



## **Malaria in Pregnancy in Rwanda Report of a Prevalence Study**

Conducted by

**The Malaria and Other Parasitic Diseases of the Rwanda Biomedical  
Center- Ministry of Health**

**&**

**The Maternal and Child Health Integrated Project, USAID**

**For The US President's Malaria Initiative**

### **Study Team:**

Principal Investigators: Corine Karema, William Brieger  
Co- Investigators: Jeremie Zoungrana, Aline Uwimana, Beata Mukarugwiro,  
Rachel Favero, Elaine Roman, Barbara Rawlins, Irene Umulisa,  
Noella Umulisa, Angeliqum Mugirente, Jovite Sinzahera,  
Veneranda Umubyeyi, Fidele Ngabo, Mulindahabi Ruyange  
Monique, Rukundo Alphonse



## Contents

List of tables.....	3
List of figures .....	3
List of Acronyms .....	4
Background.....	5
Study Aim and Objectives .....	6
Methods.....	7
Feasibility assessment.....	7
Study design.....	8
Study Population.....	8
Sample Size.....	9
Sampling Procedures .....	9
Confidentiality and Consent .....	10
Recruitment Criteria.....	11
Study Procedures .....	12
Variables .....	13
Study Personnel .....	14
Data Collection .....	14
Data Management and Analysis .....	16
Study Implementation.....	17
Supervision of Implementation.....	17
Data and Sample Management .....	19
Lesson Learned from Data Collection .....	20
Results.....	20
I. Demographic characteristics.....	20
II. Results by Objectives.....	21
Discussion and Conclusions .....	29
Policy Implications .....	29
Recommendations and Next Steps.....	30
Annex A: Rwanda MIP prevalence data collection and extraction form .....	31
Annex B: consent form .....	32
Staff at the clinic may test your blood for malaria the next time you come for ANC.....	33
Annex C: supervision check list .....	37
References.....	38

## List of tables

Table 1: intended sampling in selected districts .....	8
Table 2: Sampling calculation .....	9
Table 3: Intended Study schedule .....	13
Table 4: Demographic Characteristics .....	20
Table 5: Positive Predicted Value (PPV).....	22
Table 6: PCR Falciparum results versus RDT results .....	23
Table 7: PCR Falciparum results Vs Microscopy results .....	23
Table 8: RDT positive results versus PCR .....	24
Table 9: PCR results per parity groups .....	26
Table 10: Hemocue Results and Malaria Test Results .....	27
Table 11: Malaria prevalence according to hemoglobin results .....	27

## List of figures

Figure 1: Percentage of positive malaria test per district by type of test .....	22
Figure 2: Percent tested positive for malaria according to ITN use .....	24
Figure 3: Chi-square test for association of prevalence and ITN use .....	25
Figure 4: Malaria Prevalence according to the parity using PCR test .....	26

## List of Acronyms

ACT	Artemisinin-based Combination Therapy
ANC	Antenatal Care (aka Prenatal Care)
ASCM	Agents Santé Communautaire Maternelle
CHW	Community Health Worker
DHS	Demographic and Health Survey
HC	Health Center
HIV	Human immunodeficiency virus
HMIS	Health Management Information Systems
IPTp	Intermittent Preventive Treatment for pregnant women
IST	Intermittent Screening and Treatment
ITN	Insecticide Treated Net
JHSPH	Johns Hopkins Bloomberg School of Public Health
JHU	Johns Hopkins University
LLIN	Long Lasting Insecticide-treated Net
M&E	Monitoring and Evaluation
MCHIP	Maternal and Child Health Integrated Program
MIP	Malaria in Pregnancy
MOH	Ministry of Health
MOPDD	Malaria & Other Parasitic Diseases Division
NMCP	National Malaria Control Program (later changed to MOPDD)
PBF	Performance Based Financing
PCR	Polymerase Chain Reaction
PI	Principle Investigator
PMI	US President's Malaria Initiative
RBM	Roll Back Malaria
RDT	Rapid Diagnostic Test
SRS	Simple Random Sampling
USAID	United States Agency for International Development



although true incidence or prevalence data were not available. Sievers *et al.*<sup>vi</sup> found that, “both admissions for malaria and laboratory markers of clinical disease among children may be rapidly reduced following community-based malaria control efforts,” including community case management and ITNs. They stressed the importance of confirming cases parasitologically, especially since reduced prevalence of malaria does not mean reduced cases of febrile illness. In the second study, Otten and colleagues<sup>vii</sup> reported that, “In-patient malaria cases and deaths in children < 5 years old in Rwanda fell by 55% and 67%, respectively,” after these interventions. Karema and al.<sup>viii</sup> shown a more than 50% decline in confirmed malaria cases, admissions and deaths at district hospitals in Rwanda since 2005 followed a marked increase in ITN coverage and use of ACT.

Currently, there are no accurate national estimates of the prevalence of malaria among pregnant mothers in Rwanda. Evidence has suggested that cases of malaria are falling, but more reliable estimates are needed to inform policy that will help protect pregnant mothers from malaria as Rwanda moves towards its long-term goal of eliminating malaria in Rwanda.

As part of an effort to address malaria in pregnancy in Rwanda, the main aim of this research was to determine a national estimate of the prevalence of malaria among pregnant mothers who attend antenatal care services. This study was a prevalence survey during the malaria transmission season of November 2011- March 2012 in Rwanda, carried out with the support of the President’s Malaria Initiative (PMI) and the Global Fund . The research was implemented by the Malaria & Other Parasitic Diseases Division (Malaria & OPDD)-Rwanda Biomedical Center (Integrated National Malaria Control Program) and the United States Agency for International Development’s (USAID) Maternal and Child Health Integrated Program (MCHIP) at health centers in selected districts in order to achieve a representative epidemiological picture of the country. The study was based in health centers that provide antenatal care and laboratory services.

Results from this study will inform the Ministry of Health (MOH) and its partners in the design of a program to prevent and control MIP that is appropriate for and can be scaled up in Rwanda. It is intended that this study provide a foundation for further study of the effect of placental malaria at delivery.

## Study Aim and Objectives

**The main objective of this study was:** To determine the current prevalence of detectable<sup>1</sup> malaria among pregnant mothers using data from their first ANC visit in Rwanda.

**Specific objectives include:**

- To determine if reported current ITN use (frequency, duration) influences malaria prevalence (information that is normally requested on ANC cards).
- To determine if parity is associated with malaria prevalence at first ANC registration (information that is normally requested on ANC cards).

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<sup>1</sup> The study team recognizes that no test is 100% reliable in detecting malaria.

- To compare the prevalence of MIP with the three different but complementary diagnostic tests: microscopy, Rapid Diagnostic Test (RDT) and Polymerase Chain Reaction (PCR).

## Methods

### Feasibility assessment

A feasibility assessment for the study was conducted in December 2010 with the purpose of observing current ANC services including malaria in pregnancy program procedures at health center based as well as data collection and reporting processes to see how these could be adapted to achieve the study objectives. After visits to Kicukiro and Ruhango Districts, the field team determined that:

1. First ANC visits are quite high – over 90% of pregnant women access this service, yielding a representative sample for study purposes
2. ITNs are in fact provided through ANC clinics which have their separate stocks. Concurrently, nationwide distribution of nets to households is taking place
3. Malaria case management is based in clinics on parasitological diagnosis through microscopy which is less likely to detect placental malaria
4. CHWs are taught to use RDTs for community based case management, but RDTs, which are more sensitive to placental malaria, are used in clinics only when the laboratory is not open (e.g. nights and some weekends)
5. ANC cards and registers combined collect a variety of data that can be used for surveillance and thus a study on MIP prevalence
6. Electronic data reporting is enabled by submission of monthly summary forms via cell phone modem

The team also set out to determine the feasibility of MIP surveillance, appropriate surveillance tools and the potential role for IST as part of the surveillance process. The following findings were noted:

1. While nurses in theory can perform RDTs, they may feel burdened to add this to the four existing tests carried out at first ANC visits
2. Nurses would be interested if CHWs, who know how to use tests, could help perform RDTs at ANC. This could be considered in the community PBF to help the CHW cooperatives
3. PCR testing would be easy to do along with RDT as one can use the same blood sample. PCR cannot be analyzed yet in country
4. Placental malaria determination at the time of delivery would be valuable and those taking delivery could take needed samples. The challenge would be two-fold, first this would increase the length and cost of the study and secondly only 60-70% women deliver in the facility where they had ANC

Finally the team consulted with key partners to determine their interest and willingness to collaborate in the study. The results of these discussions included:

1. The Ministry of Health was clearly interested in combining a MIP prevalence study with a service like intermittent screening and testing (IST) in order to increase its ability to protect pregnant women and continuing to bring down malaria prevalence
2. The Ministry also expressed great interest in including follow-up to delivery and collecting placental parasitemia data
3. USAID was supportive at this point for establishing first the true prevalence of MIP in different transmission settings
4. USAID was willing to entertain the idea of an intervention research on IST and placental parasitemia as a follow-up, but expressed caution about taking on too many tasks for the initial study.

Based on these findings, the study protocol and methods were finalized based on the findings

### Study design

This was a cross-sectional study which involved six districts in Rwanda in different malaria transmission zones. Six health facilities were randomly selected from each district. The total sample was divided equally among the six districts. Sample size for facilities within a district was assigned proportionate to the average monthly attendance. We consecutively enrolled all pregnant women coming to the facility for their first ANC visit during the study enrolment period until each health facility had enrolled its targeted number of participants in order to establish prevalence of MIP.

### Study Population

The study population was women registering for the first time for ANC services for their current pregnancy at health centers in six districts of Rwanda during the period November 2011 to February 2012, the known malaria transmission season in the country. The 2010 DHS preliminary results indicate that 98% of pregnant women do register at ANC, and thus, the population of pregnant mothers registering for the first time during their current pregnancy should be representative of the population of pregnant mothers and formed the target for study. The districts were selected because they represent a mix of high, medium and low malaria transmission. Health centers were prioritized since the majority of pregnant mothers in Rwanda seeking ANC services do so at a health center. Further hospitals were excluded from the study as they largely provide ANC services for complicated referral ANC cases, rather than routine ANC clients.

Table 1: intended sampling in selected districts

District (Region)	Endemicity Level	MCHIP Program	Number of Health Centers Selected	ANC Clients to be Recruited
Gakenke (N)	Low	Yes	6	670
Karongi (W)	Low	No	6	670
Ruhango (S)	Moderate	Yes	6	670
Kicukiro (K)	Moderate	Yes	8	670
Gisagara (S)	Higher	Yes	6	670
Nyagatare (E)	Higher	No	6	670
TOTAL			38	4020

## Sample Size

The aim of the study is to estimate the prevalence of malaria among pregnant women registering for antenatal care for the first time in their current pregnancy. Based on DHS and HMIS data, we assess the prevalence of malaria in this population to be 1 to 5%. The sample size calculations are based on achieving adequate precision of the prevalence estimate. The basic calculations assume simple random sampling (SRS). The study utilized cluster sampling defined as visits by pregnant women to the given facility on the same day. Therefore, the sample size calculated assuming SRS needs to be multiplied by the design effect of 2 (ratio of the variance for the cluster sampling and the variance of SRS). Table 2 below summarizes the sample size estimates for various levels of malaria prevalence and their precision at 0.05 level of statistical significance.

**Table 2: Sampling calculation**

Prevalence level (%)	Precision* (%)		
	0.5	0.7	1
1	3,043†	1552	761
3	8,943	4563	2,235
5	14,598	7448	3,650

\* Defined as the distance from the estimated prevalence in either direction for the 95%CI (for example: 1% prevalence and 0.7% precision translate into 1% (95%CI: 0.3 to 1.7%)

† This sample size estimates include the design effect

Based on the above assumptions we estimated the sample size as follows:

- The most recent population based estimate of malaria prevalence in Rwanda is the 2007-08 DHS
- Using DHS figures and knowing that HMIS data show declines in cases, we estimate a possible 1% prevalence currently in pregnant mothers nationally
- We used a precision of 0.5% and assumed a potential clustering effect when taking multiple samples from the same health facility
- Sample size estimates thereby ranged from 3,043 to 3,628
- Adding 10% in case of error, we were ultimately aiming for a sample of 4,000 pregnant mothers who were registering at ANC for the first time in their current pregnancy
- Considering the variation of reported malaria cases across the country, we intended to sample from ANC clinics in districts where HMIS shows relatively higher, lower and moderate proportions of malaria cases among total case load.

## Sampling Procedures

In five of the study districts we randomly chose six health facilities and for the sixth district all eight health centers in the districts were considered. If a health center without a functional laboratory facility was selected, a replacement was chosen. After the health centers in a district are chosen, we determined their monthly average new ANC attendants. Since we sampled approximately 670 pregnant women per district, we divided that number among the six chosen

health facilities proportionate to the size of their monthly ANC new registrations. Once the study started, all new ANC attendants were enrolled sequentially until the number of clients reaches the number assigned to that health center.

## **Confidentiality and Consent**

### ***Data security, record keeping, and access to data***

The team affirmed that privacy, anonymity and confidentiality of data/information identifying one as a participant was strictly maintained. Since we had only one contact with each participant, we used identifiers that did not link with personal information and records. Dissemination of the results will be done after the data has been analyzed and conclusions drawn and will not identify any of the participants in the study. It is envisaged that the final dataset that will be used for the analysis described in this document will be de-identified data. However, during the process of building this dataset from the data as they currently exist, it will be necessary to be able to identify subjects in order to match their PCR results and anthropometric history with their socio-demographic and educational attainment data. Once this process has been completed and checked for errors, the data will then be de-identified for further analysis.

### ***Identifiers***

The study team collected (extracted from ANC records) non-identifiable data on a separate data sheet including socio-demographic and clinical characteristics such as age, gestational age, parity, anemia, and health center location. Participants were assigned an identification code for the sole purpose of linking PCR findings with the data that are collected during the ANC visit. There was a register with identifiers that was locked by the ANC staff that links the study ID with the client's health center registration number for the purposes of validating data, matching it with a PCR result and undertaking further research on the effects of MIP at delivery.

### ***Plans for identifiers***

The data recording form did not include client identifiers. A non-linkable study number was assigned to each participant and recorded on that person's form and parasitological test materials (RDT, filter paper, and slide). Since there was only one contact with each client, and malaria treatment was based on immediate RDT results, there was no need to retain client names for future study.

Records will be stored in a locked room in the MOPDD office in Kigali, Rwanda. A protected computer with no access to the web will store local data.

Stored blood samples will be held for not more than five years and are kept for proof of any queries arising out of the research findings

## ***Risks***

There were no major risks involved except the minimum pain experience during the performance of finger pricks while collecting blood samples. Staff were trained to conduct finger pricks in a manner that minimizes discomfort. They also maintained sterile conditions to prevent infection. Participants could refuse to provide specimens at any time.

Participants may feel uncomfortable answering questions. To minimize this discomfort most information collected was the normal questions asked during ANC registration and a normal ANC visit. Clients can refuse to answer any question for any reason at any time during the study. The length of interaction with a participant will be that of a normal ANC visit with the addition of approximately 15 minutes, the time needed to get an RDT result and prescribe treatment if needed.

## ***Benefits***

There are benefits for the individual as well as the community. Any individual who was RDT-positive was treated with the recommended first-line therapy. The larger community benefit will occur when the Ministry of Health uses the findings to design more appropriate malaria prevention and treatment services for pregnant mothers. We ensured that each woman also received an insecticide-treated bed net.

## ***Consent process and documentation***

Local customs of requirement for community consent was followed carefully. Before initiation of the study, courtesy visits were paid and meetings were organized for local political and administrative leaders via our local research team. Community Health Workers also helped provide public information on the nature and purpose of the study. At the end of each study period, a senior investigator will hold a series of informational meetings to report on the outcomes of studies to the community.

Consent was acquired by a trained health worker staff, under the supervision of the study investigators. The staff informed the potential participants attending antenatal care clinic about the study. If interested, a detailed description of the study was provided both verbally and in writing, following the informed consent forms, in appropriate languages used locally. Issues emphasized were the voluntary nature of the study, uncompromised medical care that remained regardless of study participation, the ability to refuse participation in the study at any time during the ANC visit, confidentiality of medical and laboratory information, and a careful explanation of the risks. Subjects were required to read the consent if they able or it was read to them if the subject was unable.

## **Recruitment Criteria**

### ***Inclusion criteria:***

- Registered at ANC for the first time for the current pregnancy
- Living within study districts catchment areas
- Informed consent signed

***Exclusion criteria:***

- Very sick or in a situation where woman cannot be interviewed
- Not staying in the catchment area of the district

**Study Procedures**

Women's consent was obtained by the ANC nurse during routine ANC with each client. On the first ANC visit for a particular pregnancy the nurse included explanation of malaria testing procedures along with other information she normally provides about ANC services. The client was informed that malaria parasitological diagnosis is being added to the routine tests given to pregnant mothers on their first visit for which she may opt out. Those who opt out were informed that they will continue their routine ANC services. The nurse explained how the tests were used and that they are accepted malaria diagnostic tools in Rwanda and pose minimal risk when clean/new lancets are used and minimal blood was needed.

We obtained a finger prick sample of blood to put on filter paper to perform PCR, on a slide for microscopy and on a cassette for RDT all at the same time. The client was informed that if the RDT was positive/shows presence of malaria, she will receive free treatment according to established national guidelines. For women with negative RDT but had an elevated temperature, the treatment decision was made in conjunction with the microscopy result. Women also received the normal treatment for other febrile illnesses.

The client was also told that a drop of blood was used for additional PCR testing as a comparison with the two other tests. It was explained that PCR takes some weeks to be analyzed and it was not realistic to share those results since they would come too late to influence treatment decisions.

Field supervisors ensured quality performance of these tasks by nurses and laboratory staff. They also collected data forms and PCR test papers on a weekly basis and brought them to the MCHIP office in Kigali where data entry and storage occurred by trained data entry personnel under the supervision of the project coordinator. The Kigali office sent the filter paper for the PCR test to JHSPH on a monthly basis through courier services. All test results were recorded on a special project data sheet as seen in Annex A.

In order to ensure that malaria test results became part of the woman's medical history, her RDT and microscopy results were also recorded on her ANC card along with any treatment provided. This enabled health center staff to provide continuity of services.

***Number of study contacts or visits required of participants.***

Only one visit to the ANC clinic, the first for a particular pregnancy for each participant, was required given that the study aims to determine the prevalence of MIP at the first ANC visit.

## ***Study Timetable***

The entire study period was planned for one year as outlined in the Table 3 below.

**Table 3: Intended Study schedule**

Description of activities	Period (2011-2013)	Remarks
Writing Protocol	July-September	
Ethical clearance	October	
Training and Preparation	October-November	Testing study tools
Data collection	November-February	
Data entry and cleaning	November-April	
Data analysis	May - December	
Reporting and dissemination of results	Jan-March 2013	Preliminary RDT results were shared
PCR Test Analysis		PCR was more involved than planned and extended until late 2012

## ***Referring subjects to care outside the study, if needed***

Severe cases of malaria were referred to respective district hospitals based on normal procedures operating in the Rwandan health system. Other problems observed will be referred using existing protocols in the health centers.

## **Variables**

There are four sets of variables, 1) basic demographic characteristics of the women, 2) clinical variables, 3) malaria related behaviors of the women, and 4) their malaria status as determined by parasitological diagnosis.

Basic demographic variables include woman's age, age of pregnancy, gravidity and parity.

Clinical items that are normally obtained during routine ANC visits will be collected and include recent reports of fever/malaria, hematocrit for anemia using Hemocue, HIV and current temperature.

Behavioral items include treatment sought for fever/malaria and sleeping under a bednet. Three measures of malaria prevalence were used as follows:

- a. RDTs- Each pregnant mother who fulfilled enrolment criteria was given a rapid diagnostic test. This was a first response LDH Pf pan and supplied through the NMCP. A small amount of blood was being drawn from the finger of the woman for the RDT tests. Test results were obtained in approximately 15 minutes for each ANC client. If the woman tested negative for malaria, her ANC care continued according to national policy and protocol in

Rwanda. If the woman tested positive for malaria, she was given malaria treatment according to malaria treatment guidelines.

- b. PCR – When blood was being taken for RDT, a drop of blood was also being placed on Protein Saver 903 Filter Paper to be preserved for PCR testing for molecular markers of *Plasmodium falciparum* at the Johns Hopkins University (JHU) Bloomberg School of Public Health. Preservation includes placing filter paper card in small plastic bag with desiccant added beforehand. Up to 100 small plastic bags were placed in a larger plastic bag. All bags were stored out of direct sunlight. PCR samples were shipped to JHU for testing.
- c. Microscopy – All primary care health centers where the study was based have basic laboratories. Any client or patient suspected of malaria was sent to these laboratories to confirm parasitaemia. In the case of this study, all first time ANC attendees were also tested with microscopy.

## Study Personnel

The field staff listed below was key personnel supporting the study. Their role is briefly described.

**Research Coordinator-** He had overall responsibility for coordinating the study team; specifically, keeping the team on track according to the study timeline (Table 3). He also worked closely with the PI and Co-Investigators and contributed to the collection and analysis of data and provided written and oral reports related to the analysis.

**District Coordinators-** S/he was responsible for: a) collation of data; b) liaison between clinic staff and Research Coordinator; c) support to clinic staff to address any complications (e.g. maintaining supplies; consent forms); d) forwarding all data forms. The District Coordinators collected PCR samples monthly and delivered them to the Research Coordinator for final shipment to JHU.

**Existing ANC staff-** S/he was responsible for providing each patient with information about the study and give ANC clients the consent form for signature (among those who do not opt-out). S/he administered the RDT and PCR and collected samples for microscopy (described above) as well as provided malaria treatment for pregnant mothers testing positive for malaria.

Existing laboratory staff in the selected health centers performed microscopy.

Two Data Managers entered data at the Kigali office.

## Data Collection

ANC clients at the study facilities received parasitological testing for malaria using microscopy, rapid diagnostic tests (RDT) and Polymerase Chain Reaction (PCR). These tests were chosen to accurately determine malaria prevalence among registering ANC clients. Although microscopy is somewhat standard at the health facility level since the RDT is fairly simple to use, it was a good comparison test to microscopy. Also, it could potentially be incorporated fairly easily into routine ANC care considering its simplicity. Finally, PCR was used for final comparison as it is considered the most sensitive of all three tests in detecting low levels of parasitemia.

Existing government ANC clinic staff were responsible for collecting, interpreting and recording the MIP test results and informing the client about the microscopy (slide) and RDT results. The PCR specimens were analyzed at the Johns Hopkins Bloomberg School of Public Health laboratory facilities. Lab material transfer agreement was signed between the Malaria Division and JHU. All target health center laboratory and ANC staff were trained in study procedures and data collection procedures including extraction of relevant information from ANC cards and registers and informed consent. Community Health Workers, who regularly provide health education, malaria treatment and referral, were also involved in mobilizing newly pregnant women to register for ANC.

At present most of the key variables are already recorded in the existing ANC cards and ANC registers. RDT was the only additional item. The study had a one-page individual client data form that copies the routine data from the ANC cards and registers and adds the RDT results and will show if the filter paper is taken for PCR testing or not.

Data were collected on a one-sheet form as seen in Annex A. This information will be gathered during the normal ANC visit by existing nursing staff who will be trained for this purpose. Thus the process is integrated into routine ANC in order to be least disruptive of clinic schedules and client visits. The first section of the form extracts information from the Antenatal Clinic Register and Card including District, Health Center name, date of ANC visit, age of mother, parity, age of pregnancy in months, anemia, and HIV status.

The second section includes questions asked of the mother about malaria interventions as follows:

- Have you had a fever/high body temperature since the current pregnancy started?
- Have you received any Malaria treatment since you knew you were pregnant? Have you received any malaria treatment in the past 2 weeks?
- Does your family own an ITN? If Yes, how many?
- Did you sleep under an ITN Last Night?
- In the past month would you say that you slept under an ITN ... (none, few, some, most, all)

The third section records test results and treatment provided (if needed based on RDT results). These include RDT, Microscopy and PCR results and provision of ACTs and/or Other Antimalarial medicines. Again, the existing staff nurses collected the blood samples for the three tests, interpreted and recorded the RDT findings, made and sent slides to the clinic's laboratory where these were examined by existing laboratory staff and preserved the PCR test papers for collection and transmission to JHSPH.

## **Data Management and Analysis**

### ***Data Management***

Data were double entered in EPI INFO version 2006 by the data entry clerks at the project office in Kigali. Data validation was done by comparing the two data sets and making corrections where discrepancies existed using source documents by an independent individual not involved in data entry.

### ***Data Analysis***

Analysis of cleaned study data was done using STATA V.12. Information on demographic characteristics including gestation age, use of malarial control interventions including antimalarial medicine in the previous four weeks and frequency of ITN use were tabulated. In addition the prevalence of MIP was determined. Kappa analysis was carried out to establish concordance of the different malaria diagnostic tests (RDT, PCR and Microscopy). Chi-square was used to establish the risk factors for MIP including gestation age, parity, age of the mother, and use of control interventions.

Data analysis was carried out by project co-investigators in Kigali. Study findings will be shared with the Integrated National Malaria Control Program for strategic policy and programming decision making regarding prevention of malaria in pregnancy.

#### **Reporting and Validating**

Plans for reporting test results to participants are described below. Medical tests and results were validated (e.g., conducted in a CLIA certified laboratory). Results of rapid diagnostic tests were reported as they were performed as point of care diagnosis. Since results of PCR tests were not available until months later, these were not reported to participants as they would have had no clinical relevance for treating acute malaria. A positive test by PCR does not necessitate malaria treatment. Microscopy results were not used to determine treatment measures. Treatment was based on the RDT which was positive in clinically relevant case presentations.

### ***Dissemination***

The results of the study will be disseminated through conferences, written reports, and other available media (e.g. journals, radio). The key audience for dissemination is the Rwandan Ministry of Health whose responsibility it is to use the results for decision making based on the disease burden and risk factors established from the study. Such decisions may include determining that current interventions are adequate to prevent malaria in pregnant mothers, or whether additional strategies may be needed to hasten movement toward elimination and save lives.

Dissemination among national malaria partners will also take place so that they may use the results to guide programming. It is planned that publications and presentations with international journals and conferences will also help the global malaria community track and plan for malaria elimination.

## ***Oversight plan***

Study sites consist of both MoH and districts/clinics supported by the USAID's Maternal and Child Health Integrated Project in Rwanda and additional clinics/districts chosen by the Rwandan MOH. The same project staff hired and supervised by Jhpiego/MCHIP will supervise all field sites.

William Brieger, Jhpiego's Senior Malaria Adviser and Professor in the Department of International Health at Johns Hopkins School of Public Health, is the Principal Investigator of the study, while Dr Corine Karema, Head of the Rwanda NMCP is co-PI on the ground. William Brieger provided technical guidance to the MOH while Dr Karema ensured that the study is implemented according to the protocol and provided ongoing guidance on the ground. Dr. Brieger conducted 2 visits to Rwanda over the course of the study to support the training of clinic staff and participate in advocacy meetings as well as dissemination of results. Jhpiego's Country Director, Jérémie Zoungrana, provided supervision and worked closely to support the Study Coordinator, the NMCP and the PI to facilitate the study protocol. This included liaison between Rwanda and Baltimore. The Study Coordinator assisted Dr. Karema in ensuring the study is moving forward according to plan.

## **Study Implementation**

The study began in mid-December 2011 shortly after final approval from the Rwanda Ministry of Health Institutional Review Board. Meetings were organized with district leaders to inform them on the beginning of the study, study materials were distributed to the sites and data collection by health center began. District coordinators conducted weekly supervisions.

During the study implementation phase the team focused on two major issues: 1) supervision and quality control of data collection in the field and 2) quality control in data management including data entry and collection of samples. The supervision activity had two major components including meetings with the field coordinators and direct onsite supervision in the ANC clinics.

## **Supervision of Implementation**

The first meeting among District Coordinators/Supervisors, MCHIP and MNMCP was held in mid-January 2012. In the 4 weeks since the study had started, 25% of the approximately 4,020 targeted pregnant women had been recruited into the study.

District Coordinators revealed a misunderstanding about inclusion/exclusion criteria and thought clinics were only supposed to recruit from their catchment areas, when in fact sample targets were calculated based on actual ANC attendance. They were encouraged to relay back to their clinics that all clients from within their district (not neighboring districts) could be included.

A number of problems with the data entry forms were identified during review. In particular some had blanks where microscopy results should have been filled. We recommended that Coordinators should not collect any incomplete forms and that a few lab staff were reluctant to assist with the study or were slow in completing the tests. We also discovered in a few cases that the PCR papers were not numbered correctly. Some problems were voiced about adequacy of

study materials, in particularly the desiccants to include in each PCR packet. We discovered that these came in batches of 40, not 100, hence under-ordering had occurred

The team conducted selected site supervision visits to observe data collection in the field and make recommendations for improvement. Supervisory visits were made to two clinics in each of the six study districts during the January 2012. A district supervisory checklist was developed prior to the field visits. Objectives of the supervision were as follows:

1. Check data collected up to the day of the visit, check how the forms were filled out and discuss achievements and challenges
2. Observe nurses doing enrolment
3. Observe district coordinators providing feed back
4. Provide feedback to both providers and district coordinators
5. Meet with HC managers to discuss challenges and provide recommendations to the nurses
6. Discuss with district coordinator on the district rate recruitment
7. Return data sheets to the district coordinators that needed correction before data entry

We found that trained ANC staff at different clinics had developed different ways to integrate the study procedures into their ANC routines. One did all blood collecting activities – study tests, HIV tests and hemoglobin tests in one room. Others integrated study procedures into all the regular ANC procedures for ‘one-stop-shopping.’ One of the clinics set up a separate space just for study procedures and required all clients to wait each time for different activities.

The problem of inadequate study materials was partly due to lack of clear planning in distribution. Some clinics had more than they needed and some had less.

Again the lack of cooperation of a few laboratory staff was identified. An unexpected opportunity was learning about the role that MCHIP-trained Agents de Santé Maternelle – maternal community health volunteers – played in encouraging pregnant women to register for ANC. We were able to visit two ASMs who had been trained in mid-2011 and learn about their work.

Based on the field experiences, a second District Coordinators meeting was called on 27 January. Recruitment had increased overall to 51%, but three districts fell below the average.

In February, from the 14th to the 21th, the National Team conducted a second supervision in the districts. Two health centers among the 4 health centers by district that were not visited in January were visited. We observed that four districts were on track for the recruitment and some health centers had reached their targets. But the two remaining districts were not on track for the recruitment. For many health centers, services were well organized, data sheets were filled out well and the registers had information needed for the study.

Some health centers encountered challenges related to provider work overload which at times led to long patient waiting times. In some facilities, only one of the two trained staff was available to provide services while the other was taking care of other health center duties or was absent from the site.

A meeting was organized on February 27<sup>th</sup> at the MCHIP office in Kigali that included the 6 district coordinators as well as staff from the NMCP and MCHIP. The district coordinators

brought PCR samples that had been collected. The members of the meeting recommended that the study team focus the March supervision in the two districts.

The recruitment was at 77% with two districts that were still at 60% recruitment rate. It was recommended that those districts recruit at outreach ANC sites and request the CHWs to continue mobilizing mothers to attend ANC.

A last supervision by the National team was conducted in March for the two districts that were behind in recruitment. The trained staff for the study were recruiting all clients and were assigned to ANC outreach. Some health centers thought the targets were over estimated. Since the estimates were based on reported attendance figures, the team discussed the reasons why such figures may be incorrect. We discovered that the figures represented all attendees, while the ANC staff were recruiting participants only from the immediate catchment area. Supervisors were told to inform recruiters to include all their clients since that was the basis for sample selection.

A final meeting was organized the 5<sup>th</sup> April in MCHIP office. After doing a quick analysis of the recruitment, the team discussed how to manage the extra clients for some districts. Because the district of Karongi did not reach its targets, the team proposed to keep the extra clients in the study.

### **Data and Sample Management**

Planning for data entry formats and procedures also took place in January 2012. After reviewing the submitted data forms, the Data Manager and Principal Investigator designed a data entry template. Four data entry clerk candidates were interviewed by the study team, and two were chosen to undertake the double entry process. During the test data entry process we identified some additional improvements needed in the data template and corrected those. We developed a series of data entry guidelines for the clerks, who were then oriented.

Efforts also focused on organizing procedures for shipping PCR samples to JHSPH for testing. Careful review of all PCR samples was conducted and any with inappropriate client numbering were corrected, and any incorrectly packaged without an individual desiccant were re-packaged. A list of all samples collected during the first District Coordinators' review meeting was compiled.

The team determined that FedEx had experience in shipping medical samples and thus, they were contacted for assistance. Appropriate clearance papers were obtained by Dr David Sullivan of JHSPH whose lab was used for PCR analysis.

The team discussed dissemination plans for the study findings and follow-up activities. NMCP staff participated in Kigali-based meetings, but was unable to undertake field visits due to timing of other Ministry activities. The NMCP agreed that the proposed RBM Malaria in Pregnancy Working Group would be an ideal time to present preliminary results and also requested that we explore other upcoming conferences.

Data entry continued with the two data clerks who were recruited in January under the supervision of the study coordinator and MCHIP M&E officer. All data sheets and samples were

reviewed and hand over from the district coordinators to the national team was done. Data entry continued and was completed when the PCR analysis was finished in September 2012.

### Lesson Learned from Data Collection

The field experience yielded lessons for both conducting studies as well as the potential inclusion of malaria monitoring within antenatal care settings. The team identified the following lessons:

- Sensitization helps the recruitment to go well
- Integration of the study counseling and sample collection in other ANC activities facilitated the recruitment with less waiting time for clients
- At sites who are conducting focused antenatal care with ANC every day, recruitment was easy because of a small number coming in ANC sessions
- Combining study activities with HC activities can have impact on the quality of recruitment because it can place too much work on health center staff. When feasible, it could be better to recruit special staff for the study.
- Training of all staff involved in the study helped everyone to understand the study in the same way

## Results

### I. Demographic characteristics

A total of 4,037 participants in the six districts were recruited during ANC. Table 4 provides brief information on their background characteristics.

Table 4: Demographic Characteristics

Characteristic	Number, (%) N=4,037	Characteristic	Number, (%)
<b>Age (Years)</b>		<b>Parity</b>	
21-25	1,486 (36.8)	0	1,119 (27.7)
26-35	2,025 (50.2)	1	1,031 (25.5)
36-45	510 (12.6)	2	679 (16.8)
46-56	16 (0.4)	3	488 (12.1)
		4	341 (8.5)
		>=5	379 (9.4)
<b>District</b>		<b>Family owns ITN</b>	
Gakenke	672 (16.6%)	Yes	3,261 (80.8)
Gisagara	678 (16.8%)	No	776 (19.2)
Karongi	612 (15.2%)		
Kicukiro	730 (18.1%)	<b>Slept under an ITN last night</b>	
Nyagatare	672 (16.7%)	Yes	3,000 (74.3)
Ruhango	673 (16.7%)	No	1,010 (25.0)

	Not certain	2 (0.05%)
	Missing	25 (0.6%)
<b>HIV</b>		
Positive		134 (3.3)
Negative		3,740 (92.6).
No Test		34 (0.84)
Missing		129 (3.2)

A total of 4,037 were recruited. Roughly one-third of women were between 21-25 years of age. Twenty-one is the age of majority in Rwanda and was used as the lower recruitment limit, making pregnant married women who were less than 21 years of age ineligible for the study. This created another challenge in meeting recruitment targets in a timely manner.

The parity reveals that more than half of respondents (53.2%) were having their first or second child, a group that in most circumstances is considered at highest risk of malaria in pregnancy and its consequences.

HIV prevalence was low (3.3%) compared to other countries in the region. Among pregnant women HIV positive, 6.7% (9/134) were both HIV and PCR positive; 1.4% (2/134) were both HIV and RDT positive and 2.2% (3/134) were positives to HIV, RDT and microscopy.

Most homes (80.8%) owned at least one bed net, though only 74.3% of pregnant women slept under nets.

## II. Results by Objectives

*To determine the current prevalence of detectable<sup>2</sup> malaria among pregnant mothers using data from their first ANC visit in Rwanda.*

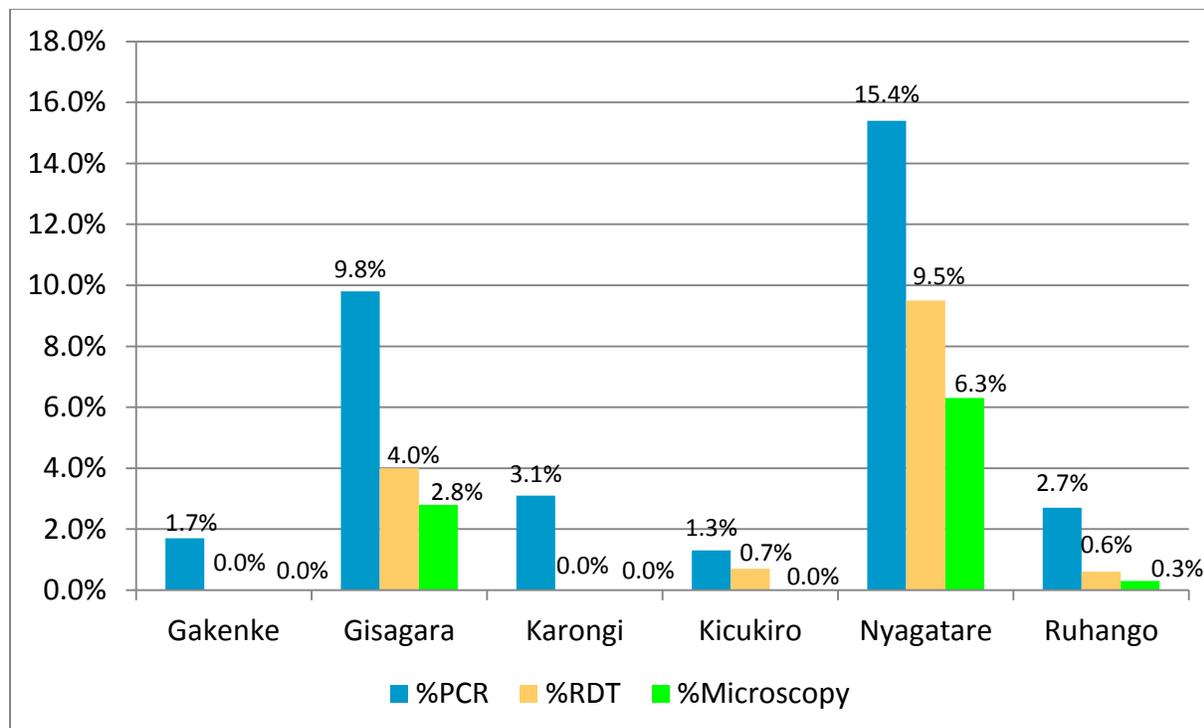
Comparisons were made between the various test results and variables such as bednet use, HIV status, parity, age and anemia. Since there were recorded only 7 women whose temperatures at the time of visit were recorded at 38C and above, this variable was not considered. There was no association with any of the tests and either HIV status or parity. Increasing maternal age was associated with negative tests for only microscopy and RDT.

<sup>2</sup> The study team recognizes that no test is 100% reliable in detecting malaria.

- **Malaria Test Results by district**

Three tests have been used in the study to compare prevalence of MIP in all the six districts.

**Figure 1: Percentage of positive malaria test per district by type of test**



**Table 5: Positive Predicted Value (PPV)**

Test	Compared to	Test	PPV
RDT	→	PCR	69%
Microscopy	→	PCR	81%
Microscopy	→	RDT	84%

As shown in Figure 1, in considering the average result from the 6 districts, the highest overall positivity (5.6%) was found using PCR, followed by 2.5% for RDT and 1.6% for microscopy. The prevalence by district also reflected results of national case reporting from health centers showing proportion of slides testing positive. Hence Nyagatare and Gisagara were in the ‘high’ prevalence areas, as expected, Gakenke and Karongi in the ‘low’ prevalence areas and Kicukiro and Ruhango were classified as ‘moderate’.

- **Comparison of three malaria tests results**

The prevalence of MIP was compared with the three different but complementary diagnostic tests: microscopy, Rapid Diagnostic Test (RDT) and Polymerase Chain Reaction (PCR).

Based on study design, it was planned to perform 3 tests to each pregnant women recruited in study. During the study, 100% of study participants received RDT test (4,037) among them hundred (100) were confirmed RDT positive; 99.8% (4,029/4,037) study participants were tested on malaria using microscopy and only 63 were confirmed malaria positive and 93.6% (3,781/4037) PCR readings carried out among them 246 positives including 213 falciparum and 33 malariae.

Based on PCR results, malaria vivax do not exist in Rwanda and 9 PCR were both Falciparum and malariae positive thus the number of women with malaria confirmed by PCR is 237.

Based on all positives test during the study, 6.2% (251/4037) pregnant women/cases were confirmed with malaria among them 6.3% (16/251) had fever (temperature  $>37.3^{\circ}$  C) the day of test.

Below tables show comparison of malaria tests.

**Table 6: PCR Falciparum results versus RDT results**

RDT Results	PCR Results			Total
	Positive	Negative	Indeterminate	
Positive	69	27	4	100
	<b>32.40%</b>			
Negative	143	3538	249	3,930
		<b>99.20%</b>		
Indeterminate	1	3	3	7
Total	213	3568	256	4,037

Table 6 compares PCR and RDT tests. While specificity is high (99.20%), sensitivity fares poorly. Only 32.4% of positive RDT results were also positive with PCR.

**Table 7: PCR Falciparum results Vs Microscopy results**

Microscopy	PCR Results			Total
	Positive	Negative	Indeterminate	
Positive	51	8	4	63
	<b>23.90%</b>			
Negative	162	3,560	252	3,974
		<b>99.80%</b>		
Total	213	3,568	256	4,037

Table 7 compares PCR and microscopy tests. Here the specificity is high (99.80%) but sensitivity remains low with only 23.9% of positive microscopy results also positive with PCR.

**Table 8: RDT positive results versus PCR**

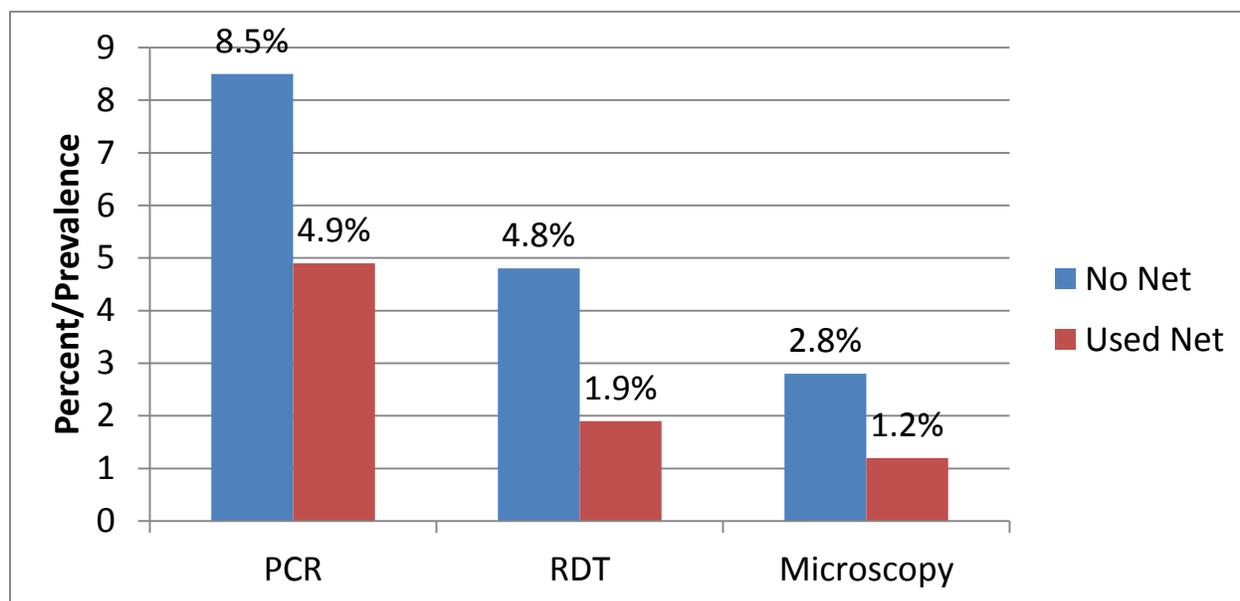
Microscopy	RDT Results			Total
	Positive	Negative	Indeterminate	
Positive	52	10	1	63
	<b>52%</b>			
Negative	48	3,920	6	3,974
		<b>99.70%</b>		
Total	100	3,930	7	4,037

Table 8 compares RDT and microscopy tests. Here the specificity is high (99.80%) while the sensitivity is slightly higher with 52% of positive microscopy results also positive with RDT.

- *ITN use and malaria prevalence*

The study was looking to determine if the reported current ITN use influences malaria prevalence.

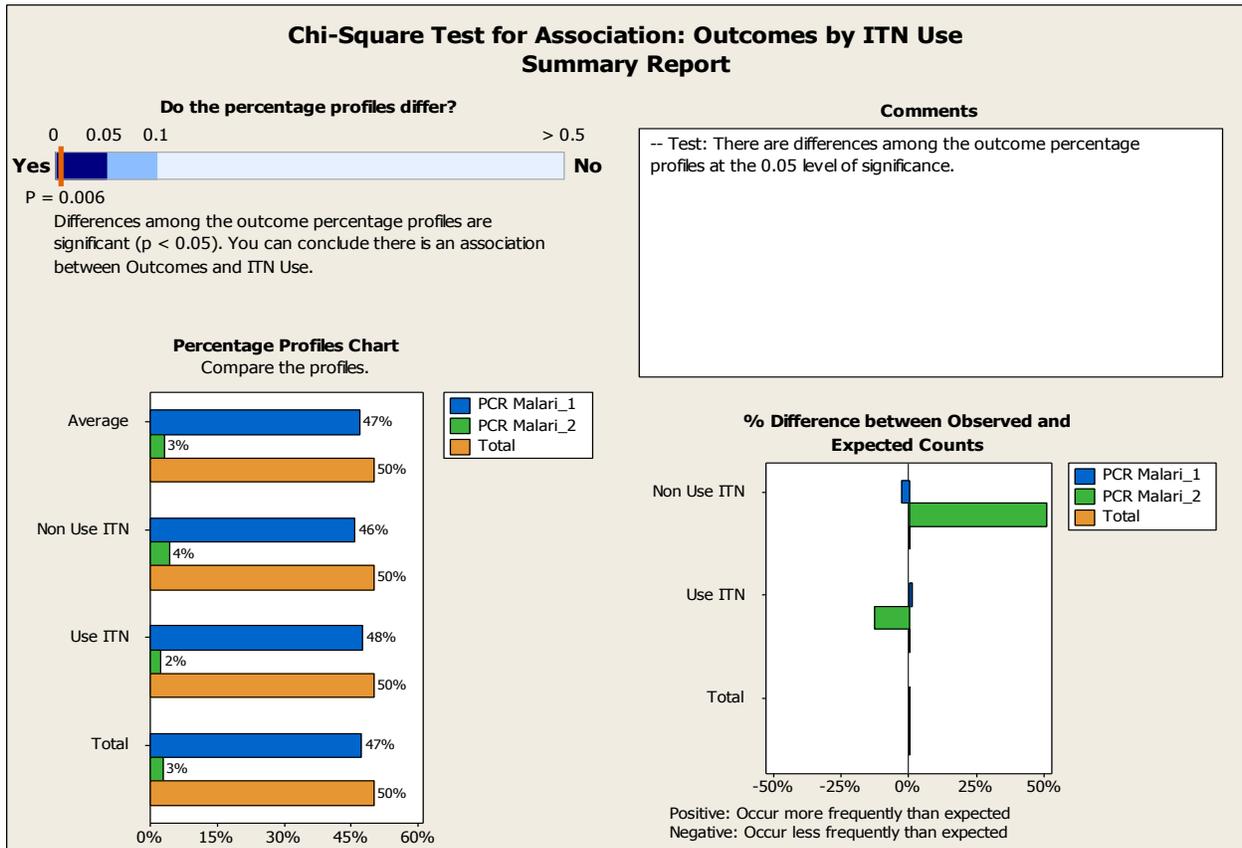
**Figure 2: Percent tested positive for malaria according to ITN use**



Use of bed net the previous night was associated with negative results on all three tests as shown in figure 2. Generally there were double the proportion of positive test results among those who did not sleep under a net, than among those who did. The strongest association was demonstrated with PCR results where 8.5% who did not sleep under a net the previous night tested positive compared to 4.9% who used their nets. Of course nets did not offer complete protection because the indicator was only referring to the night before the interview, a standard approach in Demographic and Health Surveys.

The following Chi- square test in figure 3 is confirming significant association between malaria prevalence and ITN use.

Figure 3: Chi-square test for association of prevalence and ITN use



All samples are large enough to obtain expected counts. The P-value is less than 0.05; thus, there is a statistically significant difference in the falciparum outcomes between those who used an ITN last night and those who did not.

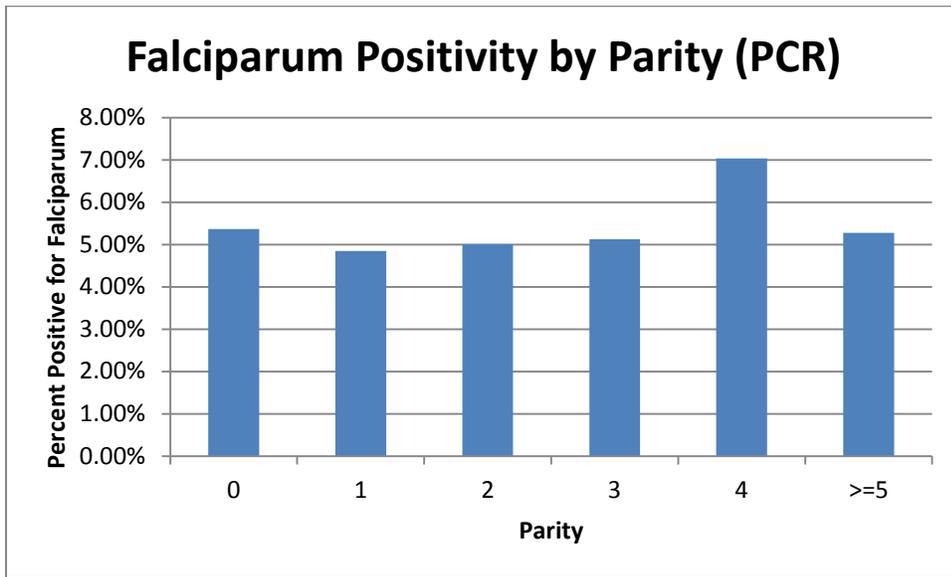
- ***Parity and malaria prevalence***

The study aimed also to analyze the association between the parity and the malaria prevalence.

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Figure 4: Malaria Prevalence according to the parity using PCR test



There was no association between MIP prevalence and parity even when parities are grouped as shown in the table below. This is confirmed by the Chi-square tests results.

Table 9: PCR results per parity groups

Parity group	PCR results		Total
	Negative	Positive	
Parity 0- 2	2665	164	2829
Parity 3- 5	960	65	1025
Parity 6 and above	175	8	183
<b>Total</b>	<b>3800</b>	<b>237</b>	<b>4037</b>

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.183 <sup>a</sup>	2	.553
Likelihood Ratio	1.242	2	.538
Linear-by-Linear Association	.006	1	.937
N of Valid Cases	4037		

- *Anemia and malaria prevalence*

Hemocue test was made to determine the anemia and later associated with malaria prevalence.

**Table 10: Hemocue Results and Malaria Test Results**

Malaria Test Results	Hemocue Results			T test, P values
	Mean	Variance	Median	
<b>Microscopy</b>				
Positive	11.1	3.438	11.0	T = 7.800
Negative	12.7	2.439	13.0	P < 0.000001
<b>RDT</b>				
Positive	11.4	2.963	11.5	T = 7.940
Negative	12.7	2.439	13.0	P < 0.000001
<b>PCR</b>				
Positive	11.8	3.940	12.0	T = 8.885
Negative	12.7	2.353	13.0	P < 0.000001

Those testing positive on any malaria test had significantly lower Hemocue blood test results (the measure of anemia). The average and median values of Hemocue are seen in Table 10. In all cases there is an average of 1.5 points higher Hemocue score for those testing negative compare to positive.

**Table 11: Malaria prevalence according to hemoglobin results**

**Case Processing Summary**

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Hemoglobin test	4016	99.5%	21	0.5%	4037	100.0%

**Malaria results and anemia status**

Hemoglobin group	Malaria Results		Total
	Negative	Positive	
Anemia: Hgb <11g/dl	508	85	593
Hgb Normal: >11g/dl	3235	188	3423
Total	3743	273	4016

**Chi-Square Tests<sup>c</sup>**

	Value	df	Asymp. Sig. (2- sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	62.365 <sup>a</sup>	1	.000	.000	.000	
Continuity Correction <sup>b</sup>	60.977	1	.000			
Likelihood Ratio	50.977	1	.000	.000	.000	
Fisher's Exact Test				.000	.000	
Linear-by-Linear Association	62.349 <sup>d</sup>	1	.000	.000	.000	.000
N of Valid Cases	4016					

According to WHO and Rwanda standards, hemoglobin below 11g/dl is considered anemia for pregnant women. Study results indicate five hundred ninety three women (14.7%) have anemia. 31% (85/273) of women are confirmed malaria associated with anemia. The results of Chi-Square Tests in this case, the significance value displayed is very low.000 (<0.05) , which means it appear that the anemia is related to malaria positivity.

## Discussion and Conclusions

The overall goal of documenting the prevalence of malaria in pregnancy at the first antenatal care visit of a current pregnancy in Rwanda was found feasible using the three malaria tests. The results conformed to the pattern of suspected malaria prevalence across the country as determined by slide positivity rates from health center laboratories. The highest prevalence districts bordered neighboring countries that themselves have higher malaria prevalence.

Although use of RDTs has been the job of community health workers for the prompt detection and treatment of malaria in children, the study showed that it is feasible for trained ANC staff to perform RDT as part of routine ANC, especially considering that the process was easily integrated into blood sampling normally undertaken for HIV and anemia testing. Since there were extremely few other indicators of malaria in these women, e.g. current elevated temperature, the process of testing was valuable in identifying and promptly treating infected women.

The main concern with the study is the low agreement between typical clinic and field based test procedures – RDTs and microscopy – and PCR, a process that as yet is not adapted fully to real time testing needs.

The factors associated with positive test results have important implications for pregnant women. First, all three tests indicated concern about anemia for those testing positive for *Falciparum* malaria parasites. Anemia in pregnancy is a major cause of other complications including pre-eclampsia and maternal death. Thus the need to prevent or detect and treat malaria promptly in pregnant women remains an important goal of malaria control activities in the country, even as prevalence drops in the general population.

The value of sleeping under insecticide treated bed nets is reinforced by the results. The fact that 20% of women did not use nets is of great concern considering that nets are the only way to prevent malaria in the early stages of pregnancy. The fact that some pregnant women did not have access to or did not use nets prior to their first ANC visit needs to be addressed in upcoming efforts to achieve universal ITN coverage.

### Policy Implications

Currently national malaria policies and strategies for addressing malaria in pregnancy focus on two main interventions, use of insecticide treated bednets and appropriate case management of malaria in pregnant women. While the former appears to be well implemented with each pregnant woman receiving a new ITN on her first ANC visit, one can see that prior to first ANC attendance when a pregnancy is very vulnerable, 25% of the women studied did not sleep under a net. Even in households where ITNs are available, 8% of women did not use them. Therefore greater emphasis is needed in national policies and strategies concerning universal net ownership, net hang up and net use so that women are protected from malaria whenever they may become pregnant and not wait until they reach ANC some few months into their pregnancy.

While policies give guidance about the procedures for case management of malaria in pregnancy, the actual process is relatively passive. Should a nurse/midwife suspect a pregnant woman has malaria, she will send the woman to the laboratory for testing. Treatment will ensue after positive microscopy. The results of this study indicate that more aggressive guidance is needed. Not all women who tested positive with any of the three tests used would have been suspected cases. Studies have shown that asymptomatic and submicroscopic malaria infection still contributed to anemia and threatens the pregnant woman and the fetus. Widespread screening of all pregnant women may not be warranted for cost reasons, but based on the results protocols for identifying higher risk women may be in order such as those who are anemic or do not report ITN use. Considering the variety and availability of community health agents in the country, greater community level vigilance and referral could also be instituted.

The findings that the districts with the highest slide positivity rate from HMIS reports are also the ones with the highest malaria test rates have implications for policy at two levels. Living in such a high risk area may be another indicator for the need for more intensive screening of pregnant women living there. Also since these two districts are on the border with other higher endemic countries (Tanzania, Uganda, Burundi), there are implications for policies that promote closer cross-border collaboration on malaria control.

### **Recommendations and Next Steps**

As Rwanda moves toward malaria elimination we can see that pregnant women are still at risk. In fact considering the very low level of elevated temperature, many infections may be asymptomatic. With this in mind, the Ministry of Health and specifically the Malaria and Other Parasitic Diseases Division should consider the following actions:

1. Ensure that all households receive adequate supplies of ITNs; since it is not possible to detect the time when a woman actually becomes pregnant and that the early weeks and months of pregnancy are the most vulnerable, it is crucial that all women of reproductive age have access to bed nets.
2. Improve malaria in pregnancy case management protocols stressing better identification of high risk clients.
3. Strengthen the role of Agents Santé Communautaire Maternelle (ASCMS) in malaria control. Through supportive supervision encourage them to intensify efforts to remind pregnant women in their villages to use the bed nets every night. Provide training for them in prompt malaria case detection and referral.
4. Organize cross-border collaboration with countries nearest to Rwanda's higher burden districts.

## Annex A: Rwanda MIP prevalence data collection and extraction form

Section	Items	Response/Information
<b>PART A</b>	<b>Information from ANC Register/Card</b>	
<b>LOCATION</b>	District	
	Health Center	
	Date	
<b>WOMAN</b>	S/N or ID	
	Age of Woman	
	Parity	
	Gravidity	
	Age of Pregnancy in Months	
<b>Routine ANC Tests</b>	Anemia (Hematocrit/Hemocue)	
	HIV Status	
	Current temperature	
<b>PART B</b>	<b>Information Asked from Woman</b>	
<b>FEVER</b>	Have you had a fever/high body temperature since the current pregnancy started?	G Yes    G No    G Not Certain
	Have you received any Malaria treatment since you knew you were pregnant?	G Yes    G No    G Not Certain
	From whom or where did you get this treatment/medicine?	G CHW    G Govt. Clinic    G Private G Not Certain G Other _____ (tick all mentioned)
	If yes, what was the name, brand, type of medicine used?	
<b>NETS</b>	Does your family own an ITN?	G Yes    G No    G Not Certain
	If Yes, how many?	
	If Yes, where did you get the nets?	G Campaign    G Routine    G Private    G Not Certain (tick all mentioned)
	Did you Sleep Under an ITN Last Night?	G Yes    G No    G Not Certain
	In Past Month would you say that you Slept under an ITN ...	G no nights    G a few nights G some nights    G most nights G All nights
<b>PART C</b>	<b>Malaria Test and Treatment Information</b>	<b>Result/Dose</b>
<b>Malaria TEST RESULTS</b>	RDT	
	Microscopy	
	PCR	
<b>Malaria Treatment Provided</b>	ACT	
	Quinine	
	Referral/Reason	

## Annex B: consent form

### **PARTICIPANT CONSENT FORM / RESEARCH PROJECT Cross-sectional Survey of Malaria Prevalence in During Pregnancy in Rwanda**

**Study Title:** A Study to Determine the Current Prevalence of Malaria Detectable Among Pregnant Women Registering for the first ANC (Antenatal Care) in Six Districts in Rwanda

**Principal Investigators:** Corine Karema and William Brieger

Before recruiting into the study, the study subject must be informed about the objectives, procedures, and potential benefits and risks involved in the study. Details of all procedures must be provided including their risks, utility, duration, frequencies, and severity. All questions of the subject must be answered to his/ her satisfaction, indicating that the participation is purely voluntary. The subject must indicate her acceptance of participation by signing or thumb printing on this form.

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#### **What you should know about this study**

You are being asked to join a research study. This consent form explains the research study and your part in the study. Please read it carefully and take as much time as you need. We can also read it to you. You are a volunteer. You can choose not to take part and if you join, you may quit at any time. There will be no penalty if you decide to quit the study.

#### **Purpose of research project**

We and the Rwanda MOH (Ministry of Health) are working together to learn about malaria in your community. We want to know how much and what type of malaria is present among pregnant women, who get malaria, what things can increase the risk of malaria, and how malaria changes overtime. These information will help us control malaria in this area and understand what we can do to treat and prevent the illness well. We cannot get any of the information without your participation, and we greatly appreciate your time and effort in this study.

Malaria is an illness caused by parasites, tiny little organisms that cannot be seen with your naked eyes. They have to be magnified to be seen or we can detect them by looking for their proteins or genes. Proteins and genes are parts of the parasites. You get infected with the parasite when you are bitten by a mosquito infected with the parasites. Sometimes you get very sick, sometimes you do not, but even without feeling sick, the parasites can sit in your blood quietly, and cause your blood levels to be low which is not healthy. If you are pregnant, the parasites can go into your placenta, and cause harms to your baby.

#### **Why you are being asked to participate**

We cannot see all people in the district. We chose you because of where you live. You are one of about 670 women of this district we are asking you to participate because you live in this village where we are trying to learn about malaria and you are exposed to the malaria parasites often. Because the number of people who get malaria is not the same throughout the country –

we are visiting different districts to learn more about the disease. Your district and health center were chosen so we can learn about malaria in this particular setting. We hope test as many pregnant women as possible in this clinic over the next two months, and since you are a client of this clinic, we hope you will be willing to be tested for malaria.

### **Procedures**

If you agree to join this study, you will be asked first to sign this consent form, and you will become part of our study. If you cannot read or write, we will read this consent form to you. You can place a 'left thumb impression' mark as a proof that you have consented to this study if you like. We will ask a witness to sign the form to show that you understood the explanation of the study.

Today you will receive your routine antenatal care. In addition, after getting your consent, we will ask you about your recent experiences with treating and preventing malaria. We would also like to take your temperature with a thermometer. We will also like to collect information from tests that are part of your normal ANC such as level of blood in your body and HIV (if you agree to that test), and the ANC medicines you are given, not something we collect separately. All this questioning and measuring will take about 25 minutes. We would also like to take a small sample of blood from your fingertip. It will be no more than a few drops of blood. Getting blood from your finger and finding out if you have malaria or not will take about 15 minutes. You might have had a malaria test in the past and the small prick on your finger should not feel different than that.

We will collect drops of blood from your fingertip for three malaria tests. We will put some on a small piece of paper. We will dry it, and store it in a safe place. We will later look for the malaria gene in your blood using a special technique called PCR (Polymerase chain reaction). This will be done about 2-3 months from now. We will use one drop of your blood to look for malaria proteins. Evidence of malaria disease in your blood? Using Rapid Diagnosis Test (commonly known as RDT (Rapid Diagnostic Test) and used by our CHWs (Community Health Workers) in the community for children) which will show you a change in color if you have malaria. We will also examine the blood in the health center laboratory by microscope by putting a drop of blood on a slide. If the RDT test shows malaria, we will provide you with the standard malaria treatment according to the national guideline.

Staff at the clinic may test your blood for malaria the next time you come for ANC.

. If we complete our research and no longer need to keep the specimens, we will destroy them.

### **Risks/discomforts**

You may be familiar with blood tests to find out if someone has malaria – maybe you yourself have had a malaria test or one of your relatives. If so, you know that there are minimal risks associated with finger sticks including pain, very rarely infection and fainting. We will take all precautions and follow procedures to minimize these risks for both participants and investigating team. Study team members are well trained to do this procedure and will use very small needle so that pain will be minimal. Every attempt will be made to avoid infection or hematoma from finger stick. Skin will be cleaned with alcohol before the procedure and pressure will be applied

until bleeding has stopped. The amount of blood taken during the study is very small, and the danger of developing a low blood level (anemia) is very low.

### **Benefits**

If we find that you have malaria in your blood, you will benefit from treatment. Otherwise what we learn from testing you and other women is the level of malaria infection in this community and Rwanda generally. This will help the National Malaria Control Programm develop better services for all pregnant women. You will not benefit directly from the study, except that we may catch your malaria without ill symptoms and you will benefit from getting medical treatment early which may correct your low blood levels and prevent further worsening of the illness. This benefit is even more valuable if you are pregnant. Although you don't benefit directly from this, you will be part of this intensive effort to understand malaria, and find a way to improve treatment and prevention of this disease which is so common in your community.

### **Payment**

You will not be paid to participate in this study since this is part of normal ANC. You will receive treatment if needed and an insecticide-treated bed net.

### **Protecting data confidentiality**

The data collected and generated from this study will be kept confidential in documents and electronic forms that will be locked and that have limited access to only authorized investigators and research staff.

### **Protecting subject privacy during data collection**

Your hospital number will be recorded so that the various malaria test results can be entered on your card and so that during your next ANC visits, staff will have a good history of your malaria experiences and be able to treat and advise you appropriately. Your hospital number will be removed from the study record form when the study is over. These study record forms will be kept in locked file cabinet in the Senior Clinical Officer's locked office when not in use. After enrollment, all laboratory specimens will have only an identification number (Not your name) in order to protect confidentiality. Only authorized investigators and research staff will have access to these files.

### **Alternatives to procedures or treatments**

Alternative to study procedures is NOT to participate in the study. You will receive your routine medical and pregnancy care, regardless of your participation in the study.

### **Biological specimens**

The blood and data collected from you during this study are important to science. You will not own the blood or data after you give it to the study. You will not receive any financial benefit from any product or idea created by the investigators using the data or materials collected from you.

### **Cost of participation in the study**

You will not be charged for participating in this study.

**What happens if you leave the study early?**

If you decide to stop participating in the malaria tests and interview, nothing will happen to you. Your medical care at any health centers will not be compromised for leaving early.

**Sharing your health information with others**

We will not share your individual health information with others without your permission.

**Conflict of Interest**

We do not conduct this research project for any financial and material gain, and declare no conflict of interest.

**Payment of treatment costs for injury or illness from study participation**

We do not have a program to pay you if you are hurt or have other bad results from being in the study. However, we will take care of you according to our national standard of care and you will not be billed for the cost of any treatment you receive for study-related injury or illness.

**Choice for Future Research Use and Future Contact**

Please read each statement below and think about your choice. Check either yes or no for each statement.

My samples/data may be used for future malaria research by the National malaria Control Program

Yes     No

I agree to be contacted for future studies regarding the future use of my samples/data, when appropriate. I understand that in certain circumstances, the PI will not need to recontact me to use my samples/data in future studies.

Yes     No

**Address to Contact the Investigators****Who do I call if I have questions or problems?**

- Call the principal investigator or other investigator following the above address if you have questions, complaints, or get sick or injured as a result of being in this study.

Dr Corine Karema  
Tel: 0788303915

Bwana Jeremie Zoungrana  
Tel: 0788306678

- Call or contact the Rwanda IRB (Institutional Review Board) Office if you have questions about your rights as a study participant. Contact the IRB if you feel you have not been treated fairly or if you have other concerns. The IRB contact information is:

Wana Justin  
Tel: 0788500499

Dr Emmanuel Nkeramihigo  
Tel: 0788557273

**Permission to Proceed (For oral consent)**

**Is it okay to proceed with the survey, questionnaire, and blood draw listed above?**

\_\_\_\_\_  
Print name of Person Obtaining  
Consent

\_\_\_\_\_  
Signature of Person Obtaining Consent

\_\_\_\_\_  
Date

**What does your signature on this consent form mean?**

Your signature on this form means:

- You have been informed about this study's purpose, procedures, possible benefits and risks.
- You have been given the chance to ask questions before you sign.
- You have voluntarily agreed to be in this study.

Thank you for your Cooperation.

◆ \_\_\_\_\_  
Print name of Adult Participant

\_\_\_\_\_  
Signature of Adult Participant

\_\_\_\_\_  
Date



Give your Left Thumb Impression in this box if you are unable to sign the consent. This mark will be taken as your consent to the study.

## Annex C: supervision check list

### District Supervisory Visits by National Team form

District: \_\_\_\_\_ Heath Center:  
\_\_\_\_\_

Date of supervision: \_\_\_\_\_

Item to observe	Observation	Comments
Date of starting of Data collection		
Target for the HC		
Enrolled to date		
Missed new registrants?		
Checking of ANC cards for enrollment		
Ask ANC staff if it is a normal enrolment		
Observe the HC nurse enroll ( 2- 3 clients), use data collection sheet as check list to see if she follows procedures		
Observe the coordinators doing supervision of nurse		
Appreciate the Coordinators feed back to the nurse		
National supervisor feed back		
Review all data forms and PCR samples collected since last visit by District Coordinator for accuracy and completeness		
Encourage the District Coordinator to identify strengths and weaknesses in the completed data and PCR forms before providing your own feedback. Make a note of strengths and deficiencies on the data forms by the nurse and the ability of the supervisor/coordinator to guide her.		
Get the nurse's opinions of what she enjoys about the project and what she finds challenging. Ask if she has figured out ways to overcome any challenges on her own. Ask her how the supervisor/coordinator has been helpful.		
Get Coordinator's opinion of strengths and weaknesses of the health center's performance in the study. Ask him/her to explain any efforts made to reinforce good practices (with examples) or correct deficiencies (with examples)		

## References

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- <sup>ii</sup> World Health Organization. A Strategic Framework for Malaria Prevention and Control during Pregnancy in the African Region. WHO Regional Office for Africa, 2004. AFR/MAL/04/01.
- <sup>iii</sup> Ministry of Health of Rwanda. Rwanda Interim Demographic and Health Survey 2010. Kigali, Rwanda.
- <sup>iv</sup> Van Geertruyden JP, Ntakirutimana D, Erhart A, Rwagacondo C, Kabano A, D'Alessandro U. Malaria infection among pregnant women attending antenatal clinics in six Rwandan districts. *Trop Med Int Health* 2005;10(7):681-8.
- <sup>v</sup> Rwanda Malaria Program Performance Review 2011. Malaria Unit/TRACPlus – MoH, External Review Team
- <sup>vi</sup> Sievers AC, Lewey J, Musafiri P, Franke MF, Bucyibaruta BJ, Stulac SN, Rich ML, Karema C and Daily JP. Reduced paediatric hospitalizations for malaria and febrile illness patterns following implementation of community-based malaria control programme in rural Rwanda. *Malaria Journal* 2008, 7:167.
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- <sup>viii</sup> Karema and al. (Trends in malaria cases, hospital admissions and deaths following scale-up of anti-malarial interventions, 2000–2010, Rwanda published in *Malaria Journal* 2012, 11:236 doi:10.1186/1475-2875-11-236)