje for having run this second part of our group so well. The only point where we really could not come to any conclusion was the problem of placebo. I cannot conceive of any scientifically or ethically acceptable placebo in comparison with an active vaccine. What would be a placebo to an active vaccine? The only placebo I can perhaps think of is an inactive vaccine. But will that be a true placebo? Any other substance whether an adjuvant without a vaccine or another injection will not be a scientifically acceptable placebo. Perhaps Dr. Winter could explain why this situation of "no placebo at all" may not be acceptable to the powers that be.

SALAKO: I do not want to comment on the placebo control. I think this would go on and on because there were as many opinions as there are

scientists here. I support the contingency by Dr. Kaseje that another group may need to look into these matters critically. The question I am interested in is that of informed consent. How does one obtain informed consent from rural communities? I think that whatever trial we do must conform to international guidelines on the use of human subjects for experimentation. But here we run into practical difficulties. Why do we say "informed" consent? How does one get informed consent? Should the consent be given by the village head or, if we use a school, by the headmaster, or should we ask every person whether he or she wants to participate and to indicate their choice by either standing up or sitting down? These are the practical issues that we encounter each time we do field studies. I would welcome the view from people who have run into this difficulty and who wanted to comply with the international requirements for experimentation on human subjects.

CAMPBELL: I would like to make one comment. As I recall, during the country presentations there were some who mentioned that they had already instituted an ethical review board. I recall the comment from Professor Mugambi that the Kenyan Medical Research Institute already had such a group. I would like Dr. Winter to make a comment here. I do not know whether Professor Mugambi would be prepared to offer some views from the perspectives of an institution at the national level that has already created a body prepared to consider ethical issues.

WINTER: I think that Professor Bruce-Chwatt and I are not in as much disagreement as might be apparent. With respect to placebo, I think that we have an ethical obligation in phase III trials of this, as yet hypothetical, vaccine to conduct as rigorous a scientific comparison as possible to assure that whatever trial design we have is as well controlled as can be done under the circumstances. I think the discussion about placebo or nonplacebo, what the placebo might consist of, or what the control groups are may really relegate itself to a discussion of semantics. I think we are all aiming at the same purpose.

MUGAMBI: Mr. Chairman, with regard to informed consent I think that Professor Salako's concern is a correct one. I think there is no truly informed consent as such. There is always an element of a lack of true and correct information for the individual that is going to be tested. However, I think what you do is to make an attempt to explain as much as possible in the simplest language possible, including an attempt to make the technical terms understood. I believe if you have taken the trouble of explaining the trials in the local context as completely as possible you have done the best you can for passing the relevant information. If that is coupled with a review committee that has already examined the procedures and has ensured that there is no abuse, then I think this would be

considered adequate. Of course, in the case of children the guardians, including the parents, will have to understand the risks and benefits as best as possible and sign the consent form on behalf of their children.

SHEPARD: My question is to Dr. Kaseje. How far do you think we have come in dealing with such complex questions as those pertaining to a vaccine trial? Do you think we should start now with trying to inform people about the vaccine and to do that without raising false expectations?

KASEJE: I think that one of the points our group has made was the fact that communities in which vaccine trials will be implemented should be those that we have known well. This means that they are communities that have already been involved in other primary health activities and that have made their own decisions regarding the health care provided to them. So, at the time when the vaccines eventually become available, it will be unnecessary to make hasty preparations for the trials. Our recommendation was that possible trials sites should be identified well in advance of their actual conduct.

NUSSENZWEIG: I would like to address the question of placebo as a scientist. I see the question on two levels, as a moral and ethical issue, and as a scientific issue. With the ethical issue I have problems. If an effective vaccine has been developed on the basis of preliminary clinical trials, I would have trouble deciding which part of the village will receive the vaccine and which the placebo. For the scientific plan the issue is much simpler for me. If we deal with a synthetic peptide vaccine, there is a very obvious placebo. The malaria vaccine to be injected is a synthetic peptide of the malaria parasite hooked on to a carrier protein., i.e., tetanus toxoid given together with alum as the adjuvant. In this case the placebo would be tetanus toxoid plus alum. The former has been used widely in vaccine programs while the latter has been investigated thoroughly and is safe when given in a reasonable dose. From an ethical point of view, I have problems in not giving the placebo because it should not harm at all, but it might bring some benefits in this particular case.

GREENWOOD: I think Dr. Kaseje's group has done a good job in putting forward the sort of problems that one has to consider when dealing with vaccine trials. I think it is important that some of the people who are not working in the field should not go away thinking that there is something terribly special here because there are quite a number of vaccine trials going on in Africa at this very moment (e.g., rotavirus, hepatitis, a new measles vaccine, and a new pertussis vaccine). I am sure that all of those had to face the same problems, especially that of community participation. All of these trials are progressing well. There will be special problems that will have to be considered about a malaria vaccine. But I

think it is important to realize that it is not something completely new. There are a number of research groups in Africa who have had a lot experience in the areas where these trials are going on. They have been accepted by the communities and appropriate ethical procedures have been worked out. I cannot envisage that there are going to be major problems in sorting out an appropriate way of doing malaria vaccine trials. In fact, some of the other vaccines had to deal with ethical and other problems that have been even more difficult than those discussed here. So, I do not know what Dr. Kaseje feels about that. I think we do know quite a lot about how to handle some of the problems of carrying out trials with new vaccines.

CAMPBELL: Before you sit down, could you give us an example of a vaccine trial going on at this point where a placebo trial is underway and where there is a risk of exposure?

GREENWOOD: Rotavirus trials have been carried out that way. All of the ethical review committees have accepted them without restriction. There is a completely inactive placebo.

CAMPBELL: There are a number of other questions. But could we turn back to Dr. Kaseje for just a minute and let him tell us about dealing with communities on a variety of what are sometimes difficult issues. What is your feeling about a placebo trial with the perspective of a community in which you have been working for a number of years?

KASEJE: Well, that is a very difficult question for me to answer because I have not had to face that situation. I think I will find it very difficult to see how a community would participate in a situation where I, as one who is supposed to be advising them in the area of health, would give something that I know to be totally useless or ineffective in comparison with something I know to be useful in a situation where the whole community is exposed to the disease. I do not think it would be easy to convince the part of community that is going to receive the placebo to give their full consent. I am sure that they are giving their consent only if they do not fully understand what it actually means. On the other hand, if the placebo is another useful material like tetanus toxoid, then it lessens the problem. I think in that situation a placebo trial would be effective.

CAMPBELL: There is time for one more question.

OSTER: I want to address the question that Dr. Nussenzweig has raised about the ethical use of placebo. I think that withholding an efficacious product form those people who will receive a placebo is an issue we are all concerned with. But the design of trials is to show efficacy. If we knew that it was efficacious, then we would just give it to all. But we do not know the answer and that is why we are conducting trials. So, I do not

think that there is a problem in withholding an effective product until it has been proven to be so. The other aspect of this issue is that you do not just vaccinate people and turn them loose; you have to provide a system to care for the people in your trials. It becomes a matter of following these people very carefully. We follow our children on a daily basis. We take care of them when they are sick. We treat them for their illnesses. We do not let them die of malaria. We see them the first day they have malaria and give effective treatment. I think you have to take responsibility as a physician conducting such trials to provide adequate medical care so that those members of the group who receive a placebo as well as those who receive a vaccine that may not work 100% are going to be protected should they develop malaria while in your trial.

CAMPBELL: Well, obviously we will not resolve this issue today. But I think it is clear from the discussions that there are various approaches and that there is a clear issue that needs to be considered. The important issue that Dr. Kaseje has raised for us is that this is not a matter we want to postpone until we have a vaccine in our hands. It is not too early to develop close relationships at the community level that will instill trust and ensure that no matter how we design the vaccine trials they will be done both in a scientifically valid and in an ethically appropriate manner. We will now adjourn the meeting for a break.

# Workshop 3: Vaccines

N. ROSE
*Moderator*

R. REESE
*Rapporteur*

CAMPBELL: It is obvious that our morning session stimulated a great deal of interest. We would like to continue with the third of our working groups, which deals with specific issues relating to the vaccines themselves. Dr. Reese served as the rapporteur of that group and will present a summary of the recommendations made by the working group.

## MINUTES OF THE WORKSHOP

The following issues were discussed:

1. Research leading to the development of sporozoite and asexual erythrocytic stage vaccines should be emphasized.
2. Vaccines that reach phase I and II clinical trials should be evaluated to scientific completion.
3. The immune response of individuals to vaccine candidates and to *Plasmodium* infection should be studied intensively with respect to humoral and cell-mediated immunity.
4. Research on the development of new vaccine candidate antigens should proceed unabated and independent of current studies of antigens in clinical trials.
5. The highest priority should be given to the development of safe and effective adjuvants suitable for human use.
6. Methods for the evaluation of humoral and cell-mediated immune responses that correlate with and are predictive of protective immunity should be developed.
7. The vaccine potential of candidate asexual erythrocytic stage antigens should be studied singly and in combination in both monkeys and humans.
8. The development of diagnostic assays for the monitoring of the

presence of parasites during vaccine trials should be continued.

9. The potential adverse effects of immunization with candidate vaccines during clinical trials should be investigated.

## DISCUSSION

KILAMA: Why are transmission-blocking vaccines omitted from your considerations?

REESE: The group considered candidate vaccines presently developed by U.S. AID and those that are near to formulation. That does not mean that transmission-blocking vaccine should be neglected.

MEUWISSEN: The fact that the transmission-blocking vaccines are left out of the recommendations made by a scientific group like this may intimate that this approach is scientifically invalid. This interpretation may have a negative effect on administrators of institutions that have supported research on the development of malaria vaccines.

BRUCE-CHWATT: [I support] Professor Meuwissen. It would be a tragedy if this meeting would say anything adverse about practical possibilities of transmission-blocking vaccines.

COLUZZI: Many aspects of transmission-blocking immunity are not yet fully explored. One example is the possible interference of migration of sporozoites to the salivary gland of *Anopheles gambiae*

NAJERA: [I support] what has been said about transmission-blocking vaccines and that they should be incorporated in the recommendations.

NUSSENZWEIG: [I suggest that] the transmission-blocking vaccines be included in the research strategy.

CAMPBELL: Transmission- blocking vaccines may be viewed as favorably as sporozoite and blood stage vaccines, which offer personal protection to the recipients. However, there have been precedents for similar immunization campaigns with other agents.

NAJERA: The transmission-blocking vaccine that has the community as its target can be added to the other vaccines that render personal protection of the vaccinees. Insecticides are used widely for malaria control. They do not protect the individual but the community.

DEGREMONT: Curative medicine is also a part of primary health care. As malaria control can only be done effectively through primary health care, which emphasizes disease prevention, there is no contraindication to promote transmission-blocking vaccines as well.

MARTINEZ: [*Underscores Najera's statement from the point of the TDR program.*] The scientific working group for Research of the Immunology of Malaria (IMMAL) is now supporting all laboratories that work in this area. With regard to the acceptability of such a vaccine it appears highly probable that the transmission-blocking component of a vaccine would be combined with others that confer protection to the individual.

REESE: [I suggest that] statement No. 1 from the summary of recommendations be modified to include all vaccines.

BRUCE-CHWATT: [I suggest that] statement No. 1 be expanded to include all potential vaccines. The somewhat negative results of the first observations of the still ongoing first trial of a sporozoite vaccine in volunteers should be de-emphasized. In spite of disappointments work should continue on all malaria vaccines.

CAMPBELL: I think we have to move on. As the discussions have shown, there remains a lot that we do not know. I hope that none of the participants will go away feeling disappointed, but rather we should feel challenged at this stage. Let us now move on to hear the report from the epidemiology section. I think we will have several recurrent themes in terms of study design and evaluation methods that will pick up some of the issues that were raised in the first two workshops. Dr. Buck will present the report as rapporteur of the epidemiology section.

# Workshop 4: Epidemiology

B. GREENWOOD
*Moderator*

A.A. BUCK
*Rapporteur*

In the spring of 1985, the first broad guidelines for malaria vaccine field trials were issued jointly by WHO/TDR/MAP and major research institutions and donor organization (WHO document). Since then, considerable progress has been made in our understanding of the natural development of cirumsporozoite (CS) *Plasmodium falciparum* antibodies in the indigent population of malarious areas in sub-Saharan Africa. Seven projects are now in progress in which new circumsporozoite antigens of *P. falciparum* have been employed in epidemiologic studies of populations with different transmission patterns and ethnic compositions. Unfortunately, different antigens for determining CSab levels have been used in these studies. Moreover, information exchanges between the research teams in the different African countries have been deficient.

New knowledge about the magnitude of microepidemiologic variations in the antisporozoite antibody levels to *P. falciparum*, even in communities located only a few miles apart, has made it necessary to review the research strategies and study protocols for clinical and epidemiologic evaluation of new candidate malaria vaccines. In a cooperative effort, WHO/MAP and TDR have issued a document entitled "Guidelines for the Epidemiological Evaluation of *Plasmodium falciparum* Sporozoite Vaccines" (WHO 1986). These guidelines were examined in the light of new questions that have arisen from recent epidemiologic studies in Africa. The review of epidemiologic factors that are relevant to the complex study of malaria immunity in endemic areas has been the basis for the recommendations made by the workshop on epidemiology of this conference.

## ISSUES FOR CONSIDERATION

What factors of the human host, the malaria parasites, their vectors, and the environment must be controlled to prevent bias from confounding

variables when interpreting the results of malaria vaccine trials in endemic areas? How will these factors be measured and evaluated? Will a given vaccine be equally effective and safe when used in different geographic and ecologic regions?

These and other factors were examined in detail by the workshop on epidemiology. In addition to the variables usually controlled by matching or stratification in comparative epidemiologic studies (e.g., age, sex, residence), other determinants of malaria immunity need to be considered. These include genetic factors, the nature and level of malnutrition, feeding habits, coendemic parasitic infections, concomitant disease, and others.

Do multiple infections with different plasmodia constitute a problem and how can this evaluated? Will *Plasmodium malariae* become a greater problem if *P. falciparum* is reduced by immunization? What are the parameters that will yield optimal information about the efficacy of a vaccine (e.g., measurements of protective immunity under field conditions)? What is the sequence of repetition of the different types of clinical and community-based vaccine trials (phases I–III) in population groups with different risks (e.g., pregnancy, infancy, childhood, etc.) and in human volunteers of nonendemic areas of malaria (e.g., in the countries of the vaccine manufacturers, among travelers to endemic areas, etc.)?

In Africa, there are pronounced dissimilarities in the epidemiologic patterns and clinical presentation of malaria. The workshop has made a reassessment of the steps to be taken for preparing multidisciplinary epidemiologic evaluations of malaria vaccines in different ecologic settings. Table 1 comprises, in matrix format, essential data requirements for phases I-III vaccine trials. Figure 1 is a modification of the schematic diagram of malaria vaccine development shown in the original WHO document "Principles of Malaria Vaccine Trials" (WHO 1985).

## RECOMMENDATIONS

The recommendations include specific proposals for targeted research on protective immunity to malaria infection and morbidity. This can be achieved most efficiently and economically through cooperative research. Elucidation of the complex pathophysiology of malaria immunity is a goal that requires imaginative, multidisciplinary team research. It is truly an international endeavor. Following are the workshop's recommendations:

1. The methods recommended for obtaining the essential information for the different phases of the vaccine trials (Table 1) must be standardized and should be used by all investigators who participate in a multicenter network of malaria research. Initially, this should

Table 1. Information requirements for malaria vaccine trials.

| | Phase I | | | | | Phase II | | Phase III |
|---|---|---|---|---|---|---|---|---|
| | Safety, Dose Finding, Immunogenesis | | | | | Efficacy Pilot Trials | | |
| | Nonmalarious Areas | | Malarious Areas | | | | | |
| TYPE OF INFORMATION | No Previous Experience | Previous Experience | Perennial Transmission | Seasonal Intense | Mesoendemic | Nonmalarious Areas | Malarious Areas | Expanded Efficacy Trials |
| GENERAL CHARACTERISTICS OF AREA & POPULATION | Adults | Adult males (e.g., foreign students) | 1. Adult males<br>2. Children likely to be vaccinated in phase 3<br>3. Pregnant women<br>4. Other high-risk group (e.g., HIV) | | | Adults | 1. Children<br>2. Pregnant women<br>3. Adults | Larger population samples as in phase 2 (double- blind with placebo) |
| MALARIA MORBIDITY | | | | | | | | |
| 1. Species specificity | | | | | | | +(*) | +(*) |
| 2. Parasitemia and fever | | | | | | + | +(*) | +(*) |
| 3. Anemia | | | | | | | +(*) | +(*) |
| 4. Splenomegaly | | | | | | | +(*) | +(*) |
| INFECTION (Parasitemia c&s symptoms) | | | | | | | | |
| 1. Species density | | | | | | | +(*) | +(*) |
| 2. Incidence of *P. falciparum* | | | | | | | +(*) | +(*) |
| 3. Drug resistance to *P. falciparum* | | | | | | | monitor | monitor |
| PREVACCINATION IMMUNE STATUS (Stage and species-specific antibodies | | +(*) | +(*) | +(*) | +(*) | +(*) | +(*) | +(*) |
| ENTOMOLOGY: Sporozoite inoculation rates | | | +(*) | +(*) | +(*) | | +(*) | +(*) |
| COMMUNITY ATTITUDES | | | | | | | | |
| 1. Acceptance of study | | | +(*) | +(*) | +(*) | + | +(*) | +(*) |
| 2. Antimalarial drug usage | | +(*) | +(*) | +(*) | +(*) | +(*) | +(*) | |
| 3. Vector control measures | + | +(*) | +(*) | +(*) | +(*) | +(*) | | |
| 4. Feeding practices | + | + | + | + | + | | | |

Table 1. Continued.

| | Phase I | | | | | Phase II | | Phase III |
|---|---|---|---|---|---|---|---|---|
| | Safety, Dose Finding, Immunogenesis | | | | | Efficacy Pilot Trials | | |
| | Nonmalarious Areas | | Malarious Areas | | | | | |
| TYPE OF INFORMATION | No Previous Experience | Previous Experience | Perennial Transmission | Seasonal Intense | Mesoendemic | Nonmalarious Areas | Malarious Areas | Expanded Efficacy Trials |
| CONCOMITANT HEALTH CONDITIONS | | | | | | | | |
| 1. Other malaria species | | | +(*) | +(*) | +(*) | | +(*) | +(*) |
| 2. PCM | | | + | + | + | | +(*) | +(*) |
| 3. Genetic factors | + | + | +(*) | +(*) | +(*) | + | +(*) | +(*) |
| 4. HIV infections | + | + | +(*) | +(*) | +(*) | + | +(*) | +(*) |
| 5. Endemic *S. mansoni* | | | + | + | + | | + | + |
| LOCAL HEALTH DELIVERY | | | | | | | | |
| 1. EPI: what, when? | + | + | + | + | + | | + | +(*) |
| 2. Antimalaria programs | | | + | + | + | | + | +(*) |
| 3. Anemia RX with iron | | | | | | | + | + |
| VACCINE CHARACTERISTICS | Vaccine: Antigen & Vehicle & Adjuvant | | | Placebo: Vehicle & Adjuvant | | | | |

(*) Information is mandatory.

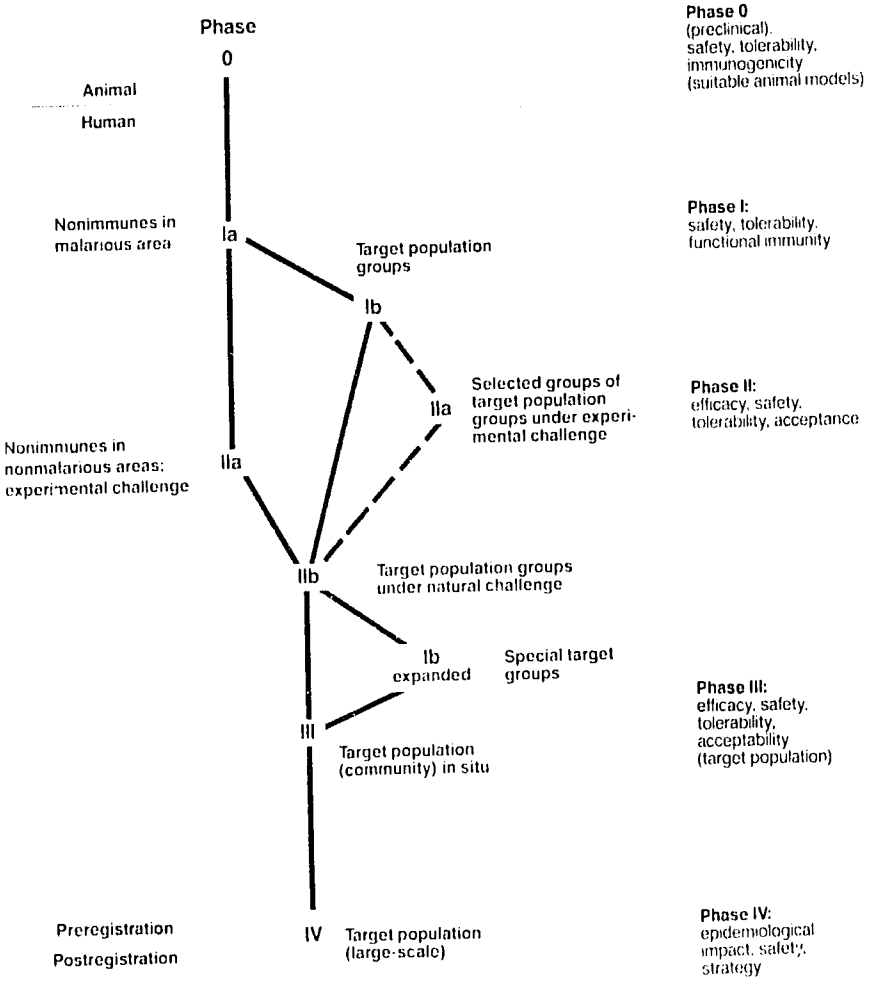


Figure 1: Schematic diagram of malaria vaccine development. (Modified from WHO document, TDR/IMMAL/FIELDMAL/VAC/85.3 Geneva, 1986.)

include the comparative evaluation of diagnostic capacities of new immunologic tests in different settings.

2. Unequivocal methods for assessing changes in malaria morbidity must be developed and validated under field conditions. Clinical malaria must be defined for malaria vaccine trials, especially in hyperendemic and holoendemic areas where parasitemia rates in children are very high.

3. A system for the regular supply of antigens and reagents to participating research centers should be established as soon as possible. Quality control of test performance should be an essential component

of the system and should include monitoring test accuracy and detecting geographic variations of the diagnostic capacity (sensitivity and specificity) of the field laboratory procedures.

4. There is a dearth of relevant scientific information in Africa. This is mainly due to poor communication between malaria researchers in different countries. To remedy the situation it is recommended that:

a. A clearinghouse should be established to facilitate rapid dissemination of important new scientific information to all malaria research teams in Africa.

b. Reports and scientific documents from regional and subregional research organizations and institutions in Africa should be abstracted and organized into scientific reviews on malaria by discipline. These summaries should be distributed regularly to all those concerned with malaria research in Africa. Examples of such reports include those prepared by regional and subregional organizations (e.g., AFRO, OCCGE, OCEAC, TDRC, EPI, SHDSI), as well as the excellent annual reports on malaria by national authorities and institutions supporting bilateral malaria research projects in Africa.

5. Predictive mathematical models for malaria control and transmission should be further developed to include malaria vaccines and potential impact in malaria control programs.

6. Annual meetings, informal consultations, workshops, and on-site visits to important field projects should be facilitate through special funds.

The members of the workshop noticed that there were various important areas of missing technology. These include, but are not limited to the following:

Proper species identification in blood sample with low-grade parasitemia;
Adequate tests to measure cell-mediated immunity to vaccine candidate antigens;
Measures of malaria morbidity;
Methods for monitoring the use of chloroquine and other antimalarials in the field;
Functional tests of the role of the spleen in malaria.

## DISCUSSION

CAMPBELL: The floor is now open for discussions of those points that you believe should be included in the recommendations. I recognize Dr. Meuwissen.

MEUWISSEN: From a scientific point of view I would like to include in the recommendations an aspect of the epidemiology of malaria that is still missing. Since Thompson's studies in West Africa in the 1950s, there has been no further work on the gametocyte reservoir in the community. We do not know how long an infected individual remains infectious for mosquitoes in the absence or presence of antibodies. I think that knowledge about this aspect of malaria epidemiology is essential for any vaccine that will be employed in the future.

COLUZZI: The point Dr. Meuwissen has raised is included in the recommendations of the workshop on vectors. I think we can return to this point after Dr. Collins' presentation.

CAMPBELL: Are there other comments? Perhaps Dr. Wurapa might be able to comment on the issue that was stressed several times here, about how we are going to work together and adequately share information. This exchange process is so critical at this time when so many good things are going on. Perhaps all of us had to come to Washington to learn about them.

WURAPA: Thank you Mr. Chairman. Actually I was going to place my remarks in the conclusion section. But I am glad that you gave me the opportunity to present my views at this time. I think that it is heartwarming in many respects to have seen the recommendations, particularly by the epidemiologic group. I can only say that the Regional Director of AFRO is quite aware of this particular problem. Our regional office in Brazzaville has been notorious over the years for not having done enough in this respect. Recently, the program manager responsible for disease control has asked all of the research institutions in the Africa region to supply the regional office with information about ongoing research activities in malaria and other parasitic diseases. I can say that we have been getting a very good response to this request. I hope that after completion of this inventory AFRO is going to get its research coordinating committee into a more effective gear to do exactly the job the epidemiology group is suggesting, create a clearinghouse. My only request to my African colleagues is that as we go back to our countries, each of the scientific institutions represented here should, please, make regular reports available to the regional office of WHO through their ministries of health. Please, do not let us have to worry about the fact that in the past you have provided these reports, but we at WHO have not coordinated the information. We want to make a new start. This is my plea. Thank you.

CAMPBELL: That was certainly a very candid and appropriate statement. I think we should all make certain that we do not forget this plea when we go back home. I know that Dr. Björkman was mentioning to me in the hall

over coffee that there were a variety of ways of looking at the complicated issues of study design that we will be facing in phase III vaccine trials. Dr. Björkman, would you care to come forward and repeat some of your comments to let other participants have the benefit of your thoughts?

BJÖRKMAN: For us in the field the ethical problem is not confined to placebo per se but rather what we have to offer to all of the subjects of a study community regardless of placebo. I want to stress two things that may be obvious but should always be remembered, namely that malaria is different from a virus disease and that it can be treated effectively. So, severe malaria may be the result of the patient seeking medical treatment or due to the delay of the doctor to give early treatment. The critical ethical issue in a vaccination trial is to minimize these events. There should be no difference in the treatment of subjects between the placebo and nonplacebo groups.

BREMAN: From the epidemiologic presentation I am impressed with the desire to develop better definitions. On the list of recommendations we go into mathematical models. We go into a lot of things that are important. But what I do not discern from the report, which is reflective of the state-of-the-art is that outcome variables in terms of vaccine efficacy have not been crisply defined. I think Dr. Molineaux's scheme is excellent but when we give a vaccine we are all concerned whether parasite acquisition is most important, or clinical illness, or mortality, or a change in immunologic indicators. I would like to ask a question of Dr. Molineaux who has written up eloquently the Garki study, which does have some analogies to reflect on these questions. Dr. Kaseje has mentioned that we should have active involvement of primary health care in these communities. We have heard of the studies in the Gambia where 41 villages were involved and of similar studies in Liberia and Burkina Faso, etc. Are these populations large enough for proper evaluation? Do we need to have active case detection with systematic specimen collection in these groups to get the right answers? Otherwise I am concerned that it will be an evaluation of immunologic responses only.

MOLINEAUX: In fact, I do not know whether I should be the person trying to answer that. The working group went into some length of discussion of the parasitologic, immunologic, clinical, and demographic end points. The one you would like to have may differ from one type of vaccine to another. If we are looking at the sporozoite vaccine then the first thing you are interested in is the presence of parasitemia. Of course, we would also like to know whether the vaccine will eventually decrease the burden of illness in the community. But in clinical trials there has to be a very close follow-up of the population under study with early diagnosis of infection and disease. So, there will be, and quite rightfully so, very little

opportunity to try to measure the severity of illness. To me it seems clear from Dr. Buck's summary that on the problem of clinical assessment of malaria we seem to be better at identifying the sequences of genes than at deciding who is and who is not sick from malaria. But some people have been working on these aspects. I thought that the working group has suggested that there should be a special way to facilitate the cooperation and sharing of experiences between investigators working on clinical assessment of malaria.

CAMPBELL: Thank you Dr. Molineaux. We have time for one last comment or question. Dr. Nussenzweig.

NUSSENZWEIG: It is a comment, and I should like state it as strongly as I can and against what has been repeated in document after document, namely that with a sporozoite vaccine you will look only at the presence or absence of parasitemia. This concept may be based, in part, on our own studies. So, let me try to put things the way we see them. We got either complete protection in animals and in humans or partial protection, which was expressed by a delay of the onset of the infection. So it is not by necessity an all-or-nothing phenomenon. The question has been raised many times. If we cannot protect completely, is there a difference in the course of infection? We have tried to reason with it because there are not much data. But if we continue to look at the issue the way this is being repeated here then we shall never get the data. I think logic tells us that if you inoculate an infectious agent in a smaller dose than you should have a more prolonged prepatent period and possibly also, a milder infection. The delay and reduction in the intensity of infection should provide the individual with an opportunity to mount his own immune response. Therefore, I do not think it is reasonable to accept the dogma that unless a vaccine protects 100%, it must be considered a complete failure. I think this is particularly important at this meeting where we are talking to people from endemic areas. Of course, in a trial of nonimmunes or very sensitive individuals you do not want to expose them and wait to see what happens before you treat. At least one should not say that it is impossible to expect that a vaccine against malaria sporozoites may not produce a partial effect.

CAMPBELL: I think it is an enormous challenge to us in terms of study design because, obviously, the study design to measure the clinical impact of a vaccine is considerably different from one that would measure sterilizing immunity only. So, it is a very, very important issue that Dr. Nussenzweig has pointed out to us. Dr. Molineaux can have the very last comment.

MOLINEAUX: I would like to reassure Dr. Nussenzweig that in our guidelines document we have considered the possibility of having delayed

the incubation and prepatent periods. It is specifically mentioned in the document that this might allow the immune response to build up . At times we may appear to be too dogmatic in our documents, but I think that with regard to the sporozoite vaccine it was not excessively dogmatic.

CAMPBELL: May I take a 10-second prerogative as chairman of the session and say that Dr. Nussenzweig has raised an extremely critical issue. There is a very real possibility that even in the early phase I trials in nonendemic areas there will be a great deal of attention to even anticipating parasitemia before it should become clinically apparent. If that is the end point that is set up in the early trials, how in the world are we going to ever see those clinical illnesses? How will these vaccines ever receive their due attention in the process of triaging among the myriads of potential vaccines and formulations that could come along. I think this is not just an issue that we can postpone until we get to phase II trials. In my mind, this represents an extremely critical issue right now, even as we move into the early, very tightly controlled phase I trials. We must move. I cannot resist, Dr. Greenwood, please.

GREENWOOD: I have just one bit of evidence that is perhaps relevant to be put here because it strongly supports what Dr. Nussenzweig is saying. Because of our studies with impregnated bed nets, which may have an effect on reducing sporozoite inoculation similar to that of a sporozoite vaccine, we see, in fact, a significant reduction of morbidity but not of parasitemia. I really think that it is terribly important that one does look at morbidity in this sort of study.

CAMPBELL: I will turn over to my co-chairman, Professor Coluzzi, to introduce the vector group. Dr. Frank Collins is the rapporteur.

# Workshop 5: Vector Biology

M. COLLUZZI
*Chairperson*

F.H. COLLINS
*Rapporteur*

Entomologic variables will be relevant both in planning vaccine strategy and in subsequently assessing vaccine efficacy. Measurement of these variables can be used to calculate the challenge faced by vaccination, the impact of vaccination on transmission, and the degree of natural exposure to sporozoites. The workshop members offer recommendations in the following five areas of entomologic measurement.

## HUMAN-VECTOR CONTACT RATES

The all-night biting collection using human baits is generally the method of choice in estimating the effective vector density. The difficulties involved in organizing reliable night-biting collections should not be underestimated. Alternative methods should be considered, such as early morning pyrethrum spray catches and double-net sleeper-type catches.

Pyrethrum spray collection coupled with bloodmeal identification is a useful method only where the vector is endophilic, where typical houses and rooms permit the use of this collection method, and when low rates of outdoor sleeping are present in the area. Double-net sleeper-type traps could be very useful, provided that possible biases due to mosquito behavior are carefully evaluated.

Whatever methods are used, collection sites should be widely distributed over the study area to deal with what are often very high levels of intravillage heterogeneity in vector activity.

## BLOODMEAL IDENTIFICATION

Commercial, species-specific, anti-IgG antibodies are readily available and have been used as the basis for a number of independently developed bloodmeal assays. ELISA methods seem to offer the most compat-

ibility with other assay techniques that will probably be used in vaccine field trials. Most methods seem to be equally effective. However, standardization should ultimately be an objective, especially where two or more studies are to be compared.

## SPOROZOITE RATE DETERMINATIONS

Continued use of both salivary gland dissections concurrently with immunological assays is recommended, at least in the preliminary stage of any major field study to determine the actual comparability of the methods. The ultimate measure of interest is, of course, the proportion of potential vectors with sporozoites in their salivary glands.

Because of observations such as those from Burkina Faso reported at this meeting, which suggest that some proportion of the vector population may not be physiologically competent to actually transmit parasites, laboratory research on the physiology and genetics of *Plasmodium-Anopheles* host parasite relationships should be supported.

Emphasis should be placed on the importance of performing sporozoite assays in manner that permits determination of sporozoite loads. Laboratory studies should be undertaken to determine the relationship (if any) between sporozoite load and size of the sporozoite inoculum.

The gonotrophic status of all mosquitoes analyzed for sporozoite antigen should be determined. Perhaps only blood-fed or biting mosquitoes should be analyzed, as empty or fully gravid mosquitoes may not accurately represent the typical, actively blood feeding component of the population. The sporozoite ELISA methodology for *Plasmodium falciparum* and *Plasmodium vivax* should be made available to the research community as rapidly as possible.

## IDENTIFICATION OF VECTOR SPECIES

The *Anopheles gambiae* complex poses a major problem that cannot be overlooked because of the presence within this complex of six morphologically indistinguishable sibling species with differing degrees of involvement in transmission. Often, two or more are sympatric. Therefore, assays such as sporozoite ELISA and or bloodmeal analysis should be performed in conjunction with species determination. Chromosomal studies will remain a basic tool, especially as they provide information in addition to speciation (i.e., association of inversions with certain patterns of vector behavior). However, this method is compatible only with certain collection techniques such as pyrethrum catches.

The cuticular hydrocarbon method of analysis may also prove valuable in a research context, as this character appears to be related to mating-recognition systems. It will not be useful as a basic epidemiologic assay, however.

A DNA probe-based method has recently been developed that can distinguish between at least four member species in the complex. The method is sophisticated and demands the support of a major research center. However, it can be applied to dried specimens of all life stages and both sexes. Furthermore, it is compatible with both sporozoite ELISA and bloodmeal determination. All these assays can be performed on a single dried mosquito. DNA methods should be further developed and adapted to nonradioisotopic labels.

Immunologic species determination assays are also being investigated. One method, which relies on antibodies to the mosquito vitellogenin protein, may prove feasible in the future. Such methods offer the distinct advantage of simplicity, and further research should be supported.

## RESERVOIR OF INFECTION

Since a transmission-blocking component is being considered as part of the vaccine development strategy, it is important that information be obtained that describes the reservoir of infective gametocyte carriers. Very little information is currently available. This information would be important both in targeting that component of the population that would most benefit from association and in assessing a vaccinated population for the effectiveness of the transmission-blocking component.

The most direct method of assessing the reservoir would be by directly feeding clean, laboratory-raised mosquitoes on members of a study community and then assessing the mosquitoes for the presence of circumsporozoite antigen after a suitable holding time. Standard sporozoite ELISAs could be used, and the reservoirs of all *Plasmodium* species present in the community could be assessed simultaneously. Blood and serum sample would be obtained from each individual concurrently with the feeding, to allow for later assessment of correlations of infectivity with gametocytemia and possible serological measures.

The nature of such a study poses a serious problem of community participation that could only be dealt with by very careful planning combined with a major emphasis on community involvement. If such a direct approach does not prove feasible, use of alternative methods, such as membrane feeding could be explored. Laboratory investigation of gametocyte-infectivity-related phenomena and sex- or stage-specific antibodies should be encouraged.

We would like to offer a final, specific proposal that the Agency for International Development consider sponsoring a workshop sometime in the future at which epidemiologic methods, both entomological and others, be the principal focus. The participants in such a workshop would be those groups involved in development and/or field applications of methods that will be used in vaccine field trials. The objective would be the development of/or agreement on standard epidemiologic methods.

# Workshop 6: Malaria Control

H. N'jie
*Moderator*

P. Taylor
*Rapporteur*

## VACCINE PREREQUISITES

The first considerations were given to the minimum requirements of a vaccine before it can be used in control of malaria:

1. The vaccine must either control mortality and morbidity in the individual or population or, through the stimulation of a sterile immunity, prevent the development of parasitemia.
2. The usefulness of a vaccine will be very clearly related to the duration of efficacy. A short-lived immune response may require frequent revaccinations with consequent risks of high implementation costs and low community compliance.
3. The acceptability of a vaccine by the population and the likelihood of successful coverage of the target group will be adversely affected by increasing the dose required to achieve the desired immune status.
4. The cost of the vaccine must be realistic and compatible with present resources available in malarious countries.
5. The candidate vaccine must be safe for the determined target groups.

## MALARIA CONTROL PROGRAMS AND VACCINE TRIALS

Vaccine trials should be carried out taking into consideration the different strategies of malaria control already in operation. Trials may therefore be recommended to be undertaken in areas where morbidity and mortality are presently controlled by the use of drugs and in areas where they are controlled both by the use of drugs and vector control methods. Areas with drug-resistant malaria may also provide useful study localities. This will provide information on the potential of vaccines to support malaria control operations in both favorable and less favorable conditions.

## INTEGRATION OF VACCINES INTO MALARIA AND EPI PROGRAMS

Due to the presence of a cold chain and the infrastructure to deliver vaccines down to the peripheral level in most countries, it is likely that malaria vaccine delivery to the periphery would make use of the Expanded Program of Immunization (EPI) system.

Delivery of the vaccine to the target population from the peripheral EPI unit may be through the existing malaria services or through the EPI services. However, it would be preferable to integrate malaria vaccination programs into the existing EPI system and strengthen this system where necessary.

The stability of the vaccine, the required revaccination frequency and the target age groups could all have a significant impact on the feasibility and potential application of malaria vaccination for malaria control.

When malaria vaccines become available, they should be studied for compatibility with other EPI vaccines.

## ISSUES RELATED TO IMPLEMENTATION OF VACCINATION

The ideal vaccine would achieve sterile immunity for a long period of time. However, this may not be feasible and a vaccine that allows the persistence of parasitemia but reduces morbidity may be acceptable and useful, especially if targeted to high-risk groups.

The epidemiology workshop report will elaborate on the requirements for measuring success of vaccination in vaccine trials. Vaccination as part of malaria control operations can be evaluated by routine or intermittent assessment of mortality and incidence of malaria, parasite load, splenic index, and infectivity of vectors. These are considered to be feasible in most endemic countries. Changes in the population perception of malaria as a disease may provide useful additional data on the changing status of malaria as a community problem.

The integrated nature of malaria control programs may preclude the routine assessment of the contribution of individual parameters such as drug availability or individual prophylaxis, which may require specific in-depth studies.

It is recommended that immunological studies be undertaken on the immunosuppressive effects of *P. falciparum* infection as this may affect the host response to a malaria vaccine in a person with concomitant infection. The role of AIDS, maternal antibodies, malnutrition, and pregnancy in immunosuppression needs to be examined in the context of a malaria vaccine.

For the evaluation of malaria vaccine trials special attention may need to be directed to the criteria required for a diagnosis of malaria. The variation in symptoms of malaria, e.g., by age and immune status is recognized, however, it remains an important objective to improve the criteria for the diagnosis of malaria especially at the peripheral level of the health service.

It is unlikely that a vaccine alone will control malaria; thus, it is appropriate to conduct vaccine trials alongside other existing control measures. A properly designed field trial in such a situation should control for variables such as existing drug availability and vector control programs so that the vaccine is the controlling variable.

It is recommended that further studies need to be carried out on the immunopathology of malaria in pregnancy, especially primigravidae, and its role in severe or complicated malaria.

The standard procedures for the evaluation of safety and toxicology of vaccines shall be followed.

## BASELINE DATA REQUIRED BEFORE FIELD EVALUATION OF VACCINES

The following baseline data are required before initiating field evaluation of vaccines:

1. Well-defined populations with clear epidemiological profiles are essential.
2. Determination of hematological disease, seasonal patterns of incidence, mortality, morbidity, spleen index, vector populations, and inoculation rate is desireable. (Modern techniques should be used.)
3. The attitude of the population to malaria and vaccination programs should be elucidated through sociological and socioeconomic studies.
4. The population in study areas should be motivated by health education prior to commencement of trials.

## DISCUSSION

BRASDING-BENNETT: A point not considered by the epidemiology workshop is the need of correlating the vaccine trials with EPI and other health programs. An example of possible interference will be that from maternally acquired antibodies. So, we cannot assume that we will get an adequate immunological response in very young children during the first year of life when we would like to give the vaccine. These are issues that need to be addressed in the vaccine field trials.

HARIDI: I wonder whether we should think of ways of analyzing the cost benefits of using a malaria vaccine for malaria control versus conventional methods of intervention.

TAYLOR: We did not actually consider this topic per se. Although cost is obviously a very important factor, it is very difficult to measure it at this time. It will all depend on how the vaccines will be delivered in a given country, whether vertically or to target populations through an already existing EPI program.

CAMPBELL: Professor Kilama, representing one of the largest countries in Africa, could you give us some comments from your large experience; whether it is even appropriate for us at this point to think about a delivery schedule for a malaria vaccine we have never seen and do not know what it will look like?

KILAMA: I think before delivery one should seriously consider the establishment of a network of health services that would be in a position to deliver the vaccine readily to the target populations within a walking distance of, say, one-half hour. The strengthening of health services for the regular and equitable distribution of the vaccine would be of paramount importance. The question was also raised about integrating this service into a regular expanded program of immunization. I think that this would be absolutely essential. We would face tremendous problems if we were thinking of having a health delivery system that would have to rely on mobile services.

SHIFF: Certain priorities must be established when providing immunization to people with different levels of malaria transmission. It seems that in areas with unstable malaria the definition of illness due to infection is not too difficult. It is, maybe, into those areas that the first priorities for use of a malaria vaccine should be directed.

IMBOUA-BOGUI: Before addressing the logistics of a national malaria vaccination program, I think that we should be able to define the properties of the vaccine. Most of the time vaccines are developed in northern countries where temperatures are very different from those in our own countries. I think we have to give this due consideration and must discuss the preservation and transport of vaccines in tropical countries.

N'JIE: In our discussions of how a new malaria vaccine will fit into the national disease control program we have to decide what will be given to whom and how to use the new vaccine within the context of the existing health infrastructure and financial constraints. In a country like the Gambia there is only a certain amount of finance available. So if a new immunization procedure becomes available, the government will proba-

bly have to decide whether it has to give up a part of an existing program and to load another new antigen onto an ongoing EPI program without the risk of reducing its credibility in the population.

TEKLEHAIMANOT: The approach and means of a malaria vaccine delivery may vary from country to country. The EPI infrastructure may not be adequate and the malaria service should become involved.

TAYLOR: Vertical programs may be used to strengthen horizontal programs. Under conditions of unstable malaria as exemplified by situations in Zimbabwe and Ethiopia malaria vaccines could strongly improve malaria control. A malaria vaccine may have limited applications and could be employed as one of various control measures to specific target groups.

BJÖRKMAN: I agree. In areas with stable malaria a blood stage vaccine may be preferable, while a transmission-blocking gametocyte vaccine could be helpful in unstable regions.

# Workshop 7: Manpower and Training

J.H.E. Th. MEUWISSEN
*Moderator*

R. LEKE
*Rapporteur*

Manpower development is fundamental to the success in any aspect of malaria control. The group therefore feels that it is of paramount importance that each country committed to malaria control should have a multidisciplinary team of professionals and a support staff of specialized technicians.

The task of the team wherever feasible may be the direction of an experimental malaria control program using vaccine but primarily it should be the nucleus of a national team to coordinate, direct, and implement malaria control operations as part of a comprehensive national health program.

The training of the team, inclusive of curriculum development, should be under the auspices of an international board.

## COMPOSITION OF A TEAM OF PROFESSIONALS

The team of professionals should be composed of the following:

1. Director with basic training in public health with emphasis on management;
2. Epidemiologist;
3. Biostatistician;
4. Clinician;
5. Entomologist;
6. Parasitologist;
7. Immunologist;
8. Social anthropologist;
9. Medical laboratory technologist.

The team would be officially appointed by the national government prior to its training. It should be understood that ultimately this team should be

duplicated in order to guarantee full continuity of the program. The second team could be allocated to a specific task in the national program.

### Training of Professionals

The training program would be composed of two parts: (a) A specialized training in the team member's particular discipline, which would be followed by (b) a comprehensive course in the various fields represented by the team. It is envisaged that this combination would lead to an internationally recognized degree in public health.

### Specialized Training

It is preferred that specialized training be given wherever possible at a center of excellence in Africa. These centers would be linked to institutions of international repute that are abroad; these institutions would actively participate in the specialized course and especially in the comprehensive course.

For this purpose, an international board should be set up to identify and accredit such centers in Africa. Selection and accreditation of centers abroad would also be carried out by the board.

### Comprehensive Course

The comprehensive course would be given at centers in Africa selected by the board. Admission to such a course should be on a team basis. This is emphasized because of the need that the group function be identified as a multidisciplinary body. The instructors from centers abroad should be requested to participate in the relevant parts of the comprehensive course.

### Training Time

It is estimated that the training program will cover a two-year period. This would include 1 1/2 years of specialized training, which would be followed by a 6-month comprehensive course. All aspects of the training should be practically oriented.

## COMPOSITION OF A TEAM OF TECHNICIANS

The size of the support staff will be governed by the extent of the program covered. However, we recommend that specialized technicians be available in the following fields:

1. Maintenance engineers;
2. Demographers and cartographers;
3. Computer programmers;
4. Nurses and health assistants;
5. Entomology technicians;
6. Immunology technicians;
7. Parasitology technicians;
8. Field assistants for social anthropology;
9. Secretaries.

### Training of Technicians

The international board should identify and accredit centers in Africa where such personnel can be trained. This training should cover a period of approximately 6 months to 3 years, depending on the skills required.

## FINANCIAL SUPPORT

Costs should be allocated according to requirements in the following areas:

1. International board;
2. Institution support, including all aspects of instruction;
3. Consultants;
4. Student stipends;
5. International and in-country travel;
6. Curriculum development.

## DISTRIBUTION OF TASKS

Table 1 is a comprehensive summary of the relative tasks of the members of the team of professionals and the support team, with regard to field operations, laboratory studies, and management of the program.

## DISCUSSION

Kakoma: We have been talking here about the planning of delivery systems almost at the assumption that the vaccine is just around the corner. While I would like to share in that optimism, I am afraid that the

Table 1. Relative tasks of the members of the team of professionals and support staff.

| | Field | | Laboratory | | Management | |
|---|---|---|---|---|---|---|
| Discipline | 1 | 2 | 1 | 2 | 1 | 2 |
| Directorate | + | – | + | | + + + | a/b |
| Epidemiology | + | c | + | d | + + | – |
| Biostatistics | + + | c | + | d | – | – |
| Clinic | + [a] | e | | – | + | – |
| Entomology | + + | f | – | f | + | – |
| Immunology | + | – | + + | g | – | – |
| Parasitology | + | h | + + | i | + | – |
| Socioanthropology | + | j | | – | + | – |
| Clinical Chemistry | | – | + | k | – | – |

Relative levels of effort by professionals (*1*) and support staff (*2*) will range from none (–) to high (+ + +).

[a] Maintenance engineer
[b] Secretaries
[c] Demographers and cartographers
[d] Computer programmers
[e] Nurses and health workers
[f] Entomology technicians
[g] Immunology technicians
[h] Microscopist
[i] Parasitology technicians
[j] Field assistants
[k] Laboratory technicians

very infrastructure that Dr. Leke proposes would probably have to wait for a long time before it is realized in most parts of Africa. I am not at odds with Dr. Leke's comments regarding the specified periods of training. I am only saying that they are very hard to realize and that there are organizations in Africa that can coordinate the training.

LEKE: The document emphasizes the importance of training in African institutions whenever possible. That is the priority. When such centers are not available there will be links with other centers abroad. I think that aspect of it was made very clear in the paper.

SHEPPERD: Pursuant to our training area we have done a survey of about 29 institutions in Africa to look what could be done in terms of strengthening them to the point that the kind of program described by Dr. Leke could be carried out. It is true that there are some centers of excellence, but we found that many of them could use a great deal of strengthening. It seems to me that if a malaria vaccine is about three to five years away we have to start now to prepare the institutions and the governments to be

able to handle vaccine trials and whatever may follow. The combined training abroad and in Africa is a good suggestion.

KILAMA: Are we proposing that the specialists listed in the document would be trained for each and every African country that has malaria as a major health problem?

LEKE: Yes. We are proposing here that these people form the team for malaria control in each country.

KILAMA: In six years of efforts we have been unable to build up such a strong team. How will this be possible for every country? Could this be limited to a few countries where this would be feasible?

MUGAMBI: I think, Mr. Chairman, I must defend some excellence. I think that it should be our aim to build strong institutions and create appropriate mechanisms for implementing effective disease control programs. Whereas nobody is saying that within the next five years you should have a list of experts as is given, I believe that one should move in that direction in stride, seeking the necessary assistance as much as possible to build up a really good team. Because I do not think we can accept second rate situations in Africa.

WURAPA: I just like to point out here that as in the case of epidemiologic information gathering, coordination is what is needed most. I think that this training workshop group has made recommendations that should be considered, at least from the standpoint of the Regional Office of WHO. We do already have regional centers. I would like to mention here particularly the Special Program for Research and Training in Tropical Diseases and their training program in Africa. What I am suggesting between what Professor Mugambi has proposed and Professor Kilama has said is the need for continuation of support for each center in our region, regardless of whether they are in malaria, leishmaniasis or in non-tropical diseases. For me the practical and useful thing to do now lies between the two extremes, i.e. centers of excellence and sufficient resources to build and maintain them. In malaria we can probably only have two or three centers for training. Other centers will concentrate on other diseases and health problems. I think that this suggestion reflects the present opinion of the Regional Office. We have a long way to go and we must encourage the present institutions to continue to improve. By all means, let us keep the information flowing between yourselves, the research centers and institutions , as this very meeting on the African problem of malaria has made possible for the last 4 days.

BREMAN: Few countries have malaria coordination bodies. The staffing of malaria activities is important to address. But vertical structures are very expensive and may be beyond the financial capacity of a country.

SALAKO: There are locally trained people that could be made available for additional instruction and development of new skills quite inexpensively in African institutions through TDRC.

CAMPBELL: Could I take the prerogative just to ask Dr. Najera if he could share with us his views about the priorities of specialized training and institution building with respect to malaria in Africa in general and the perspective of malaria vaccines in particular.

NAJERA: I do not think that I can speak for Africa. We have heard Dr. Wurapa telling us about their plans of building a network of collaborating institutions that should be strengthened and made available for training. From the point of view of malaria control we, definitely, have to create a nucleus of expertise to support government development and planning. Due to the lack of uniformity of the epidemiological features of malaria and the availability of resources in Africa the constitution of this expert group will vary with local conditions.

MEUWISSEN: In the deliberations of our working group there was unanimity about two major goals for the training of the malaria expert groups: the concept of team work and the high quality of technical support to the national malaria control programs.

# Workshop 8: Diagnostics[a]

T. REESE
*Moderator and Rapporteur*

A need for diagnostic tests for malaria was given a high priority by all. Assays must be developed that detect parasites in the blood (disease) and other assays that determine the antibody levels to specific antigens (to determine the level of immunity). Because of the concern with handling blood samples (because of AIDS), consideration of urine assays, etc., were mentioned.

The assays should:

1. Provide quantitative data;
2. Be standardized against absolute standards so that assays from different laboratories can be compared in an objective fashion;
3. Be approximately as sensitive as the slide assay (i.e., detect one parasite in $10^6$ RBC's);
4. Be simple to perform and cheap;
5. Require minimal equipment;
6. Be specific for different stages of the parasite.

This last consideration is particularly important for workers to be able to detect and differentiate circulating gametocyte from asexual blood stage parasites. DNA probes are already being tested in some areas. Alternatives to 32p need to be developed to make this approach more appropriate.

[a]This summary is based on discussions of a small group of interested persons who convened an informal workshop after working hours. The discussions were not recorded.

SECTION V

# CONCLUSIONS AND RECOMMENDATIONS

I. SALAKO AND B. GREENWOOD
*Chairpersons*

# Perpectives for Malaria Control in the Next Decade

DAVID F. CLYDE

In 1984, the most recent report year, there were said to be around 5 million cases of malaria among a population at risk numbering 385 million in African countries south of the Sahara. The actual number of infected individuals is considered to be at least 78 million. Assuming unimproved malaria control methodology, a projection of the reported incidence suggests that by 1995 the reported number will exceed 6 million and the actual number (based on a 3.3% annual increase in the population at risk) will be at least 114 million and probably very much more—the incidence being at least 202 per 1000 at risk.

While the main increases in malaria incidence naturally affect the indigenous population, many people are to a considerable extent protected by infection-induced immunity developing since early childhood. The individuals principally endangered are infants and small children, and pregnant women whose immunity is temporarily reduced.

It may be expected that residents of highland districts or of cities, where transmission is at lower levels than in the savanna or even forested areas, will also be [illegible]nhanced risk should they travel. Among nonimmune visitors from [illegible]tries outside Africa, there has been a steady increase in *Plasmodium falciparum* malaria, an increase that may be projected steeply. For example, most of the malaria developing in Americans traveling abroad (44% of the cases) is being acquired in Africa, and since the overwhelming majority of the transmission in Africa related to *Plasmodium falciparum* it is this most serious type of the disease that most frequently occurs. *P. falciparum* malaria has been diagnosed to the following extent in Americans returned from Africa: in 1983, 99 cases; in 1984, 109 cases; in 1985, 158 cases. In view of the diminishing efficacy of the prophylactic antimalarial drug generally taken, chloroquine, this incidence may certainly be expected to increase in the future.

Indeed, the effect of the spread of chloroquine-resistant *P. falciparum* malaria across Africa from east to west is already apparent among visitors. In 1985, when this type of infection had not yet reached West Africa, the *P. falciparum* attack rates in Americans visiting one or the

other coast were analyzed. Of those visiting Kenya, where resistance has become established, 0.5% developed *P. falciparum* malaria whether or not they were taking chloroquine prophylactically. Of those visiting Ghana or Nigeria, nearly 1% not taking the drug developed the disease, whereas only 0.06% of those taking the drug acquired malaria.

## PROBLEMS OF MALARIA CONTROL

In Africa more than in any other region the extent to which malaria may be controlled depends not only on the efficacy of the methods that are or will be available or on the ability to apply these methods in the many variable situations that exist, but also on the availability of funds to support the operations directly and to build up the network of primary health care facilities on which the operations must be based.

### Financial Consideration

It is accepted that the control of malaria in the rural African environment must depend on operations undertaken through primary health care centers, and the full involvement of these centers and the communities that they serve is essential to the success of the antimalaria operations. The principal need is for improving diagnostic and treatment facilities, as well as educating the community in methods of personal protection from the vectors and the disease. A lack of accurate diagnosis of people suffering from fever is responsible at present for enormous wasted expenditure on antimalarial drugs; in some places as many as 80% of the fever cases given these drugs do not actually have the disease (although virtually all will have parasitemia in very low density).

The very large expenditure on antimalarial drugs notably chloroquine may be estimated from the cost incurred by purchases made by national malaria control services. In 1984, for their programs in Africa these services purchased 159,000 kg base of chloroquine (68% of the antimalaria services purchases of 4-aminoquinolines worldwide) for distribution as presumptive or prophylactic treatment of fever cases (this figure does not include the quantities obtained for use by the basic health services nor the amounts purchased privately in the marketplace). The cost of the 159,000 kg of chloroquine was U.S. $10.6 million. With respect to quinine, 4066 kg base (48% of the world's purchases by the antimalaria programs) was obtained by the African services at a cost of $0.5 million. Considerable variations exist in the quantity of drugs used in individual countries. In 1984 or 1985, the countries reporting the greatest use of chloroquine were Tanzania (22 tablets per head of the population annually), Malawi and

Zambia (17 each), Senegal (8), Nigeria (6); distribution of the drug in these and the other countries depended on malaria control policy as well as incidence of the disease.

Expenditure on antimalarial drugs will be markedly increased should parasite resistance to chloroquine force the introduction of alternative compounds, all of which are considerably more costly. Based on the use of these drugs in properly diagnosed cases of malaria, the cost of chloroquine needed to treat every case in sub-Saharan Africa in 1984 would have been 3.9 million. (Note the discrepancy between this figure and the much higher one for provision of chloroquine for presumptive treatment of fever cases and for prophylaxis, indicated in the preceding paragraph.) By 1996, in parallel with the expected increase in population at risk (and ignoring inflation), it would rise to $5.7 million. The corresponding costs of alternative compounds in 1984 and 1996, should chloroquine have to be replaced, are as follows: amodiaquine, $9 million rising to $13.3 million; sulfadoxine-pyrimethamine (Fansidar), $15.6 million rising to $22.8 million; quinine, $60.5 million rising to $86.4 million; quinine with tetracycline, $77.2 million rising to $112.9 million: and mefloquine with sulfadoxine and pyrimethamine, $129.9 million rising to $189.8 million.

A similar escalation in cost applies to malaria control situations in which residual insecticides are used: as the vectors become less susceptible to DDT, alternative more expensive products must be employed. However, with respect to Africa such measures are applied on a limited basis usually in periurban situations or in special development areas, as are measures directed against mosquito larvae.

The rising costs of measures to combat malaria have to be weighed with those required to maintain other essential health services. In the 31 low-income countries, the average expenditure per person annually for health care in general ranges between $1.75 and $4 (1 or 2% that of industrialized countries). This is quite inadequate to pay for the social infrastructure needed to satisfy such basic necessities as sanitation, water supplies, housing, or food supplements, let alone provide the network of primary health care centers through which endemic diseases including malaria may be tackled. In many countries less than 5% ($0.20) of these health funds are allocated for each of the 90% of the population living in the rural areas where malaria is transmitted, while the overwhelming balance of the funds goes to maintain hospitals and health services in the cities.

Compounding the shortfall of funding of rural health services is the rate of population growth and its attendant damage to the fragile African ecosystem, an 80% increase in population being projected between the years 1980 and 2000. This has already led to intensive livestock grazing,

cultivation of marginal land, and deforestation, all of which result in spread of desert condition alternating with flooding and pooling, providing a plethora of new mosquito breeding sites among a progressively less adequately nourished peasantry. Projections of the malaria situation, and the costs of measures that will be required to cope with it, must take into account the unlikelihood of any dramatic reversal of the deteriorating demographic and related ecological situations that will occur during the next decade.

## Technical Problems

The principal technical problems confronting antimalaria programs in the world at large include reduced vector susceptibility to insecticides, which further complicates difficulties caused by householders refusing access to sprayers, complaints of pollution of the environment by the insecticides, and parasite resistance to antimalarial drugs. In the African situation, vector control by use of insecticides has relatively little importance, but resistance by *P. falciparum* to the drug most widely and safely used everywhere, namely chloroquine, is an increasingly serious problem.

The rapid march westward across Africa of chloroquine-resistant *P. falciparum* (CRPF) suggests that within the next decade this strain will be present in all transmission areas, although its exact prevalence in relation chloroquine-sensitive strains cannot be predicted. At the expanding periphery of its range, the grade of resistance of CRPF tends to remain low, and it is still amenable to some degree of control by chloroquine or amodiaquine. At the core areas where the parasite has consolidated, however, hybridization progressively raises the grade until chloroquine in the full recommended dosage regimen of 25 mg base/kg, let alone the commonly used (and more economical) regimen of 10 mg/kg, is often ineffectual, and cross-resistance to related compounds becomes manifest. The keystone of antimalaria work in Africa has been chemotherapy using chloroquine, and the implication of loss of effectiveness of this invaluable compound are evident. Provided, however, that CRPF stabilized at a grade of resistance compatible with clinical though not necessarily parasitological cure, or remains restricted to small and well-demarcated foci, then the place of chloroquine may be maintained.

The introduction of alternative drugs to chloroquine, should CRPF become widespread and strongly resistant to the 4-aminoquinolines, will have to be considered. The increase in cost has already been mentioned. In addition, problems of toxicity of some of the other drugs may restrict their use to clinics and hospitals, rather than at the periphery of the primary health care system. A further problem is that *P. falciparum* is beginning to show resistance to some of these drugs.

### Population Access and Popular Acceptance

Lack of communication and inaccessible populations have been major physical constraints to the inclusion of most sub-Saharan African countries in the malaria eradication campaigns of past years. While communications have greatly improved and will no doubt continue to do so, utilization of the expanding network of rural primary health care centers increasingly facilitates access to communities. Acceptance by the inhabitants of antimalaria measures based on early diagnosis and treatment of infections, and personal protection against mosquito biting, requires an intensification of the health education activities of primary health care center staff and village health communicators.

### Epidemiologic Projections

Aside from expected increases in population, there is little reason to anticipate remarkable changes in the epidemiologic factors relating to malaria transmission. The human reservoir of plasmodial gametocytes in the individual is unlikely to vary much, reflecting the stability in levels of acquired immunity in people of different ages. Such epidemiologic changes as may occur will relate mainly to the vectors. The damage to ecosystems being increasingly perpetrated by the growing human population, in particular the deforestation and overgrazing by cattle, is resulting in multiplication of the breeding sites of major vectors especially those of the *Anopheles gambiae* complex.

The almost universal consumption of chloroquine tends to ensure the dominance of *P. falciparum* over the other plasmodial species, since this drug and its chemical relatives destroy the gametocytes of the other species but not those of *P. falciparum* whether sensitive or resistant to the 4-aminoquinolines. The impact on transmission of malaria control measures applied though the primary health care system may be expected to be limited, but deployment of a successful vaccine could affect the epidemiologic situation particularly in mesoendemic or epidemic areas.

It will be necessary to keep abreast of changing epidemiologic situations by undertaking demographic and malariometric surveys. While the simplest of the latter will continue to be the morbidity returns arising from the clinical work of the primary health care centers (attempt should be made to focus mortality statistics more sharply, but exact causations will often remain uncertain), the present limited use of sophisticated seroepidemiological and entomological methods should be increased. Surveys to stratify epidemiologic situations, utilizing these new methods, should be undertaken by the specialist personnel supporting national programs. The entomological methods, particularly those relating to definition of sibling *Anopheles* species and those involving problems to

identify sporozoite loads and species, will be essential components of malaria vaccine trials and of programs involving operational use of the vaccines.

## APPROACHES TO MALARIA CONTROL

Of the two general approaches to malaria control, one is widely applicable in sub-Saharan African countries at this stage in their economic development. The two approaches are based on local epidemiological situations, tempered by available financial resources and logistic feasibility.

The first approach has three components:

1. Improvement of the primary health care system to ensure prompt diagnosis and early treatment of cases, as well as protection for groups at special risk, such as pregnant women;
2. Promotion of personal and community protection through health education;
3. Strengthening of the referral and epidemiologic information systems.

The objectives of this approach are reduction in malaria mortality and morbidity, with a capability of referral of serious cases and of notification of unusual situations such as development of drug resistance or threat of epidemics.

The second approach involves establishment of a capability for long-term control of malaria transmission, with the objective of altering the epidemiologic equilibrium and maintaining it at the improved level.

In the context of most African countries, the first approach is the more feasible, although in certain periurban and special development areas programs to alter and improve the epidemiologic equilibrium may be applicable. The antimalarial activities of the primary health care centers and their dependent communities will be designed and supervised by specialists within the national public health service at various administrative levels. An essential component, therefore, is establishment of an information system between the centers and the district, provincial, and headquarters offices.

## IMPLEMENTATION OF MALARIA CONTROL

Implementation of malaria control through the first approach indicated in the preceding paragraph involves the following activities on the part of the primary health care center. Where not already being implemented, these activities should be promoted as first-line tactics in the coming decade.

### Reduce Contact Between People and Mosquitoes

To reduce human-vector contact, duties should include (*a*) instructing and advising the community on measures to avoid building houses near mosquito-breeding sites; (*b*) clearing vegetation that provides shelter for adult and larval stages of mosquitoes; (*c*) draining small collections of water in which mosquitoes might breed (for psychological reasons culicines as well as anophelines should be reduced); (*d*) deviating mosquitoes to feed on domestic animals by stabling these animals between houses and mosquito breeding sites; (*e*) preventing ingress of mosquitoes to houses by installing screens and bed nets; (*f*) destroying mosquitoes in houses by using insecticidal aerosol sprays (flitting with pyrethins) and impregnating nets, curtains, coils, or mats; (*g*) employing measures for personal protection against mosquito bites, such as protective clothing and repellents.

### Provide Antimalarial Treatment

Community health volunteers and personnel of the primary health care centers are responsible for obtaining blood films from people suffering from fever; the films are examined microscopically at the centers. Patients with clinical symptoms of malaria and parasitemia are usually treated with chloroquine, but while the dosage regiment of 25 mg base/kg may be recommended, for reasons of economy and ease of administration the quantity most widely used in Africa is 10 mg/kg. In view of the rapid westward spread across Africa of CRPF, a spread facilitated by use of subcurative doses of chloroquine, it will be increasingly difficult to justify such a policy. Complicated cases are, when feasible, referred to the next higher clinical level, which is the district hospital. Individuals at unusual risk, notably pregnant women, may be advised to take prophylactic treatment. In special circumstances, such as epidemic outbreaks, emergency vector control measures might be accompanied by mass chemoprophylaxis, including gametocytocidal treatment with primaquine.

Should it prove necessary or desirable to introduce more complex intervention methods—such as house spraying with residually acting insecticides, outdoor spraying to reduce vector populations rapidly, or mass chemoprophylaxis or vaccination campaigns—primary health care centers would be responsible for informing and mobilizing the communities and assisting experts in the work.

### Include Vaccination as Part of Control Program

The operational advent of protection-inducing malaria vaccines will follow upon successful field trials, and may be expected to occur in the next few

years. At present, phase I (tolerance and immunogenicity) trials of potential sporozoite vaccines are in progress among nonimmune volunteers in the United States. Deployment of the various types of vaccine, singly or in combination, will be limited by cost as well as by suitability for different target populations. Initially the vaccines are most likely to find a place as adjuncts to existing control measures implemented through the primary health care systems.

The three categories of vaccines that may become available, each specific for one species of plasmodium and for one stage of that species, act to produce antibodies to sporozoites (providing causal prophylaxis), to asexual erythrocytic stages (providing clinical suppression), and to gametes and zygotes in the mosquito (blocking transmission). In the African situation, the populations that would most merit protection include residents of endemic areas, among whom particular attention would be paid to pregnant women and small children (full protection of the latter having to be balanced against the possibly undesirable effects of delaying acquisition of natural immunity to asexual erythrocytic stages). Other categories include nonimmune immigrants and visitors to the endemic areas. The need for periodic revaccination may be less in areas of high transmission where the naturally induced sporozoites or the blood stages serve to maintain the stage-specific antibodies at the protective levels induced by the initial vaccination.

Use of vaccines for the control of transmission might be applicable in those parts of Africa where endemicity is relatively low, and epidemics are a threat.

## CONCLUSION

It is apparent that developing countries, whether in Africa or elsewhere, have to provide a package of health care compatible with local community needs and within extremely limited national budgets. The health package (most of which is inflexibly committed to maintaining the prestigious and costly clinical services in the cities) contains the malaria control component, support of which has to be reconciled with demands for funding other preventive services. In Africa these financial considerations combine with severe operational and technical difficulties to preclude interventions aimed at reducing malaria transmission. However, reductions in malaria morbidity and mortality will result by (*a*) expanding the primary health care network throughout rural areas of Africa, (*b*) basing the centers' malaria control methodology on early case diagnosis and treatment, (*c*) educating the community in self-protection from mosquito bite, and (*d*) establishing referral and epidemiologic information systems. This approach, integrated as it is with other public health activities, may be strengthened during the coming decade by the selective addition of vaccination programs.

# Malaria Control and Primary Health Care: A Global Concept

J. A. NAJERA

The early recognition of malaria's relation to certain environmental conditions, either as they occurred in some areas or as they developed when attempting to improve agricultural production, led humans to attempt to control the disease by environmental manipulations. Consequently, besides the classical pestilential diseases, malaria control was one of the earliest objectives of public health activities.

Since the end of the 19th century, the discovery of the specific causative agents and their mode of transmission provided more solid foundations for scientific control. Because scientific control showed considerable success from the beginning, in the 1930s malariologists started to think seriously about possible eradication of the disease. Although malaria had been progressively disappearing from Europe and North America since the 19th century and greatly diminished under active control measures, it was not till the introduction of DDT as a residual insecticide in the 1940s that actual eradication from extensive areas seemed achievable.

The new spraying techniques were soon standardized and adopted by every organized malaria control program with remarkable success. This success was attributed to the new technology, thereby underestimating the influence of the infrastructure that had applied it, and the stage of socioeconomic development on which that infrastructure was based. In 1954 the Pan American Health Organization (PAHO) and, in 1955, the World Health Organization (WHO) adopted programs for the eradication of malaria from the Americas and the world. Early successes of the global program, although slower than expected from previous experiences, were remarkable, even if the feasibility of eradication from Africa was seriously questioned and serious problems were recognized in areas of Asia and the Americas since the late 1950s.

Problems persisted and became more obvious as areas started to move into the consolidation phase of the program, when it became apparent that, although it was possible to interrupt malaria transmission under the protection of DDT spraying, the maintenance of a malaria-free

situation could not be accomplished in the absence of a solid health infrastructure and that most of the malaria endemic countries lacked such infrastructure. It was therefore recommended that malaria eradication in developing countries should be preceded by a pre-eradication program that should include "the promotion of a parallel correlated development of rural health services to assure the effective implementation of the consolidation and maintenance phases of the future malaria eradication programs" (WHO 1961).

As early as 1962 it was recognized that there were some areas, which started to be called "problem areas" (de Zulueta and Garrett-Jones 1965, WHO 1964), where eradication might not be feasible with standard methods. Such areas, which included tropical Africa, were progressively identified in the Americas and in Asia: they included such varied problems as the widely dispersed populations in tribal areas; areas of sociopolitical disturbance; areas where the vectors were not amenable to control either because of intense and multiple resistance to insecticides or because of evasive behavior; and areas where malaria parasites were resistant to antimalarial drugs. Progress towards eradication was considerably slowed down during the 1960s (Bruce-Chwatt 1969), and localized resurgences of transmission occurred in several parts of Central America and Southeast Asia in consolidation or maintenance phase to culminate, at the end of the decade, with a massive epidemic in Sri Lanka, where malaria had been virtually eradicated.

The problem was defined as one of "near success in an environment with an excess of problems clamoring for attention. As malaria recedes to a low level other pressing health and social problems exert irresistible demands for available resources" (Scholtens et al. 1972).

This situation led the World Health Assembly in 1969 to recognize that global eradication may not be feasible in the programable future in every country of the world and therefore encouraged countries not able to carry out and maintain malaria eradication, to develop malaria control programs within their health services development policies (WHO 1969).

Following the recommendation of the World Health Assembly, member states, with the support of UNICEF, AID, and WHO, organized multidisciplinary reviews of their antimalarial programs to assess the feasibility of attaining eradication with a time-limited program or to plan for malaria control, where eradication was not feasible. From today's perspective it is possible to realize that those reviews were subjected to a great variety of pressures and that their feasibility assessment was unduly influenced by the interest of most eradication programs to maintain their organization and their autonomy, supported by governments' fear to face labor problems if many routine antimalarial activities were discontinued. Perhaps even more serious than these extraneous influences, was the fact

that the concept of malaria control remained undefined. The Sixth WHO Expert Committee on Malaria had defined malaria eradication as "the ending of the transmission of malaria and the elimination of the reservoir of infective cases in a campaign limited in time and carried to such degree of perfection that, when it comes to an end, there is no resumption of transmission" and stipulated that "a malaria eradication program is a transformation of normal malaria control" with strict requirements of perfection (WHO 1957).

When, in the 1970s, a number of formally planned eradication programs were unable to meet the perfection requirements, it was assumed that their transformation into control programs could be an automatic process, without realizing that inadequate routine eradication activities could be practically ineffective and therefore quite far from serving as a base for building an appropriate control program. It was recognized that control programs must be planned on their own merits and either relevance to the problems involved, taking care not to confuse a control program with a patchwork of scattered eradication efforts superimposed over a background of uncontrolled transmission (Najera-Morrondo 1979).

## PRIMARY HEALTH CARE AND THE DEVELOPMENT OF MALARIA CONTROL STRATEGY

While malaria programs were finding difficulties in identifying control objectives and realistic approaches, the efforts for building basic health services was not very successful in penetrating towards the periphery. These efforts followed the pattern, similarly to the concept of global eradication, of promoting the adoption by developing countries of rationalized models based on the experience of developed countries; these efforts would be concentrated on technical considerations and would relegate issues of acceptability and affordability to the level of operational problems to be solved during implementation without influencing model design.

It was the formulation, in 1978, of primary health care as the approach to the strategy of "health for all by the year 2000" that provided the basis for the realistic development of health infrastructures. Primary health care has been defined as essential care made universally accessible to individuals and families in the community by means acceptable to them, through their full participation and at a cost that the community and country can afford. It forms an integral part, both of the cultural system, of which it is a nucleus, and the overall social and economic development

of the country. The primary health care approach identifies eight essential elements, one of which is the control of prevalent endemic diseases (WHO 1978).

Primary health care provided thus a basis for the formulation of a malaria control strategy, which as adopted by the 31st World Health Assembly (WHO 1978). This strategy is based on the following principles:

1. The national will to control the disease should be clearly expressed through a governmental decision to support antimalaria activities on a long-term basis.
2. Malaria control should be an integral part of the country's health program.
3. The feasibility and practicality of reducing malaria to a level concordant with the set objectives should be demonstrated.
4. The participation of the community should be a condition sine qua non, and emphasis should be placed on the community's understanding of the effects of the different methods of control, since success will greatly depend on this.
5. Wherever applicable, permanent measures for the control of malaria should be made an integral part of the relevant development programs (e.g., irrigation, drainage, hydroelectric schemes, highways) through the collaboration of the government ministries and agencies concerned.

In 1979, the 17th Expert Committee on Malaria elaborated the control strategy, particularly with respect to the range of possible valid objectives and the activities to attain them. As an illustration of this range four tactical variants were considered, covering from dealing only with reduction of malaria mortality through the appropriate provision of treatment facilities (variant I) to the complete eradication of the infection (variant IV).

An essential feature of this strategy is that, for the first time, the antimalaria strategy included proposals for action suitable for the situation in Africa south of the Sahara, which had been bypassed by the global eradication effort, as this goal had never been achieved under epidemiological conditions prevailing in tropical Africa. Antimalarial activities in Africa had been limited to trials and pilot projects for the study of combinations of attack measures in the hope of finding a feasible strategy for eradication.

Perhaps the most serious problem for the transformation of a malaria eradication program into one of control has been their opposing views of their time dimension. Eradication was based on a campaign approach to solve the problem forever, while control has no time limit. Eradication

concentrated on efforts to interrupt malaria transmission and did not provide for the improvement of the capability to live better in the presence of endemic malaria: that is to improve diagnostic and treatment facilities and epidemiological services, which could prevent mortality and shorten the incapacity caused by the disease. Control has to rest on the development of such capability on which a solid even if slow progress could be built.

## EVOLUTION OF THE MALARIA SITUATION

All the above changes and readjustments of the antimalarial strategy were motivated by the evolution of the malaria situation and its response to control efforts. To a certain degree the strategy formulations had important influences on the development of antimalarial activities and therefore in the evolution of the malaria problem.

The recent evolution of the malaria situation may be illustrated by Table 1 and Figure 1. Table 1 shows the number of cases reported to WHO by region since 1976, and Figure 1 shows global trends since 1964, which correspond to the period of maximum success of the global eradication effort. Unfortunately, such success cannot be documented on global figures since during the 1950s and early 1960s surveillance systems were being established and global figures lack comparability. Figure 1 shows how the considerable increase in malaria cases in the mid-1970s was dependent on the malaria resurgence in India. The evolution in China is also plotted separately as official reports started only in 1977. If China and India are excluded, the global picture is one of stagnation and slow

Table 1. Number of malaria cases reported by World Health Organization region (in thousands) for 1976–1985.*

| Region | 1976 | 1977 | 1978 | 1979 | 1980 | 1981 | 1982 | 1983 | 1984 | 1985† |
|---|---|---|---|---|---|---|---|---|---|---|
| African‡ | 5514 | 4881 | 7444 | 5720 | 7146 | 7972 | 5570 | 5636 | | |
| Americas | 379 | 399 | 469 | 515 | 603 | 630 | 715 | 836 | 914 | 885 |
| Southeast Asia | 7304 | 5540 | 4800 | 3664 | 3704 | 3459 | 2936 | 2529 | 2865 | 2487† |
| Europe | 41 | 119 | 93 | 34 | 38 | 68 | 66 | 71 | 60 | 31 |
| Eastern Mediterranean | 349 | 227 | 162 | 125 | 137 | 186 | 307 | 296 | 314 | 374 |
| Western Pacific | 2117 | 4457 | 3412 | 2706 | 3654 | 3458 | 2435 | 1839 | 1361 | 1065 |
| Total (excluding Africa) | 8294§ | 10,742 | 8946 | 7046 | 8138 | 7785 | 6461 | 5566 | 5514 | 4842 |

*The information provided does not cover the total population at risk in some instances.
†Provisional
‡Mainly clinically diagnosed cases.
§Excluding China.

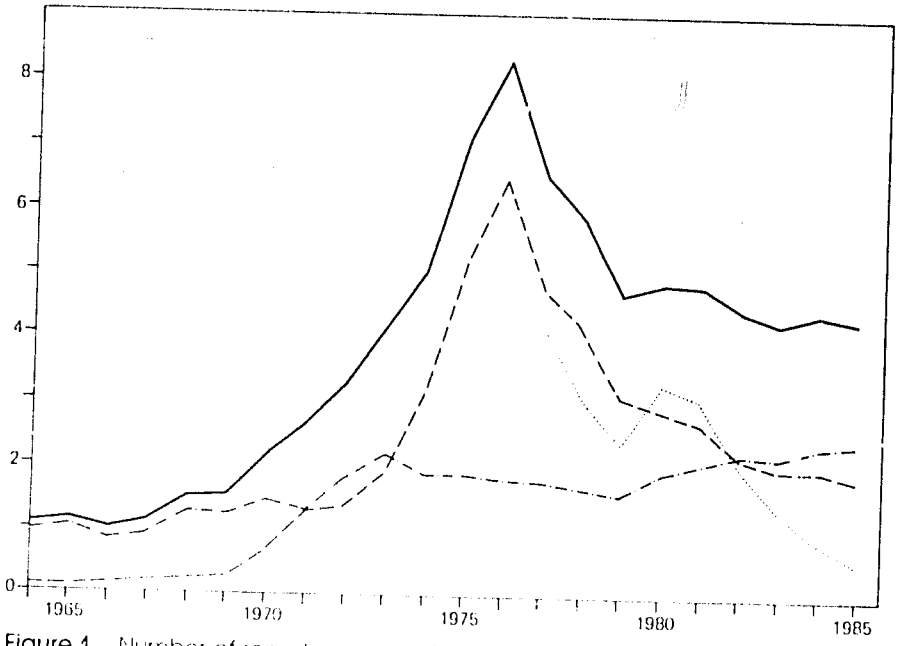


Figure 1. Number of reported cases of malaria for the period 1964–1985. *Solid line*, world total, excluding African region and China. *Dashes*, India. *Dots*, China. *Dot-Dash*, world total excluding African region, China, and India. (From WHO No. 87281.)

progressive deterioration. The case of China deserves attention because there the development of the health infrastructure, with active community participation, preceded the organization of a specific antimalarial program and the decline of malaria follows a similar pattern to those observed in the successful eradication in southern Europe, the United States, the Soviet Union, and Taiwan. In contrast most countries follow the pattern illustrated by India, where after a rapid recovery of the massive epidemic of 1976 following the mobilization of extraordinary resources for malaria, progress slowed down to reach an apparent stabilization of the situation.

The majority of antimalarial programs show a pattern of periodic deterioration leading to mobilization of resources and subsequent improvements, which seldom can be maintained and are therefore followed by renewed deterioration. This fire-fighting capability is likely to be more useful in areas where socioeconomic progress is contributing to an improved utilization of health services. At the same time, the malaria problem tends to become more and more focalized in newly developed problem areas, often out of reach of the routing activities of the program, including those areas in the early stages of economic exploitation, with highly mobile population and weak social cohesion; areas of sociopolitical

disturbances; or new settlements in periurban areas of explosive population growth.

Although increasingly focalized on the global and national perspective, the areas of concentration of malaria transmission are quite extensive, and within them actual focal distribution of the problem may change very rapidly as social and economic relations are very fluid and human aggregation and settlements are very dynamic.

These conditions have favored the transmission, dispersal, and increased selection of *Plasmodium falciparum* resistant to antimalarial drugs, and this problem has spread very rapidly during the last decade (Fig. 2). Resistance to antimalarial drugs is acquiring greater importance as a major obstacle to malaria control, as the latter becomes more dependent on the development of appropriate treatment as opposed to their widespread and indiscriminant use of mass drug administrations or chemoprophylaxis.

## PROBLEMS IN THE IMPLEMENTATION OF THE STRATEGY

The implementation of the malaria control strategy is hampered by the still unresolved problems of integration of the vertical structures of the malaria eradication programs into the national health infrastructure, or the transformation of a campaign into a service.

Programs have not been able to follow the changing epidemiological patterns and the development of new problem areas with a redeployment of resources. As a result, most endemic countries show serious mismatches between the geographical distribution of the problem and the resources for malaria control.

There still exists among some malaria specialist, as well as among some health professional, an often passive opposition to the primary health care approach, which prevents the overall revision of objective and control approaches in terms of relevance to general health development. In many places primary health care is being pursued as an additional program, competing for funds and power with other better established vertical programs, such as the malaria program. This competition is greatly influenced by short-term political demands or preferences.

Integration into the infrastructure has often been done as an administrative formal decision without a serious review of the integrated programs themselves and has led to the accumulation of duties of the peripheral health workers, without any improvement in their capacity of action.

Moreover, primary health care has in some cases a practically exclusive medical curative approach and does not appreciate the need to

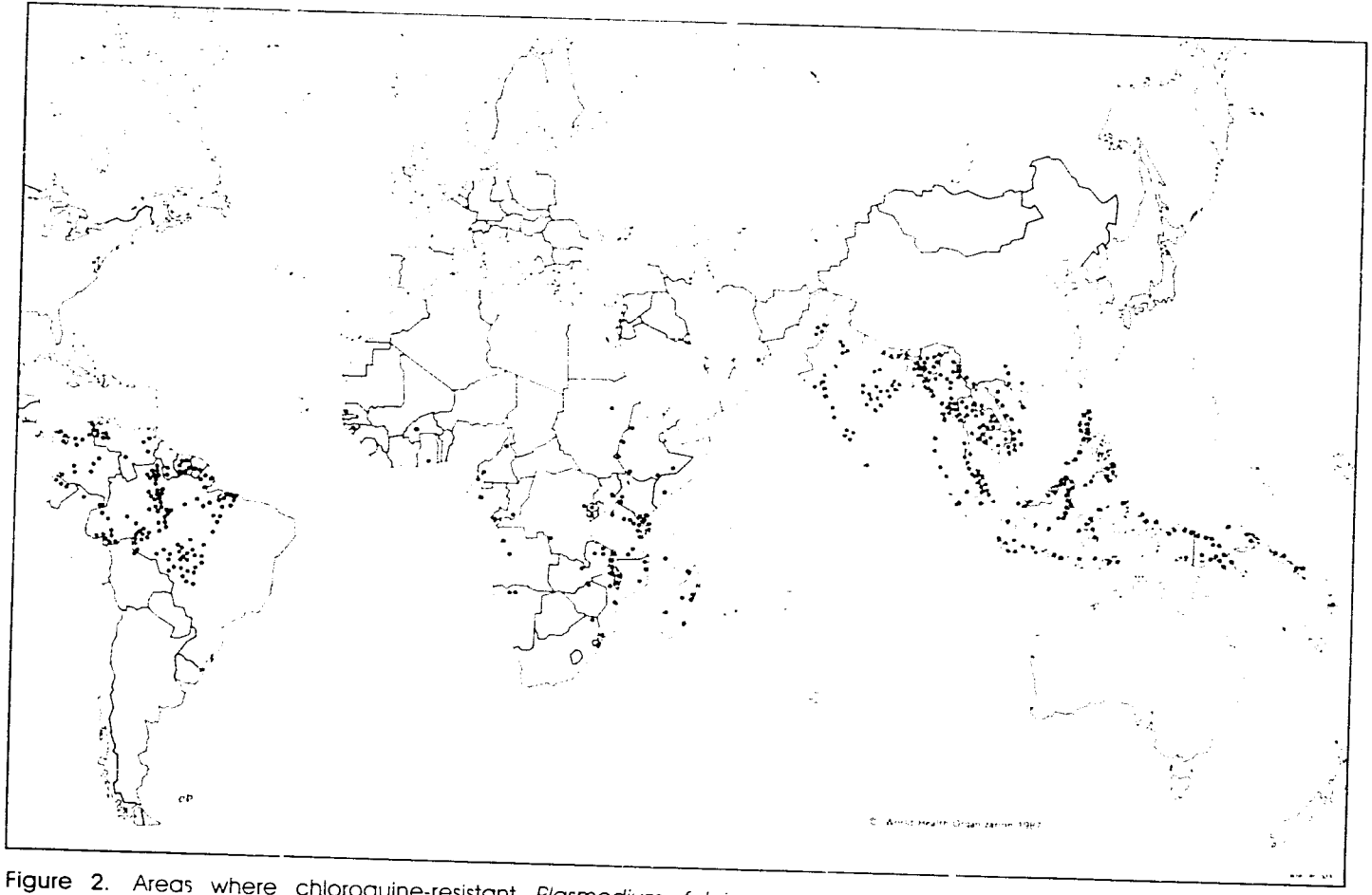

Figure 2. Areas where chloroquine-resistant *Plasmodium falciparum* has been reported. (From WHO No. 861888.)

develop appropriate epidemiological services towards which the malaria programs could make important contributions.

## PRESENT CONTROL APPROACHES

Malaria maintains its importance as a serious obstacle to socioeconomic development and endemic countries continue to expend substantial resources for its control. However, such expenditures in many instances are largely wasted because of the absence of the means to sustain long-term control.

In spite of the intensification of technical problems, present technical knowledge is adequate for controlling any existing epidemiological situation. Nevertheless, maintenance depends on the ability of the communities and their supporting sociopolitical structure to develop and sustain appropriate and viable epidemiological and response mechanisms. This gap between the available technical know-how and the capacity to carry out the necessary structural readjustments for making the best use of this knowledge has been a constant challenge for health planners.

In 1985, the 38th World Health Assembly recommended the integration of malaria control within the national primary health care systems and the urgent review of the national malaria situation and current antimalaria activities being carried out, not only in terms of their effectiveness, but also in terms of the feasibility of maintaining the levels of control being achieved.

Following the recommendation of the 38th World Health Assembly, the 18th WHO Malaria Expert Committee in 1985 reviewed the world malaria situation and recommended that the design of malaria control should be based on an epidemiological approach, recognizing that there is no single solution applicable everywhere and that the local variability of the problem and the applicability of potential interventions required a stratification of the malaria problem in every endemic country. Based on this stratification intervention should be redesigned in order to achieve the following two objectives:

The first objective would be the establishment, on the principle of total population coverage, of an appropriate mechanism for management of the disease problem in endemic areas. This is considered a necessary response to obvious population needs and should include, as a minimum, the provision of appropriate facilities for diagnosis and treatment of fever that are accessible to the whole population. These facilities should be integrated into the primary health care infrastructure, and should be supported by a referral system capable of managing the treatment of severe malaria case and the differential diagnosis of cases failing to respond to the treatment given at the periphery. This should be the basis

for monitoring the spread or intensification of parasite resistance to antimalarial drugs. This infrastructure should also provide the mechanism for the promotion of measures of individual or community protection against malaria transmission and it should be the basis for the development of the epidemiological services that should guide the application of more complex solutions to local problems. The role of malariologists is to guide the health services to ensure the best possible quality of the services provided.

The second objective is the selective implementation of organized activities aimed at the control of malaria transmission. These activities should be applied for well-defined objectives with particular emphasis given to the possibility of maintaining the benefits achieved; in other words sustained, even if slow, progress should be preferred to rapid effectiveness, which is difficult to maintain. It is imperative that the whole process of planning, implementation, monitoring, and evaluation of results be guided by appropriate technical competence.

The implementation of transmission control requires the existence of basic epidemiological services, which, as mentioned above, should have been developed as part of the referral system of the health infrastructure. It is also essential to avoid, as much as possible, the creation of specialized operational structures that may tend to persist as vertical services. Whenever such specialized services are necessary, they should have the capacity of future development into multidisciplinary epidemiological services. Perhaps the earliest objective for such services would be the control of recognized epidemic outbreaks. Further developments could provide improved systems for the recognition of high-risk situations, the evaluation of malaria endemicity in different areas, the stratification of the malaria problem in terms of health and social impact, the forecast and prevention of epidemics, and the control of malaria transmission in highly endemic areas and eventually throughout the country.

The Expert Committee's report does not present an easy method for malaria control, least of all for eradication, but it calls for a serious and realistic reappraisal of the problem, as well as of present and potential intervention, their expected effectiveness, the possibility of maintaining the improvements achieved, and, as a consequence, the possible improvements in the use of available or potential resources.

Primary health care is not viewed as a technological alternative, but as the general approach for health development; neither is the health infrastructure necessarily a prerequisite that should be fully developed in order to undertake antimalaria activities. What is envisaged is that, whatever kind of structural elements exist today, should collaborate in developing a health infrastructure for primary health care; this infrastruc-

ture should view malaria control as an essential component in endemic areas and should distribute appropriate responsibilities to general and specialized health services.

The effectiveness and limitations of available antimalaria measures are now better known than ever before, and the WHO Expert Committees on Malaria and on Vector Control, as well as specific study and scientific groups, have reviewed periodically the technological options. A careful analysis of each country's past experience will give a clear picture of local applicability, both in terms of effectiveness and of operational feasibility and social affordability. What will then be needed is a firm political decision to mobilize all available resources to redress the current deteriorating trend and often ineffective and even dangerous misuse of insecticides, drugs, and diagnostic procedures, into one of sustainable progress with improved efficiency in the use of resources.

Training and technical reorientation will be a major instrument for effective implementation of the strategy. Research in malaria is already producing promising results in the development of new drugs, new methods of diagnosis, and possible means of active immunization against malaria. However, as mentioned above, the main obstacle to malaria control has not been the lack of technology or even knowledge, but the difficulties of adapting existing knowledge to local situations and making optimal use of the resources of the health infrastructure and of community participation and intersectoral cooperation. The capacity to adapt can only be developed by a process of "learning by doing" and the appropriate documentation of those experiences paying particular attention to whatever ecological, economic, or social changes may have contributed to the obtained results or to their sustainability, besides the specific antimalaria activities or structural readjustment that were implement. A proper understanding of all those influences will enhance the potential transferability of those experiences.

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# CLOSING SESSION

# Closing Address

N.C. BRADY

I am sorry I could not be with you during your deliberations. As you know, this is the third and final in a series of AID-sponsored regional conferences to discuss malaria vaccines and future field trials.

As with other two regionally based conferences, the purpose of this meeting is to bring together two groups of individuals who are deeply involved in and concerned about the battle against malaria:

Scientists currently working on the development of malaria vaccines;
Scientists and policy makers from the nations of Africa.

You came here to develop a strategic plan that will be used in evaluating and field testing malaria vaccines when they become available for human trials. From what I have seen, and heard from my staff, this purpose has been met far beyond expectations.

During the last few years, malaria vaccine development has made a number of tremendous leaps forward. We are now conducting the human volunteer trials that Dr. Herrington discussed on Monday. At least six potential immunogens are undergoing advanced research at several AID-funded laboratories against the asexual blood stages of falciparum malaria. We hope that these can be ready for phases I and II trials in the near future.

As you are well aware, the malaria situation in Africa is particularly difficult and alarming. The speed of chloroquine resistance, discussed by Dr. Wurapa, only strengthens our resolve and commitment to develop malaria vaccines for integration into malaria control, primary health care, and immunization programs in countries with endemic malaria.

Your broad-based deliberations have touched on vaccine development and technology. They have also included the epidemiological and entomological requirement, social and cultural considerations, and infrastructure, manpower, and training needs of a malaria program. You have clearly underlined the complicated nature of this disease and the need for all involved to become fully aware of this complexity. You have also emphasized the tremendous cost of this disease as it affects human productivity and health, especially of children during their formative years.

I wish to offer thanks to each one of you, both personally and on behalf of AID Administrator McPherson, for taking time from your busy schedules to attend this conference and offer you advice and counsel. We are making progress toward the day when a malaria vaccine will add a new tool to our arsenal against this age-old, worldwide scourge of mankind.

# Closing Remarks

L. BRUCE-CHWATT

Mr. Chairman, ladies, and gentlemen. I am honored and delighted to participate in this meeting of hearts and minds of a large group of distinguished colleagues and friends involved in the problem of malaria control in Africa.

I also wish to apologize for some delay in arriving here not direct from London but via Baltimore. My wife and I decided to have a snack at the Baltimore airport before taking a taxi to Washington. A waitress came and said, "Would you like to eat something?" I said, "Yes. Could you give each of us a scrambled egg and a kind word." Within 10 minutes she brought our food. After we ate, I said, "Well, thank you very much for the eggs, but what about the kind word?" She said, "Don't eat them." Such a welcome to the United States was promising, and a look at the program of the forthcoming conference showed me the range of problems that were to be presented and this calls for a comment.

As you well remember, the feasibility of a hypothetical vaccine was promoted by the Agency for International Development (AID) in the 1960s in spite of general skepticism. The initial studies on rodents and monkeys showed the difficulty of this enterprise, but the new knowledge was of great value. You know that in the 1970s, when the dream of global eradication of malaria came to an end, there grew a need to develop fresh approaches to malaria control as the means to curb this disease in endemic tropical areas were gradually depleted.

The growing menace of resistance of *Anopheles* vectors to most of our effective (and increasingly expensive) insecticides, as also the resistance of malaria plasmodia, especially *Plasmodium falciparum*, to many therapeutic compounds and even to the good old quinine were the main reasons for the interest in prospective vaccines against malaria. The remarkable progress of modern immunology had stimulated the World Health Organization (WHO) and AID to bring about new methods of malaria control, at least against the notoriously virulent *P. falciparum*.

Two major recent international meetings, one in Geneva and the other in Honolulu, were devoted to planning of clinical and field trials of prospective malaria vaccines. It was generally agreed that these trials

should proceed sequentially through five main phases. The absence of adverse effects and high immunogenicity of the vaccine material are of primary concern to the first phase. The subsequent four phases involve human subjects and range from clinical trials on a small number of carefully selected volunteers under strict medical supervision and then gradually extend to larger groups of people in field conditions.

Many ethical, technical, and logistic problems of these trials were examined, and it appears that most of them could be solved. However, the present method of evaluation of immune response to malaria infection, whether naturally acquired or artificially induced, are still adequate. It seems that the organization, implementation, and appraisal of clinical field trials may be more difficult than the production of the vaccine.

The present achievement and the great possibilities of future scientific research in the laboratory and in the field have been well presented and discussed with much frankness during the past few days. The technical problems have been outlined and are well understood. But what emerged with great clarity at this conference is the enormous enthusiasm of a relatively small international group of all scientific and field workers, stimulated by both the challenge of the enterprise and its necessity. It stems from the initiative and support of this challenge endeavor given by AID, and other national and international bodies. This difference was stressed before, and I felt it during this week more than ever before. We had a large number of colleagues from tropical Africa, and I was delighted and impressed with the quantity of the contribution we received from all of them. This is to me one of the best memories of the past week, apart from anything else. The kindness, the tremendous generosity, the beauty of the place, the friendliness that we have experienced will be remembered by all of us for many years to come. On behalf of all of the participants, I would like to express to our hosts, to our American hosts, Dr. Chambers, Dr. Erickson, to Dorothy Jordan, and the rest of the staff our profound and sincere thanks for the marvelous week we have spent here and for the great deal that we have learned. Thank you very much.

# Closing Comments

CHARLES M. CHAMBERS

There is very little further that I could add to those kind words, except to emphasize most sincerely that we could do no less for a more deserving group. We are delighted to have you with us. Having worked in the academic profession where we value freedom of speech, I have learned that many professors consider our First Amendment not to be a right, but an obligation, so I will not try to take that privilege here and talk at great length.

It has been, as I said, our great pleasure to host you this week. We hope you found the sessions informative, stimulating, and perhaps even inspiring in terms of the magnitude of the task we all face. Disease knows no political boundaries. It does not respect cultural differences. Pain and suffering is never an individual situation. We have all devoted ourselves in our respective careers to solving very difficult health problems. I would like to extend our thanks to the workshop chairmen and to the rapporteurs. They have taken the responsibility of summarizing and collecting the findings of your discussions and presenting them in a form that can be used for future research and study. To Dr. Clyde and Dr. Najera, for their summaries; and to the Agency for International Development, which has provided and continues to provide unflagging support for the continuing worldwide efforts to control this and other diseases.

I have heard the term "billions of dollars" here and "billions of dollars" there as we talk about the needs in health care. I was at a meeting once where another group was talking about their budget requirements, which were also in the billions of dollars. One of the speakers made an effort to describe to the group what a billion dollars was in terms of the 1987 U.S. budget:

> A billion hours takes us back to the dawn of recorded history as man was just evolving culture and emerging from the cave. A billion minutes ago takes us to the time in our history when the Roman civilization was at its peak. A billion seconds ago takes us to when man first began exploring outer space. But a billion dollars ago, . . . a billion dollars ago, was only last night!

So in the scope of the needs of the world, and the efforts which we must all make, the money certainly is there. What we have been doubly

blessed with in the Agency for International Development is an indefatigable persistence with this problem. As you have heard, not only in the early days of vector control and insecticide use, but, as the technology advanced, in the forefront of immunology. As the political winds have shifted in this city, as they do every few years, it has taken a great deal of perception and wise leadership to maintain the momentum and the initiative. That, from where I sit, is the single most important thing, because it gives the funding staying power for the entire program.

You have heard a lot over these last few days; you have learned a lot about what has been done in the laboratory and reported from the field, but to my mind there is something even more important with which you will leave this meeting. Many of you knew one another in your professional work before coming here, but I am sure that there are an even greater number of you who are making first acquaintances here. One of my great pleasures has been the chance to meet many of you for the first time and learn of the very important work you are doing in your own countries. These professional bonds that you have formed, not only in the sessions, but at the social functions, and perhaps in touring around our city, will endure and will help you form a vast network of committed colleagues who can work with these problems as they continue to evolve. From our point of view, our magazine *Bioscience*, which is widely distributed in this country and is in every college library, will report on your important work here and will preserve it for the use of others in a summary form.

The conference proceedings that we will be publishing will have the full papers. I would urge optimism; the optimism that we have all heard, but tempered with a good measure of caution. It is no surprise that our nemesis *falciparum* and the other protozoa parasites are very wily critters, as they are so highly evolved. Our great promise is that in the new tools we have a sharpness and a focus to enable us to probe more precisely than ever before. In thinking of the enormity of the problem like the boulder mentioned earlier, I am reminded that if you can place a sharp instrument on a rock at just the right place, a small tap will cleave it in half. That's what we are looking for—not the sledge hammer, but the very sharp, incisive tool that the biotechnology methods are giving us.

We also owe a great measure of humility to the mammalian immune system. It of course is the paradigm under which we all operate. We are just beginning to tease and test it and to ask it to share with us more of its deep and exquisite secrets. As you go back to your homes, I hope that you will keep that faith, that you will maintain some confidence in where we are heading. You may not hear from us for six months. You may not hear from us for a year. You may not hear from us for five years. But you will hear from us, because the science is progressing and it will be at a

stage where you and all of your colleagues in the primary health care delivery systems will be able to demonstrate its safety, effectiveness, and utility in crafting a future cure for disease.

With that in mind, I ask Dr. Winter and Ms. Jordan for any administrative announcements or other details that we should share with the group before concluding the symposium. I would like to thank each one of you for coming to be with us and for sharing, not only your presence, but also your active and involved participation in the conference. On behalf of AIBS may I wish you well in your travels back to your homelands and with the permission of our session chair, Dr. Salako, I declare this Conference on Malaria in Africa adjourned.

# Program

MONDAY, DECEMBER 1
- Opening Session
- Malaria Vaccines: State of the Art
- Malaria and Malaria Control in Africa
- Evening Workshop Session

TUESDAY, DECEMBER 2
- Epidemiologic Studies of Malaria and Malaria Immunity

WEDNESDAY, DECEMBER 3
- Workshop Sessions

THURSDAY, DECEMBER 4
- Presentation of Workshop Summaries
- Conclusions and Recommendations
- Closing Session

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Bruce-Chwatt
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Oster
Poluku
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Steketee
Winter

**3. Vaccines**

Aikawa
Chulay
Heidrich
Hollingdale
Kakoma
Levine
Martinez
Miller
Perlmann
Reese
Reeve
Rose
Salako
Siddiqui
Tam
Trager
Zolg

**4. Epidemiology**

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**5. Vector Biology**

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Coluzzi
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**6. Malaria Control**

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Lamizana
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N'jelessani
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Wirima

**7. Training**

Imboua-Bogui
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Gbato
Goriup
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Meuwissen
Mugambi
Mukunyandela
Onori
Roger

**8. Diagnostics**

Reese