UNIVERSITY OF LIVERPOOL

The Burden of Malaria in Pregnancy in Madhya Pradesh, India

Thesis submitted in accordance with the requirements of
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Doctor in Philosophy
by

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LSTM

TEXT BOUND CLOSE TO THE SPINE IN THE ORIGINAL THESIS

Dedication

To my late father, Ahmed Ali Didi who was a key figure in the work that led to the elimination of malaria from the Maldives. Had he lived to see my work in malaria, this would have made him proud

To my mother, Ayeshath Moosa Didi who taught me the joy of caring, the strength of patience, to live without complaining and whose love keeps me going

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The Burden of Malaria in Pregnancy in Madhya Pradesh, India

Rukhsana Ahmed

Introduction and Method: Malaria in pregnancy (MiP) can have devastating consequences for the mother and the newborn. An evidence based framework for the control of MiP has been developed by the World Health Organisation (WHO) for malaria endemic sub-Saharan Africa. In contrast, no such framework exists for areas with low malaria transmission, such as in Asia where both *P.falciparum* and *P.vivax* are the predominant species. There is relatively little information on the burden of MiP in this region, hampering the design of rational control strategies for this high risk group. The objective of my thesis was to better define the burden of MiP in the central Indian state of Madhya Pradesh and to evaluate the performance of new rapid diagnostic tests (RDTs) that are considered as part of the new antenatal malaria screening strategies for the control of MiP. Women attending antenatal clinics (ANC module) and delivery units (delivery module) in a primary, secondary and tertiary health facility in a rural, semi-rural and an urban town in 3 districts of Madhya Pradesh were enrolled in a series of cross-sectional surveys. Pregnant women, regardless of symptoms were screened for anaemia and maternal and placental malaria using RDTs and standard microscopy. An adjusted version of the MiP rapid assessment tools developed by the US-based Centres for Disease Control and Prevention was used.

Results: 1817 and 2696 women were enrolled in the ANC and delivery modules conducted during two 6-week periods in the dry (April-May 2006) and the post-rainy season (October-November 2006). The delivery module was extended in two sites to provide year-round information. The overall malaria prevalence in antenatal women was low: 4.5% (1.9% in the dry season and 6.5% in post rainy season) with wide variation between the rural (20.7%) and urban (0.3%) sites. The prevalence of maternal and placental malaria in the delivery module, assessed by standard microscopy, was 2.0% and 1.0% respectively in the year-round survey. *P.falciparum* was the predominant species (89%), with a seasonal peak between October and December. The prevalence of *P.vivax* infections was persistently low throughout the year (<1%). Sixty-three percent of the microscopically detectable *P.falciparum* and 55% of the *P.vivax* infections were symptomatic. All gravidity and age groups were equally at risk. *P.falciparum* was associated with moderate-severe anaemia and preterm low birth weight. Babies born to women with placental *P.falciparum* malaria were approximately 20% lighter than women without malaria. Overall, 70% of the preterm births and 50% of the low birth weight in infected women were attributable to placental *P.falciparum* malaria. The corresponding population attributable fractions were 6.6% and 4.6%. Mono-infections with *P.vivax* (PCR confirmed) were also associated with anaemia and preterm low birth weight. Studies using diagnostic PCR showed that in the peak transmission season, 1-in-5 smear and RDT negative women were PCR positive for malaria; these 'sub-patent' infections were associated with mild anaemia. Similarly, placental sub-patent infection was associated with reduced birth weight (207g). No PCR data was available at delivery. The *P.falciparum* associated placental histopathological changes were mild and suggest most infections were acute, contrasting to findings in stable high transmission areas. *P.vivax* associated placental changes were negligible.

Compared with microscopy, the new First Response® pLDH-based RDT had 94.7% sensitivity and 99.3% specificity for *P.falciparum*, and 85.7% and 99.5% for *P.vivax*. However, compared with PCR, the sensitivity was only 23.2% (99.4% specificity) and 4.7% (99.6% specificity) respectively.

Conclusion: The overall prevalence of microscopically detectable malaria in Madhya Pradesh was low and markedly seasonal with a predominance of *P.falciparum*. The persistence of *P.vivax* throughout the year probably reflects relapses of hypnozoites rather than year-round transmission. In contrast to malaria endemic Africa, many, but not all, infections were associated with fever. Maternal anaemia and LBW, primarily due to preterm births were the main adverse consequences. Severe placental sequestration or placental inflammation associated with *P.falciparum* infections, were not seen. However, there was an unexpectedly high prevalence of sub-microscopic infections detected by PCR in antenatal women and this was associated with increased risk of anaemia. This suggests that the risk of exposure and the burden of MiP in this region of India may be much higher than previously appreciated and the presence of these sub-microscopic infections should be taken into account in planning appropriate control measures for pregnant women this region.

Contents

List of Tables

List of Figures

Acknowledgement

"the product that the PhD student creates is not the thesis....
the product of their study is the development of themselves"
-Sir Gareth Roberts-

Many people have contributed directly and indirectly to the project that led to this thesis and to my personal development. All are thanked for, although only some names appear in the limited space.

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I am deeply grateful to Feiko for his insightful advice and guidance, for making me see sense in data and meaning in numbers, his willingness to discuss the thesis subject anytime I knock on his door and for assigning me the responsibility of the Indonesia project. All of this has had an impact on my interest in malaria epidemiology. I hope that the substance of my thesis reflects what I have learned from both my supervisors.

At LSTM, I thank my advisory panel members Professor Bernard Brabin and Dr Imelda Bates, for showing interest in my work and keeping up my motivation. The statistical advice provided by Brian Faragher is appreciated. I offer thanks to all the colleagues at the Malaria Epidemiology Unit for their friendship and willingness to help and a big thank you to Alison for volunteering to proof read my thesis chapters.

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At the Centers for Disease Control and Prevention, Atlanta, USA I am grateful to Drs Udhayakumar, Meghna Desai, Monica Parise and Naomi Lucchi for their contributions to the project and support. I thank Dr Carlos Abramosky for teaching me to read placental histology slides.

For my network of friends spread across the world I thank for compacting distance into "Facebook"; Carole for her willingness to help, Rachel Braddock for reading through my chapters and to my PhD colleagues for the interesting discussions and late night company.

I am thankful to my large extended family, for the laughs and keeping me connected to family events via the internet. Lastly to my sisters Ihusana and Sultana and in-laws Ambari Sattar and Ahmed Maniku for their love and support and being available just a phone call away regardless of distance and time. As this chapter of my life ends, I am glad I did it and thank my parents for instilling in me the value of education.

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Declaration

This thesis is the result of my work. The material contained in the thesis has not been presented, nor is currently being presented either wholly or as a part, for any other degree or other qualification.

The field project of this study was part of a collaborative project between the National Institute of Malaria Research, India, the Centres for Disease Control and Prevention, Atlanta, USA, and the Liverpool School of Tropical Medicine, UK. The field work was conducted at three health facilities in the towns of Jabalpur, Katni and Maihar, in Madhya Pradesh, India. I was responsible for the organisation of field work and overseeing of the shared laboratory work. The laboratory work took place in the Field Station of the National Institute of Malaria Research in Jabalpur, India. My contributions to this research were as follows:

Activity	Responsibility
Field work:	
Day to day management of the study	Sole
Proposal development	Shared
Development of study manual	Sole
Training of study Staff	Sole
Participant recruitment and data collection	Shared
Blood sample collection (placenta & peripheral)	Shared
Laboratory work:	
Microscopic examination of blood smears	Shared
Placental histopathology slide examination	Sole
PCR assay	Shared
Data Management:	
Data Entry	Shared
Data cleaning	Sole
Statistical analyses	Sole
Writing up of the thesis	Sole

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Abbreviations & Acronyms

ACT	Artemisinin Combination Therapy
ANC	Antenatal Clinic
ASHA	Accredited Social Health Activist
CDC	Centres for Disease Control and Prevention
CIDR	Cysteine rich interdomain region
CSA	Chondroitin sulphate-A
DBL	Duffy-binding-link
DDT	dichlorodiphenyltrichloroethane
DHP	Dihydroartemisinin-Piperaquine
DHS	Demographic Health Survey
DNA	Deoxyribonucleic acid
DU	Delivery Unit
EIR	Entomological Inoculation Rate
FANC	Focused antenatal care
FIND	Foundation for Innovative New Diagnostics
G6PD	Glucose-6-phosphate dehydrogenase
GLM	General Linear Model
HIV	Human Immunodeficiency Virus
HRP-2	Histidine Rich Protein 2
ICMR	Indian Council Medical Research
IFNγ	Interferon gamma
IgG	Immunoglobin G
IPTp	Intermittent Preventive Treatment in pregnancy
IRS	Indoor Residual Spraying
IST	Intermittent Screening and Treatment

ITN	Insecticide Treated Nets
IUGR	Intrauterine Growth Retardation
LBW	Low Birth Weight
LLITN	Long Lasting Insecticide Treated Nets
MDG	Millennium Development Goals
MiP	Malaria in Pregnancy
MiPc	Malaria in Pregnancy Consortium
MLE	Maximum Likelihood Estimates
MTCT	Mother to Child Transmission
MUAC	Mid Upper Arm Circumference
NFHS	National Family Health Survey
NGO	non-Governmental Organisation
NIMR	National Institute of Malaria Research
NRHM	National Rural Health Mission
NVBDCP	National Vector Borne Disease Control Programme
PCD	Passive Case Detection
PCR	Polymerase Chain Reaction
pLDH	*Plasmodium* Lactate Dehydrogenase
Primi	Primigravidae
RBM	Roll Back Malaria
RDT	Rapid Diagnostic Test
SEARO	South East Asian Regional Office
Secundi	Secundigravidae
SGA	Small for Gestational Age
SP	Sulfadoxine-Pyrimethamine
SST	Single Screening and Treatment
STARD	Standard for Reporting Diagnostic Accuracy

TNF	Tumour Necrosis Factor
UN	United Nations
UNICEF	United Nations Childrens' Fund
VSA	variant surface antigen
WHO	World Health Organisation

Chapter 1

Introduction

"the world is moved along not only by the mighty shoves of its heroes, but also by the aggregate of the tiny pushes of each honest worker"

-Helen Keller-

1.1Topic overview

Malaria in pregnancy (MiP) is an enormous public health problem that affects millions of pregnant women and their babies. The adverse impacts of malaria in pregnant women primarily occur as maternal anaemia and low birth weight (LBW, <2500g) and are largely due to *P.falciparum.* Without timely treatment or prevention these complications could be fatal to the pregnant woman and her baby and contribute significantly to maternal, neonatal and infant morbidity and mortality. The problem of malaria in pregnancy gained greater attention at the initiation of the Roll Back Malaria (RBM) Partnership by World Health Organization (WHO) in 1998 (WHO 2004b). By then the susceptibility of pregnant women to malaria was well documented from studies conducted in sub-Saharan Africa. In the African region alone, malaria accounts for 3-15% of maternal anaemia resulting in 10,000 maternal deaths annually and approximately 100,000 babies are estimated to die from malaria associated low birth weight (Desai, *et al* 2007, Guyatt, *et al* 2004, Steketee, *et al* 2001).

To appreciate malaria in pregnancy an understanding of the general epidemiology of malaria is important and is described briefly in the next paragraph.

Malaria in focus: In an effort to reduce the global burden of malaria, at the start of the 21st century, the United Nations as part of the Millennium Development Goals (MDG) declared 2000-2010 as the malaria decade with a target to halve the incidence of malaria by 2015 (UN 2010). This commitment to fight malaria has seen a shift in malaria control strategies since the historic eradication efforts of the 1950s with initiation of large programmes. These control strategies recommend malaria confirmation in all suspected cases with a Rapid Diagnostic Test (RDT) or microscopy, treatment with artemisinin combination therapy (ACT) and vector control with insecticide treated bed nets (ITN) and indoor residual spraying (IRS)(WHO 2008c). The concerted efforts of the international organisations, governments of malaria endemic countries and non-government organisations (NGOs) in implementing these programmes, together with a substantial increase in donor funding are starting to have an impact, particularly in sub-Saharan Africa and in changing the global malaria picture.

After a long stagnation the number of clinical cases and deaths due to malaria are now declining in several countries. There were 247 million episodes of malaria estimated in 2006 and this decreased to 225 million in 2009. The estimated number of malaria deaths has also decreased from 1 million in 2000 to 781,000 in 2009, mainly in children less than 5 years in Africa (WHO 2010b). Countries such as Kenya, Gambia, Zambia and Eritrea recently reported large reductions in malaria prevalence in certain areas of the countries following successful vector control efforts and strengthening of community case management (Barnes, *et al* 2009, Ceesay, *et al* 2008,

Nyarango, *et al* 2006, O'Meara, *et al* 2008). According to WHO, 11 African countries and 32 countries outside the African region in 2009 showed a 50% decrease in confirmed clinical cases and deaths. The reductions in malaria incidence indicate that control measures are at least partially succeeding with subsequent falls in transmission in some parts. Thus, it is also likely to have an impact on the exposure risk in pregnant women resulting in a potential shift away from the effects seen with high transmissions, to that of outcomes associated with lower transmission.

The attention given to malaria control strategies has now shifted from control to elimination and possibly to eradication of malaria. This necessitates a reduction of malaria carriage in individuals. The ultimate tool to achieve eradication probably involves a malaria vaccine. A candidate vaccine RTS,S has proved effective in phase I and phase II field trials and is the first vaccine that has been approved for phase III trials raising expectations of malaria prevention (Aponte, *et al* 2007, Pichyangkul, *et al* 2008). Until a proper vaccine is available, it is important to prevent malaria infections using the currently available diagnostic and prevention tools that are known to be effective.

Control of Malaria in pregnancy: Malaria at present is a curable, preventable and a controllable disease. To decrease the public health impact of malaria, the RBM Partnership incorporated malaria in pregnancy control among its four strategies founded on the basis of cure and prevention. Due to the high disease burden in sub-Saharan Africa, this region has been the focus of most malaria in pregnancy research and control. However, to reduce the global burden there is a need to control malaria in pregnancy in regions outside of Africa where the burden might be lower in terms of relative exposure risk to the individual, yet the number of women at risk of malaria is considerably higher (Dellicour, *et al* 2010, Snow, *et al* 2005). There is relatively little information available on the burden of malaria in pregnancy in Asia where malaria transmission is generally lower than most African countries and where *P.vivax* co-exists with *P.falciparum*. More systematic data on the burden of malaria in pregnancy is needed from countries in the Asian region such as India to help develop a rational control strategy for the region.

The African region has benefited from over a decade of research on the use of effective control measures and the development of a strategic framework for the control of malaria in pregnancy by WHO (WHO 2004a). The framework for Africa comprises of: 1) case management, 2) long-lasting insecticide treated mosquito nets (LLITNs) and 3) intermittent preventive treatment in pregnancy (IPTp). The control programmes have been implemented in 33 out of 47 African countries according to an analysis of African national surveys (WHO 2008c). Although they are showing progress a current analysis found there was room for scaling up, with coverage of ITNs being 17% whilst 25% had received at least one dose of IPTp (van

Eijk, *et al* 2011). No such strategy has been developed for the Asian region. In most of Asia, control of malaria in pregnancy is limited to passive case detection (PCD) and inconsistent distribution of ITN and indoor residual spraying (IRS).

1.2 Rationale for the Study

This study was initiated as one of a series of studies to assess the burden of malaria in pregnancy in India, to address the gaps in knowledge on the consequences for the pregnant woman and her baby in cases of asymptomatic and symptomatic *P.falciparum* and *P.vivax* malaria. The surveys presented in this thesis were conducted in the central Indian state of Madhya Pradesh, where transmission was expected to be moderate to high (in Indian classification of annual parasite index) and seasonal. The findings will contribute much needed information for developing a control policy for malaria in pregnancy and for the planning of future research strategies.

Previous studies from India have mainly focused on symptomatic febrile cases in hospitals or on passive case surveillance and community studies among symptomatic women during malaria outbreaks (Chattopadhyay, *et al* 2000, Singh, *et al* 1998). These studies confirmed a predisposition of pregnant women to severe disease compared to non-pregnant women. They also showed an increased risk of maternal anaemia, pregnancy loss and maternal death (Das 2000, Kochar, *et al* 1998, Maitra N, *et al* 1993, Sholapurkar, *et al* 1988, Singh, *et al* 1999a). However, since these studies focused on symptomatic women, they did not allow an estimation of the relative contribution of asymptomatic infections or the scale of the problem of malaria burden in the general pregnant population.

The potential numbers at risk of malaria in pregnancy are large in India. The population of 1.1 billion has the largest number of malaria cases in South East Asia accounting for 60% of the total in the region and amounting to 10.6 million cases in 2006 (SEARO 2008, WHO 2008c). One third of India's population live in the moderate to high malaria endemic regions spread over seven states including Madhya Pradesh. Approximately 360 million people live in the high malaria endemic regions of India; this is nearly half of the 647 million potentially exposed in the 47 malaria endemic countries in sub-Sahara African. Worldwide there were 54.7 million pregnant women living at risk of *P.falciparum* malaria in 2007, and 70 million in areas of low transmission or with *P.vivax*, of which approximately 17% (12 million) were in India (Dellicour, *et al* 2010). These figures suggest that a high number of women at risk of malaria are living in India and a preventive policy is urgently needed for this population.

Although there is abundant data from the African region, it is not applicable to India because of the difference in malaria transmission patterns and species variation. Control programmes designed for Africa would not necessarily be suitable for India, and region specific data is necessary. An assessment of the burden of malaria in pregnancy in Madhya Pradesh is therefore important to capture the impact of both *P.falciparum* and *P.vivax* in this region.

At the time of conducting this study the recommended control policy by the Indian National Vector Borne Disease Control (NVDBC) programme was weekly chloroquine chemoprophylaxis during pregnancy in all malaria endemic areas (NVBDCP 2008). Additionally, intermittent preventive treatment (IPTp) with two doses of sulfadoxine-pyrimethamine (SP) was recommended in high risk areas by the Drug Policy Working Group in India. In practice however these guidelines were not implemented. The treatment regimen for febrile case management included chloroquine for all cases with the addition of primaquine for seven days for *P.vivax* cases after delivery and quinine for the management of severe cases. Although local scientific data on the effectiveness of these drugs is sparse, there is evidence of increasing chloroquine resistance in parts of India. In one study conducted in Assam in 2001, the clinical and parasitological failure rate at day 14 was 64% for chloroquine and 29% for sulfadoxine-pyrimethamine in *P.falciparum* malaria (Campbell 2006). These control regimens for malaria in pregnancy suggested there was a need for revision of policy.

1.3 Study Objectives

Primary objective: To determine the burden of malaria and main risk factors of malaria in women attending antenatal clinics (ANC) and delivery units (DU) in urban and rural health facilities in eastern Madhya Pradesh.

Secondary objectives:

1. To determine the seasonality of peripheral and placental malaria infections and the relative contribution of *P.falciparum* and *P.vivax* infections to maternal anaemia and low birth weight.
2. To determine the histopathological changes in the placenta in response to *P.falciparum* and *P.vivax* infection and their impact on fetal growth and maternal anaemia.
3. To explore the risk factors associated with low birth weight and preterm births.
4. To determine the accuracy of pLDH based Rapid Diagnostic Test (RDT) as a screening tool for malaria in women attending antenatal clinics.
5. To compare different diagnostic methods for the detection of placental malaria.

1.4 Thesis Outline & Chapters

The thesis consists of four sections. The first section covers the introductory chapters providing an overview of the topic, rationale for the study and relevant literature. The second section describes the study design and methods of the overall study. The third part consists of five results chapters corresponding to each specific objective, with a final chapter on the general discussion and recommendations.

- Chapter 1: Introduction
- Chapter 2: A literature review with relevance to the study topic and specific study objectives.
- Chapter 3: Provides an overview of the general study design, study area and population, methods and procedures.
- Chapter 4: Describes the epidemiology and burden of malaria in pregnancy in association with maternal and placental malaria by species and seasonal variation in women attending the antenatal and delivery units.
- Chapter 5: Describes the changes in placental histology associated with *P.falciparum* and *P.vivax* infections according to the chronology of infection and the association with fetal growth and of maternal anaemia.
- Chapter 6: Explores the determinants of low birth weight defined by preterm-LBW and IUGR-LBW to identify amendable factors and describes the characteristics of both entities.
- Chapter 7: Assesses the performance of the pLDH based RDT (First Response®) as a screening test in antenatal women using microscopy as the main reference test and PCR as a 'resolver' reference test. The analysis was carried as part of a modified discrepant analysis in which the RDT and microscopy discordant and concordant positives and a sub-sample of concordant negative results were assessed by PCR.
- Chapter 8: Evaluates the accuracy of peripheral blood tests in predicting placental infections and the performance of placental incision and impression smears compared with placental histology.
- Chapter 9: This chapter discusses the main findings by consolidating the results presented in chapters 4 to 8 and provides recommendations for malaria control and future research.

Chapter 2

Literature Review

2.1 Introduction

Malaria is an old disease described since ancient times by its characteristic periodic fevers. The Egyptian papyri and the scripts of the Sanskrit Sushrutha have referred to it, although the earliest description of malaria in pregnancy had been noted by Hippocrates. Much later, two 19th century physicians, Góth (1881) and Chiarleoni (1886) described low birth weight in term babies born to mothers suffering from malaria (Duffy, *et al* 2001). By the late 20th century the harmful effects of malaria in pregnancy to the expectant mother and her baby had been well documented.

In the current literature, malaria in pregnancy is dominated with studies on *P.falciparum,* particularly from the sub-Saharan Africa. In contrast, data from the Asian region where transmission is low or seasonal and unstable, as in parts of India, is limited. Paradoxically, South Asia is the origin of some of the early literature that documented the adverse effects of malaria in pregnancy. In the early 20th century, physicians in India recorded a devastating number of stillbirths during the malaria epidemic in the Punjab (Christopher 1911). Later, the classic work of Wickramasuriya (1936), based on the observations during the malaria epidemic in Ceylon (present Sri Lanka) in the 1930s provides insight to many aspects of malaria in pregnancy (Wickramasuriya 1937).

This chapter describes the current understanding of the pathogenesis, epidemiology and the burden of malaria in pregnancy in the context of this study and with reference to Asia and India. The diagnostics relevant for malaria in pregnancy and control and prevention is also reviewed.

2.2 Search Methodology

Database Searches: The information for this review was collected through searches using the Pub Med, ISI Web of knowledge, Malaria in Pregnancy library, which is a resource of the Malaria in Pregnancy Consortium and consist of a regularly updated bibliographic database of published and unpublished literature relating to malaria in pregnancy (http://www.update-software.com/publications/malaria/). In addition, theses data bases, books and individual references identified from publications and the Central Cochrane Library were also accessed.

Search Terms: A broad search was undertaken initially applying subheadings and truncations (exploding). The search was refined later using MeSH ='Medical Subject Headings' terms in various combinations and Boolean operators such as 'AND' and 'OR' and key words (Table 2.1).

TABLE 2: 1: SEARCH RESULT IN PUBMED USING MESH TERMS

Main Searches in Pub Med	Number of articles (As major topic)	Number of articles (as key word)
Malaria in Pregnancy,	700164	
pregnant OR pregnancy; AND		1142
burden OR prevalence; AND		
epidemiology:	67468	92
Africa,		1307
Asia		236
low transmission,		195
high transmission		178
India AND (burden OR prevalence)	100	5
Placental Malaria: AND	704	
P.falciparum		428
P.vivax		21
Malaria in Pregnancy: AND		
anaemia, OR	597	
moderate anaemia OR		37
severe anaemia		165
placental malaria and anaemia		160
Malaria in Pregnancy: AND		
low birth weight	351	
preterm; OR	117	
prematurity	64	
placental malaria AND low birth weight		182
placental malaria AND preterm		28
Malaria Rapid Diagnostic Tests: AND	357	
malaria in pregnancy		16
placental malaria		4
Several other searches for topics on prevention, chemotherapy, antimalarial drugs, immunology and molecular aspects were performed in different combinations with the main topic as' malaria in pregnancy' and 'placental malaria'		

Selection Criteria: Titles were scanned and abstracts of titles considered relevant to the objectives of the thesis were reviewed and retrieved to obtain full text. Electronically unavailable articles were obtained from the holdings at the libraries of the University of Liverpool and the Liverpool School of Tropical Medicine. All electronically selected references were downloaded and managed for citation initially in Endnote-X and later in version-X3. Only English language articles were used. No date restriction was applied and searches were performed throughout the thesis writing period and citation library updated regularly.

2.3 The Malaria Parasite

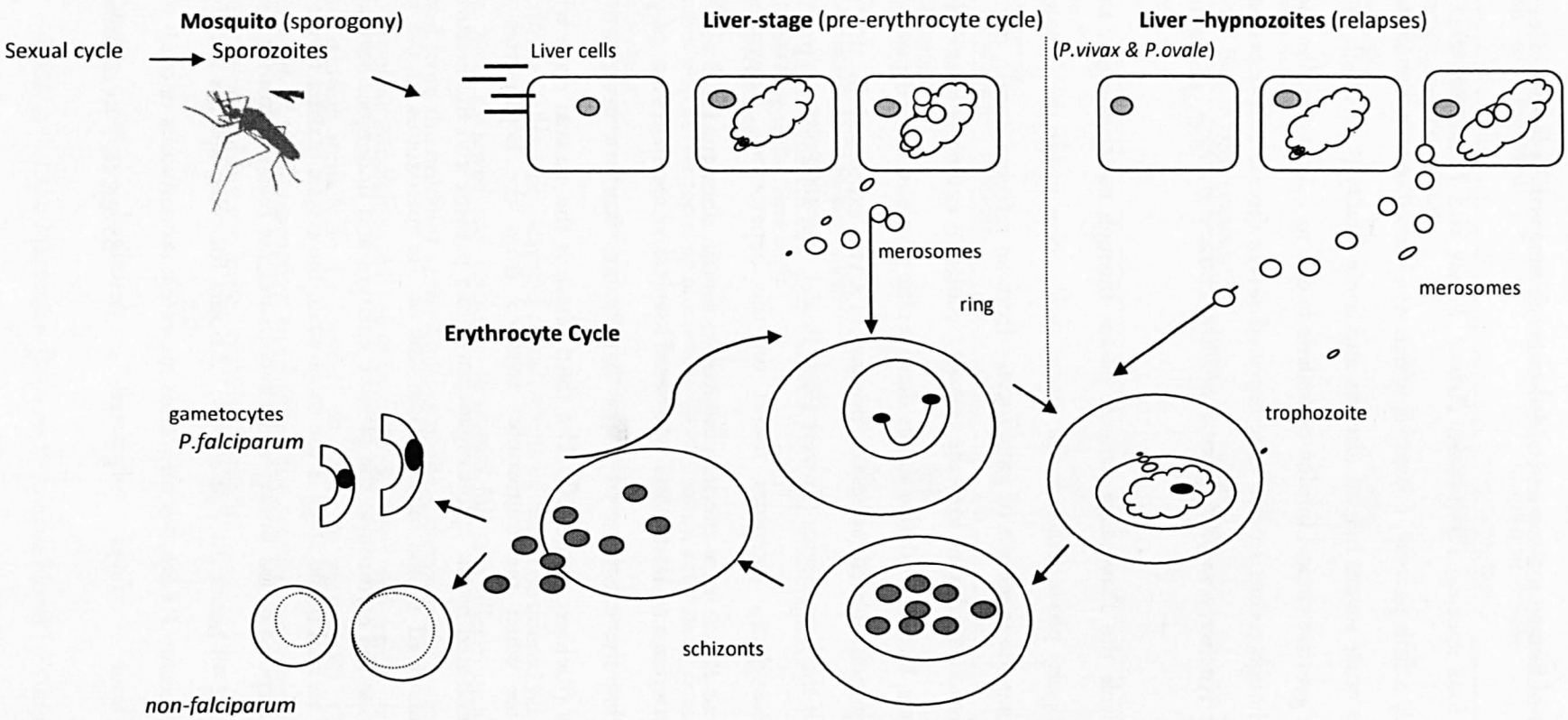
History: The discovery of the *Plasmodium* parasite in human blood by Alphonse Laveran in 1880 confirmed the single cell organism, *Plasmodium* as the cause of malaria. Seventeen years later, in 1897, Sir Ronald Ross observed *Plasmodia* in the gut of parasite infected birds and proved the

mosquito as the vector of malaria. In 1898, Grassi and colleagues in Italy described the *Anopheline* mosquito as the vector of human malaria and added to the findings of Sir Ross (Gilles 2002).

Species: Traditionally four species; *P.falciparum*, *P.vivax*, *P.ovale* and *P.malariae*, were known to infect humans. Recently a fifth parasite, *P.knowlesi*, which was known to infect macaque monkeys have been observed to cause severe human diseases and some fatality (Cox-Singh, *et al* 2008, B. Singh, *et al* 2004). The greatest clinical burden of malaria is due to *P.falciparum*, the most virulent of the five species, although recent studies have reported severe disease and occasionally deaths associated with *P.vivax* (Kochar, *et al* 2009, Price, *et al* 2009, Tjitra, *et al* 2008).

Life-cycle: In its life cycle the *Plasmodium* parasite passes through two hosts and three phases (figure 2.1). The sporogony phase which is the sexual cycle, occur inside the female *Anophelene* mosquito's gut following the ingestion of gametocytes from an infected human during a blood meal. With it the sporozoites released into the human circulation enter the liver cells and starts the exo-erythrocyte cycle. Recent evidence shows that specific proteins, circumsporozoite protein (CSP) and thrombospondin-related adhesion protein (TRAP) expressed in the sporozoites mediate the invasion of the hepatocytes (Frevert 1994, Pradel, *et al* 2002). These proteins bind to heparin sulphate proteoglycan receptors found on the hepatocytes. Conventionally, the sporozoites of *P.vivax* or *P.ovale* were species accepted to remain dormant for several months to years within the hepatocytes and later cause relapses. However, a recent review of publications since 1922 showed that the existence of *P.ovale* hypnozoites has not been proven by any biological experiments (Richter, *et al* 2010). The third phase is the asexual cycle which occurs within the erythrocytes when the merosomes released from the hepatocytes invade the erythrocytes. This is mediated by the apical organelles of the parasite and the merozoite surface proteins (MSPs) are involved in the initial attachment of the merozoites to the erythrocytes (Gaur, *et al* 2004). Inside the erythrocyte the parasite enlarges and undergoes several rounds of nuclear divisions. The asexual blood stage is the stage when the classic malaria fevers occur. The clinical symptoms correspond to the timing when merozoites are released from the erythrocytes and occur at intervals of 48 hours for *P.falciparum*, 72 hours for other species and 24 hours for *P.knowlesi*. Although in reality *P.falciparum* infections are rarely synchronous enough to cause this classical 48 hour fever pattern described in text books

FIGURE 2: 1: DIAGRAM SHOWING THE LIFE-CYCLE OF THE PLASMODIUM PARASITE



It is during the late asexual cycle that the mature trophozoites and schizonts produce protein molecules which appear on the surface of the erythrocytes. These molecules benefit the parasites as a survival mechanism by mediating sequestration in deep tissues, an action which forms the pathogenic basis of severe disease in the human host. During the erythrocyte cycle the parasite derives its nutrition from the digestion of haemoglobin.

2.4 Endemicity Measures

Malaria in pregnancy is closely related to the level of endemicity and therefore it is important to appreciate how endemicity is categorised. The traditional endemicity classification, which characterise malaria prevalence rates indicated the level of infection in a region of a country. WHO in 1950s classified endemicity based on enlarged spleen or parasite 'rates' in 2-9 year old children and categorised as follows: hypoendemic as less than 10%; mesoendemic 11-50%; hyperendemic 51-75%; holoendemic greater than 75% in 0-11 months for parasite rate (Snow 2002, WHO 1963). However many malariologists at the time found this classification sub-optimal in determining the intensity of transmission. Later Macdonald (1957) developed the classification defining stable and unstable transmissions, based on the transmission dynamics described by Sir Ronald Ross (Hay, *et al* 2008). Accordingly, stable transmission describes an equilibrium status where the population develops high levels of immunity due to continuous transmission. In unstable areas, there can be fluctuations in transmission leading to a lower immunity level among populations making them more prone to epidemics. Later, the entomological inoculation rate (EIR) was considered a better index of exposure as it provides a measure of exposure to infective bites of mosquito and transmission intensity. It is defined as the mean number of bites inflicted on an individual by mosquitoes infected with sporozoites per unit time. Based on EIR, malaria endemic regions are divided into high transmission when EIR is >10 infective bites per year and low when EIR <1 infective per year (Hay, *et al* 2000). More recently, a new transmission strategy have been derived based on parasite prevalence in children aged 2-10years (Gething, *et al* 2010, Hay, *et al* 2009). In their model Gething *et al* classed endemicity as follows: malaria free or unstable, $PfPR_{2-10} \leq 5\%$, $PfPR_{2-10}$ >5% to >40%, $PfPR_{2-10}$ $\geq 40\%$ within stable transmission margins which was assumed as 1 clinical case per 10,000 population per annum and unstable transmission as <1 clinical case per 10,000 population per annum. This classification identified malaria and non-malarial fevers corresponding to different endemicity levels. Their classification could be used as an indicator to predict malaria at varying endemicity and has potential for screening with diagnostic tool prior to treatment with antimalarial in fever cases.

The acquired pre-pregnancy immunity level is related to the transmissions level in a region where women reside with varying consequences in pregnancy.

2.5 Pathogenesis

The pathogenesis of malaria in pregnancy involves three different pathological mechanisms. These mechanisms depend on the epidemiology, transmission intensity and prevalent species in the area. The pathologies are:

1. Pathogenesis of *P.falciparum* infection of the placenta, which is considered the main cause of pregnancy malaria associated anaemia and low birth weight.
2. Severe disease and cerebral malaria that occur more frequently among pregnant women in low or unstable malaria transmission regions.
3. Pathogenesis related to *P.vivax* infection during pregnancy which is found in much of Asia where transmission is mainly low or unstable.

It is well recognised that pregnant women are more susceptible to malaria than non-pregnant women, regardless of transmission level (Desai, *et al* 2007, McGregor 1984). In high and stable *P.falciparum* transmission areas women acquire substantial protective immunity through repeated infections by the time they reach reproductive age. The acquired immunity is altered in pregnancy. Furthermore, maternal malaria and its consequences are more pronounced in young first time pregnant women independent of age due to the acquisition of pregnancy specific immune responses to malaria (Brabin 1983). This parity dependent susceptibility is less marked in areas of low transmission, as seen in much of Asia. Many theories exploring the parity dependent susceptibility have been considered and how the pregnancy independent protective immunity of adulthood is altered in pregnant women in areas of stable *P.falciparum* transmission. The mechanisms are described here with reference to the hypotheses on immune suppression and hormonal changes in pregnancy, and newer theories on humoral and cellular immunity and physiological and behavioural aspects are discussed (Beeson, *et al* 2005, Duffy 2001, Fried 2001, Rogerson, *et al* 2007b).

2.5.1 Immuno-pathology of *P.falciparum* Placental Malaria

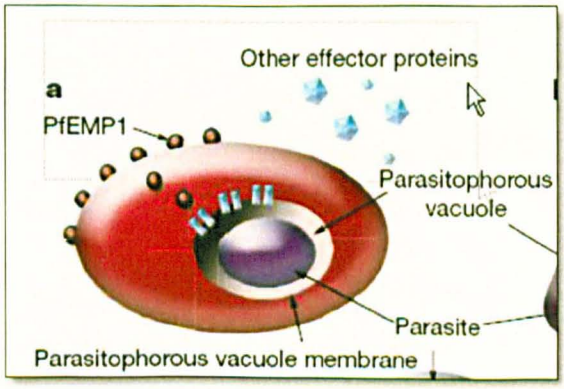
2.5.1.1 Cytoadherence of the *P.falciparum* parasite

The pathogenesis of placental malaria rests on the cytoadherence of *P.falciparum* infected erythrocytes within the placenta. Out of the five plasmodium species that infect humans, *P.falciparum* is the only species recognised to sequester in the placenta. The Italian pathologist

Bignami (1898) first observed the presence of *P.falciparum* in the placenta and in 1915 Clark described the placental preference of *P.falciparum* (Clark 1915, Duffy and Desowitz 2001). Since then much has been learned about *P.falciparum* infected erythrocytes in the placenta.

PfEMP1: During the intra-erythrocyte phase, *P.falciparum* species produce effector proteins termed Plasmodium falciparum erythrocyte membrane protein-1 (PfEMP1) that appear as knobs on the surface of infected erythrocytes (Figure 2:2) (Hviid 2007, Leech, *et al* 1984, Winzeler 2008). The PfEMP1 enables the infected erythrocytes to cytoadhere and sequester in the micro vasculature of organs such as the brain, lungs and placenta and evade splenic clearance. The adhesion of infected erythrocytes is interfered by the production of antibodies by the host immune system. The parasite in turn evades the antibody response by undergoing clonic antigen variation and is responsible for the virulence of the organism. The adhesion ligand known as the variant surface antigens (VSA) is encoded by the large family of *var* gene (Smith, *et al* 1995). The VSA can mediate adhesion to several receptors on the vascular endothelium such as CD36, intracellular adhesion molecule-1(ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) chondroitin sulphate-A (CSA) and others (Fried 2001, Rogerson, *et al* 1997).

FIGURE 2: 2: ILLUSTRATION OF PARASITIZED ERYTHROCYTES WITH PFEMP1 MOLECULES



Source: Winzeler, Nature 2008

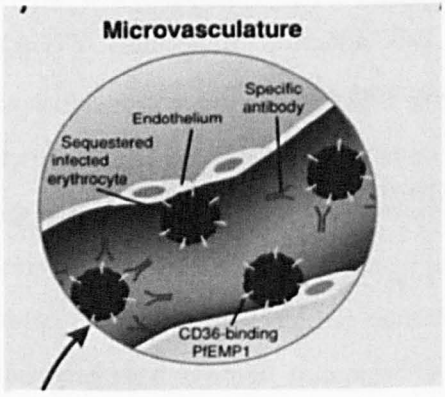
PfEMP1 binding domains of the *var* gene responsible for defined adhesions are the Duffy binding-like (DBL) and the cysteine-rich inter-domain regions (CIDR). The ability of *P.falciparum* infected erythrocytes to adhere to CSA is linked to DBLγ domains within particular PfEMP1 variants (Andrews, *et al* 2002, Kraemer, *et al* 2006).

Characteristics of parasites cytoadhering in the placenta: CSA is a low sulphated glycosaminoglycan found in the placenta, in connective tissues and cartilaginous matrix. Although other glycosaminoglycan molecules such as chondroitin sulphate-B and chondroitin sulphate-C are present, parasites do not appear to adhere to them. Since these molecules differ in the

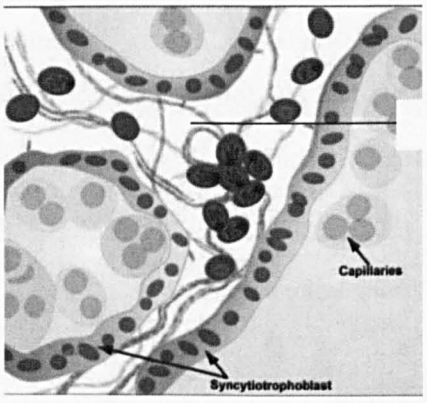
position of the sulphate bond, it is believed that sulphate bonds may play a role in binding (Fried 2001). Other molecules such as hyaluronic acid and Fc receptor have previously been implicated as receptors. However, a recent study demonstrated that infected erythrocytes in the placenta bound to CSA and not to hyaluronic acid and the role of hyaluronic acid related binding is likely to be small (Fried, *et al* 2006, Muthusamy, *et al* 2007).

The characteristics of parasitized erythrocytes in the placenta differ from isolates obtained from non-pregnant women. The parasitized erythrocytes are adherent throughout the intervillous spaces, whereas in other tissues sequestered erythrocytes are seen in apposition with vascular walls (Figure 2:3) (Andrews and Lanzer 2002, Smith, *et al* 2004). Parasite infected cells in the placenta do not appear to form rosettes with uninfected cells and agglutination is variable (Brabin, *et al* 2004, Rogerson, *et al* 2007b).

FIGURE 2: 3: THE DIFFERENCE IN ADHERENCE OF PARASITIZED ERYTHROCYTES IN THE PLACENTA AND IN OTHER MICROVASCULATURE



Source: Smith JD et al JEM, 2004



Source: Andrews KT et al Parasitol Res, 2002

The parasitized erythrocytes in the placenta adhere to glycosaminoglycans (CSA) but not to receptors like CD36 and ICAM-1, which are well recognised cytoadherent receptors of non-placental infected cells (Fried, *et al* 1996, Rogerson, *et al* 1995). These features make the placenta infecting *P.falciparum* different from parasites infecting other tissues and supports that a phenotypically distinct sub-population of *P.falciparum* infects the placenta.

Var2CSA: Two *var* genes, FCR3*var*CSA later renamed *var*1CSA and *var*2CSA were initially implicated in CSA adhesion ligands. After several experimental studies the *var*2CSA has been suggested as the principal ligand responsible for CSA binding (Gamain, *et al* 2005, Salanti, *et al* 2004). The *var*2CSA contain multiple DBL domains termed DBLX responsible for mediating cytoadhesion and lack the DBLγ and CIDR domains found in other *var* genes (Kraemer and Smith 2006). The difference of the *var*2CSA structure with the multivalent DBLX domains is

considered to have a role in placental sequestration. Also the fact that the disruption of *var*2CSA interfered with the ability to acquire CSA adhering phenotype supports the pregnancy associated relevance of this gene sub-family (Duffy, *et al* 2006).

2.5.1.2 Recent theories

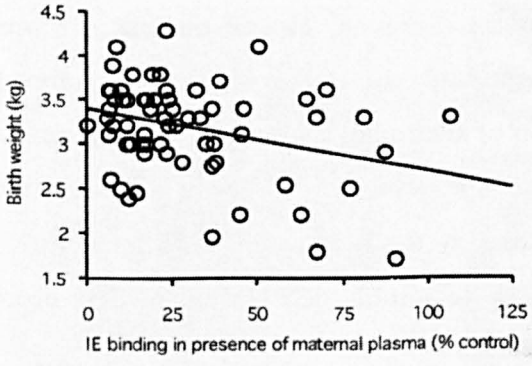
Humoral Immunity: McGregor (1984) hypothesised that "pregnancy establishes an extremely vascular organ that shields the parasite from destruction by extrauterine immune effector mechanisms". Recent studies on the plasma of CSA binding *P.falciparum* infected erythrocytes in placental isolates demonstrated an immune response after the first pregnancy. Thus, the placenta becomes a naive target in first pregnancies for the phenotypically distinct CSA binding parasites. This theory was further supported by the demonstration of anti-adhesion antibodies in plasma of multigravid women that inhibited parasite binding to CSA, but no inhibition was found in plasma from primigravidae and male adults (Fried, *et al* 1998b, Ricke, *et al* 2000). These findings explain the increased susceptibility of primigravidae and the decreasing risk of malaria in subsequent pregnancies in stable transmission regions. The same study showed variable activity among primi, secundi and multigravidae with delayed acquisition of antibodies against CSA binding parasites in sera from Karen women in Thai-Burmese border showed. The delay in acquiring humoral immunity is explained by less exposure occurring in this population associated with lower transmission. This supports the higher occurrence of symptomatic cases and less prominent gravidity-specific effect seen in low transmission areas.

Anti-adhesion antibody: *In vitro* studies performed to assess the timing of the acquisition of pregnancy associated VSA-IgG suggest that antibodies to the placenta binding isolates developed on first exposure around 20 weeks of gestation in primigravidae (Gamain, *et al* 2007, O'Neil-Dunne, *et al* 2001). The fact that antibodies are lacking early in first pregnancies also suggests that they appear after blood circulation to the placenta is established at around 10-12 weeks (Carter 1997). Further, the timing corresponds to the presence of chondroitin sulphate glycans in the intervillous space which appears by the end of first trimester and support adhesion of parasitized erythrocytes (Agbor-Enoh, *et al* 2003). These studies showed that anti-adhesion antibodies to the CSA binding laboratory isolates appear later in primigravidae (20 weeks) than in multigravidae (12 weeks). The early appearance of antibodies in multigravidae imply that after first pregnancy there is a boost to immune response with re-exposure and may also explain improved outcomes associated with malaria in multigravid women. This was observed in a study which showed increased birth weight and gestational age associated with higher presence of anti-adhesion antibodies on placental plasma isolates taken from secundigravidae in western Kenya (Figure

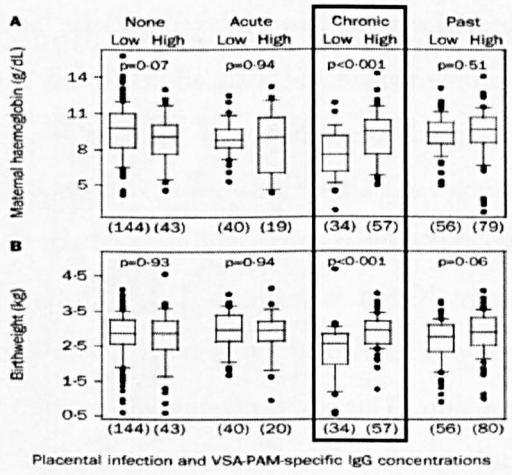
2:4)(Duffy, *et al* 2003). Another study showed low or absent VSA-PAM-IgG in histologically identified chronic placental infections was associated with a lower haemoglobin and lower birth weight (Staalsoe, *et al* 2004).

The above described studies show that there is a parity specific association in the acquisition of antibodies that inhibit adherence of *P.falciparum* infected erythrocytes to the placenta. The observations also demonstrate that the presence of anti-adhesion antibodies is protective of malaria associated adverse outcome in pregnancy. Furthermore, the protective antibodies develop from 10-12 weeks onward and become apparent at around 12 weeks gestation in multigravidae exposed in a previous pregnancy to malaria and around 20 weeks gestation in first time mothers.

FIGURE 2: 4: ANTIBODY RESPONSE AND PROTECTION FROM ANAEMIA AND LOW BIRTH WEIGHT



Source: Duffy & Fried, Infection & Immun 2003



Source: Stalsoe et al, The Lancet 2004

Immunomodulation theory: Wegmann *et al* proposed that for the 'fetal allograft' to reach term, Th2-type response is enhanced in pregnancy and Th1 mediated response is down regulated (Wegmann, *et al* 1993). On the other hand, murine studies showed that the pathogenecity of diseases controlled by Th1 immunity is increased with the switch to Th2 response in pregnancy. The study also demonstrated the presence of Th1 cytokine, IFNγ and TNFα inhibit fetal development and resulted in termination of normal murine pregnancy (Krishnan, *et al* 1996, Raghupathy 1997). Intracellular infections that require type1 response such as toxoplasmosis, leishmaniasis, tuberculosis and leprosy are known to exacerbate in pregnancy. For malaria, some earlier studies demonstrated a decrease in lymphoproliferative T cell response particularly in primigravidae (Rasheed, *et al* 1993). A likely explanation for the suppression of Type 1 response has been the increased levels of cortisol observed in pregnancy. Nevertheless the Th1/Th2 shift in pregnancy provides a dichotomous situation in the host that inhibits the type1 response

required to contest the malaria parasite, whereas strong type 1 responses induce the cascade of proinflammatory cells which are associated with negative pregnancy outcomes.

Cellular Immunity: Several studies have shown a shift of the above described Th2 bias in placental malaria. The sequestration of parasitized erythrocytes, mainly mature stage trophozoites and schizonts in the intervillous spaces of the placenta elicit a pro and anti-inflammatory response drawing monocytes and macrophages to the placenta. One placental histological study described massive monocyte infiltrates elicit a response with Th1 inflammatory cytokines, TNF-α, INF-γ, IL-2 and IL-6 (Ordi, *et al* 1998b). Other studies on placental malaria isolates have shown an elevation of TNFα was associated with maternal anaemia and both TNFα and INF-γ was elevated in the case of low birth weight (Fried, *et al* 1998a). Similarly, a study conducted among Malawian pregnant women found that TNFα, but not IFNγ, was associated with impaired fetal growth (Rogerson, *et al* 2003c). Another study conducted on *in-vitro* cytokine production found that increased production of INF-γ in uninfected placentas of multigravid women was associated with a protective effect on placental malaria (Moore, *et al* 1999). While differences in study methods may explain differences in the results, it shows that increased production of Th1 cytokines is associated with adverse pregnancy outcome. Thus, the activity of monocyte/macrophage related inflammatory mediators are a possible source of maternal anaemia, low birth weight and premature delivery in women with placental malaria.

Several chemokines such as interleukin-8, macrophage inflammatory proteins MIP 1α and MIP 1β that are chemotactic for monocytes have been associated with placental malaria. Increased levels of macrophage inhibitory factor (MIF) were found in Kenyan women with placental malaria (Chaisavaneeyakorn, *et al* 2005). Macrophage inhibitory factor is a cytokine considered to modulate protective immunity in pregnancy by activating macrophages with ability to overcome the immunosuppression of glucocorticoids. More recent immunohistochemical studies have reported changes in cytokine and chemokine expressions associated with interactions between infected erythrocytes binding to the syncytium (Lucchi, *et al* 2008). This interaction in turn is considered to stimulate migration of mononuclear cells, suggesting a direct role of syncytiotrophoblast in the pathogenesis of placental malaria. In general, histological studies have found an association between pigmented monocytes and maternal anaemia and low birth weight. Although not clearly understood, it is thought that mononuclear cells play a role in the pathophysiology of poor fetal growth associated with placental malaria. In this regard some investigators have implicated that localized inflammation trigger monocyte infiltrates which could impair fetal growth by decreased transplacental transfer of amino acids and glucose (Rogerson, *et al* 2007a). Others have suggested massive monocyte infiltrates may contribute to placental

hypoxia resulting in decreased oxygen availability to the fetus (Graham, *et al* 2000). However, a current study that used the laser capture micro dissection method could not demonstrate an association between placental malaria related hypoxia and fetal growth retardation (Boeuf, *et al* 2008, Rogerson and Boeuf 2007a).

Some authors have discussed a link between hypertension and malaria in pregnancy suggesting malaria as a risk factor for preeclampsia (Brabin, *et al* 2005). In this regard a recent study showed an association between hypertension and chronic placental malaria in young primigravidae (Muehlenbachs, *et al* 2006). The authors implied an underlying maternal-fetal conflict in the inflammatory response related to the expression of vascular endothelial growth factor (VEGF) on maternal macrophages.

Taking all the above discussed studies together, it appears that mononuclear cells (monocytes/macrophages) are the key inflammatory cells responsible for the pathogenic mechanism of placental malaria and its adverse outcomes.

Other inflammatory cells such as polymorphonuclear cells and to some degree the presence of T and B cells in placental infections have also been reported. Few studies have shown the presence of other innate cells that have a role in adaptive immune response. Natural killer (NK) cells were found absent in one study, while a more recent study showed an increased presence in placental malaria (Ordi, *et al* 2001, Sartelet, *et al* 2005). However detailed characteristics of these cells in placental malaria have not been explored.

2.5.1.3 Early hypothesis

Immunosuppression and cortisol theory: Increased susceptibility to *P.falciparum* infection during pregnancy through immunosuppression by pregnancy associated hormones has been considered previously. The studies by van Zon *et al* demonstrated a correlation between increasing levels of cortisol and loss of immunity in mice models (van Zon, *et al* 1982, van Zon, *et al* 1986). Later, studies conducted on pregnant women by Vleugels *et al* showed a causal relationship between serum cortisol levels and suppression of malaria immunity (Vleugels, *et al* 1989, Vleugels, *et al* 1987). With a renewed interest in the cortisol theory, a recent longitudinal study conducted on Gabonese women suggests that sustained increase in cortisol level underlies the increased susceptibility of pregnant women to malaria, particularly primigravidae (Bouyou-Akotet, *et al* 2005). Other pregnancy hormones, oestrogen and progesterone that influence the development of T cells and cytokine production have been suggested to increase susceptibility. Although the cortisol theory suggests the susceptibility to malaria during pregnancy, it alone does

not explain the sequestration of *P.falciparum* and the inflammatory and immune response that occur with cytoadhesion of the parasite in the placenta.

2.5.1.4 Mosquito Attractiveness

Exposure physiology: The work of Lindsay *et al* (2000) demonstrated that increase in exhaled breath and their warmer skin surface attracted *Anophelese gambiae* mosquitoes to pregnant women more than non-pregnant women. Some of the contents of exhaled breath are thought to allow mosquitoes to locate its target host. Secondly it was thought the warmer skin surface increased the release of volatile substances producing larger host signature and attractiveness to mosquitoes.

Behaviour: Changes in behaviour during pregnancy with women having to go outside more frequently to urinate at night is also thought to increase the exposure to night biting mosquitoes.

Although there is no study, we could speculate the possibility that pregnant women in rural Madhya Pradesh could be exposed to mosquitoes similar to that observed in the Gambian study. The two vectors, *Anopheles culicifacies* and *A. fluvitalis* present in Madhya Pradesh prefer to feed during early evening, but bites throughout the night until dawn.

2.5.2 Pathogenesis in low malaria endemic areas

Symptomatic malaria is more common in pregnant women in low endemic regions and if left untreated can rapidly progress to severe disease (Nosten, *et al* 2004). The data from this region suggests that pro-inflammatory response to *P.falciparum* infection mediates release of pyrogenic cytokines, which is not pregnancy specific (Hensmann, *et al* 2001). The cascade of events related to pro inflammatory cytokines is likely to lead to abortions, premature births and still births. High parasite density within the placenta and thus dense placental accumulation is rare compared to regions of high transmission (McGready, *et al* 2004). Less concentration of parasitaemia within the placenta perhaps reflect the accumulation of infected erythrocytes in other organs and increase in complications such as cerebral malaria. Alternatively the duration of the average infection may be shorter because infection become symptomatic and women could be more likely to seek care, or deliver prematurely. Although direct data on the immune-pathology of placental malaria are limited from low transmission setting, several studies on non-immune pregnant mice have shown placental infections induced pro inflammatory cytokines leading to severe maternal and fetal pathologies (Hviid, *et al* 2010).

Although the immuno-molecular pathology associated with *P.falciparum* infection in pregnancy is becoming clearer, yet the exact mechanism affecting low birth weight is poorly

understood. Whether the increased susceptibility of pregnant women and low birth weight associated with malaria in pregnancy is predominantly a parasite effect, or a host effect in response to infection or a combination of parasite-host responses remains to be investigated further.

2.5.3 Pathogenesis of *P.vivax* infection

P.falciparum placental malaria associated outcomes of maternal anaemia and low birth are also observed with *P.vivax* infections, yet *P.vivax* is not known to sequester in the placenta. This raises the question whether factors other than placental sequestration of infected erythrocytes have a role in the pathogenesis of pregnancy malaria.

Systemic infection rather than localised placental inflammation has been suggested underlying the pathology for *P.vivax* infection (ter Kuile, *et al* 2008). Additionally, biological factors specific to *P.vivax* are thought to contribute to the pathology of *P.vivax* in pregnancy. These include the preference of *P.vivax* to invade reticulocytes which may influence the decrease in maternal blood viscosity associated with reticulocytosis during pregnancy (Duffy 2001). Another feature is rosette formation which has been linked to the cytoadherance phenomenon of *P.falciparum* in non-placental tissues and has been observed *ex vivo* in *P.vivax*. The mechanisms postulated for *P.vivax* associated anaemia include oxidative damage of host mediators and cytokine related dyserythropoiesis (Anstey, *et al* 2009).

Since all the stages of the *P.vivax* parasite are detected in peripheral blood, it is implicated that it does not cytoadhere in deep tissues similar to *P.falciparum*. However, a recent study using isolates from *P.vivax* infected patients have demonstrated cytoadherence in static and flow conditions to endothelial and placental cryosections (Carvalho, *et al* 2010). The receptors implicated to cytoadhere in *P.vivax* infected erythrocytes were ICAM-1 and CSA, although the charge was weaker than that observed with *P.falciparum*. The experiment was in part mediated by the *vir* protein, which is a multi gene family specific to *P.vivax* that was reported in 2001(del Portillo, *et al* 2001). The *vir* gene which encodes immunovariant proteins are thought to have a functional role in chronic *P.vivax* infections through antigenic variation. These are interesting developments and with increasing focus on *P.vivax* malaria, future investigations are likely to unravel the pathogenesis of *P.vivax* malaria in pregnancy.

2.6 Clinical Burden of Malaria in Pregnancy

2.6.1 Maternal Malarial Anaemia

2.6.1.1 Prevalence of malarial anaemia

Anaemia is common in pregnant women in tropical countries with a prevalence ranging from 40%-60% (WHO 2008a). In these countries malaria is one of the major preventable causes of moderate to severe anaemia. Other causes of anaemia in pregnancy are iron, folate and vitamin A deficiency, helminth infections, haemoglobinopathies, HIV and other chronic infections. Anaemia in pregnancy is defined by a haemoglobin level below 11g/dL and a level less than 7g/dL defines severe anaemia (WHO 1992). Estimation of the contribution of malaria to anaemia is not easy from observational studies because anaemia is not a standalone problem in malaria endemic regions. In one analysis which looked at 26 studies in sub-Saharan Africa it was estimated that 400,000 pregnant women could have had severe malarial anaemia in 1995 with 8.2% being the median prevalence for severe anaemia (Guyatt, *et al* 2001b). A more recent review stated 5-10% pregnant women might develop severe anaemia in Africa with 26% attributable to malaria among pregnant women of all gravidities (Desai, *et al* 2007). Anaemia is more frequent in primigravidae in sub-Saharan Africa and during the rainy season when malaria transmission is higher (Bouvier, *et al* 1997, Brabin, *et al* 2001, Shulman, *et al* 2002). In Asian countries women of all gravidity are equally at risk of malarial anaemia. Similar effect is also observed with *P.vivax* associated anaemia (Nosten, *et al* 1999, Poespoprodjo, *et al* 2008). The risk of severe anaemia is reduced by 38% by prevention of malaria either with antimalarial prophylaxis or with intermittent preventive treatment (Garner, *et al* 2006). Systematic reviews of trials suggest that successful prevention of malaria in pregnancy can reduce maternal anaemia (Hb<11g/dL) by only about 10% in sub-Saharan Africa, whereas the impact on moderately severe anaemia (Hb <7 or 8g/dL) is greater (25%)(Gamble, *et al* 2007, Garner and Gulmezoglu 2006, ter Kuile, *et al* 2007).

2.6.1.2 Aetiology of maternal malarial anaemia

Maternal anaemia due to *P.falciparum* infection is caused by haemolysis of infected and uninfected erythrocytes and dyserythropoeisis. Placental infections intensify maternal anaemia with the induction of monocytes and release of TNF which suppress erythropoiesis (Moormann, *et al* 1999, Rogerson, *et al* 2003b). In pregnancy, anaemia is further aggravated by iron and folate deficiency which may develop secondary to haemolysis. Haemozoin pigment induces cytokines which mediates induction of hepcidin, an iron regulating peptide hormone (Ganz 2005, Nemeth, *et al* 2005). A recent study has reported the presence of hepcidin in placental macrophages in

cases of malaria (Muehlenbachs, *et al* 2007b). Hepcidin which is responsible for iron homeostasis may explain the interaction between malaria infection and iron deficiency.

2.6.1.3 Placental malaria and iron deficiency

Iron and folate supplements are recommended for pregnant women to improve the adverse effects associated with moderate to severe anaemia on maternal and infant health. In a Cochrane review of 40 trials the authors found women receiving daily oral iron supplementation antenatally were less likely to develop iron-deficiency anaemia at term (Pena-Rosas, *et al* 2006). However, few studies have looked at the effect of iron treatment in pregnant women with malaria. An earlier study in Papua New Guinea found more perinatal malaria was associated with parentral iron in primipara compared to multiparous women. In this study there was no evidence that parental iron had any effect on fetal maturity or birth weight (Oppenheimer, *et al* 1986). Another study that used oral iron supplements reported reduction of anaemia and iron deficiency with no increase in malaria infection (Menendez, *et al* 1994). Recent studies have reported conflicting results on the response of iron in cases of malaria in children (de Benoist, *et al* 2006, Sazawal, *et al* 2006, Tielsch, *et al* 2006). This has raised questions on the benefit of routine iron and folate supplementation in malaria endemic regions. Interestingly, a study in Tanzania found a decreased risk of placental malaria in women with iron deficiency especially in primigravid women (Kabyemela, *et al* 2008). A subsequent study that reanalysed cross-sectional data from 1998 reported a 33% reduction in the risk of *P.falciparum* infection associated with iron deficiency in pregnant Ghanian women (Danquah, *et al* 2008). Similarly, a recent study found acute or chronic placental *falciparum* malaria was less frequent in Malawian women with iron deficiency (Senga, *et al* 2011). Whether the association between iron deficiency and placental malaria observed in these studies relates to reduced parasite growth due to iron deficiency would be interesting to explore in future studies. Studies are ongoing to determine if iron supplementation in iron deficient or replete women may increase the risk of malaria (Hans Verhoeff, personal communication).

2.6.2 Low birth weight

Low birth weight defined as weight less than 2500g or 5.5lbs occur more frequently in developing[1] countries (16.5%) than in the developed countries (7.0%). The dichotomised birth weight definition is based on observations that the risk of death is 20 times higher with birth weight <2500g (MacDorman, *et al* 1998, WHO 1950). Low birth weight is therefore an important

[1] The terms developing and developed countries that appear in this thesis are based on Human Development Index (HDI) that is used by the UN to determine whether a country is developed, developing or under developed. HDI is a comparative measure of life expectancy, literacy, education & standard of living for all countries worldwide. Refer to http:/hdr.undp.org/hdr2006/statistics for more details.

predictor of infant mortality and childhood morbidity and influences chronic diseases of adulthood. However, the causal association between low birth weight and mortality in populations has been debated by some investigators (Wilcox 2001).

Multiple factors affect low birth weight which includes fetal, maternal, genetics and environmental factors. The fetal factors shows first born babies are lighter than subsequent birth order in babies and females have lower weight at birth than male babies. The maternal factors that affect birth weight are the mother's stature (height) and weight, her nutrition before and during pregnancy, socio-economic status and behavioural habits such as smoking and alcohol consumption. In addition infections affect birth weight and malaria is a major infectious cause and an important preventable determinant of low birth weight.

2.6.2.1 Falciparum endemic sub-Saharan Africa

Prevalence and risk of low birth weight: In *P.falciparum* endemic high transmission areas the risk of low birth weight is highest among primigravidae, the group most affected with malaria infection in pregnancy. The prevalence of low birth weight in primigravidae was in the range of 7% - 40.5% by one study that analyzed 33 African data-sets (Brabin, *et al* 1999). The overall prevalence of malaria associated low birth weight was in the range between 12% - 20% (Steketee, *et al* 2001).

The risk of low birth weight associated with placental infection is twice that in uninfected women in stable transmission settings (Guyatt and Snow 2004). This is different in low or unstable African transmission settings with a higher risk of low birth weight associated with malaria. For example, in stable and unstable regions of Ethiopia, the relative risk of low birth weight was 3.2 and 3.9 for the two regions (Newman, *et al* 2003). In Madagascar where the prevalence of malaria was lower in the highlands (2.1%) than in lowland clinics (26.2%), the risk of low birth weight associated with placental infections was greater in the highlands (Cot, *et al* 2002).

The types of low birth weight: Low birth weight occurs either due to preterm birth (preterm-LBW) or intrauterine growth restriction (IUGR-LBW). Studies in the 1950s described all low birth weight associated with malaria infections as preterm births (Archibald 1956, Bruce-Chwatt 1952, Cannon 1958). At the time, the simple criteria of birth weight <2500g or 5.5lbs was used to define prematurity because a method for differentiating between preterm-LBW and IUGR-LBW was not in use. Even now, obtaining precise estimates of gestational age are difficult in malaria endemic regions, because women are uncertain of their date of last menstruation. To overcome

this, researchers have used the modified Dubowitz or Ballard score to assess gestational age (Ballard, *et al* 1979, Dubowitz 1969). A system for defining IUGR was derived after (Lubchenco, *et al* 1963) demonstrated that the risk of perinatal morbidity and mortality increased as birth weight for age decreased from the 10th percentile to 1st percentile. Since then research studies have used different definitions to distinguish IUGR or small-for-gestational-age (SGA) depending on the reference population used to define the percentiles, aetiology and impact.

Placental malaria and the type of low birth weight: Determining the type of low birth weight is important in terms of the clinical burden as the impact on neonatal morbidity and morbidity differ in preterm and IUGR-LBW babies. Studies which distinguished the two types of low birth weight showed that IUGR-LBW is more common in stable malaria transmission settings; whereas preterm-LBW is more frequent in low transmission areas. This suggests the type of low birth weight depends on the malaria transmission pattern, pre-pregnancy exposure of pregnant women and thus the likelihood of symptomatic malaria and to some extent the timing of infection. One analysis that distinguished preterm-LBW and IUGR-LBW in high transmission setting reported malaria was responsible for 8-36% premature deliveries while IUGR-LBW was 13-70% (Steketee, *et al* 2001).

The grade of infection during the course of pregnancy influences whether a baby is an IUGR-LBW or a preterm birth. In this regard, one study found the presence of parasitaemia at delivery was associated with preterm delivery while parasitaemia in the antenatal period was associated with IUGR-LBW (Sullivan, *et al* 1999). Another study reported preterm birth was increased in presence of placental parasitaemia (Kalanda, *et al* 2006). These observations suggest that acute placental infections or infection occurring close to delivery may result in preterm birth. On the other hand fetal growth maybe primarily affected through chronic infection resulting in IUGR-LBW. This is further supported by placental histological studies that observed a 3 fold increase in preterm birth in cases of active placental infections whereas IUGR-LBW was associated with chronic inflammation (Menendez, *et al* 2000).

2.6.2.2 Malaria Endemic Regions of Asia

Prevalence: Few studies from the Asian region have reported on malaria in pregnancy associated low birth weight. Estimates of 16% and 18% prevalence of low birth weight associated with *P.falciparum* were reported in Thailand and Papua, Indonesia respectively (Luxemburger, *et al* 2001, Poespoprodjo, *et al* 2008). A much higher estimate of 89% low birth weight in malaria infected women compared to uninfected women was reported in a hospital based study in Madhya Pradesh, India (Singh, *et al* 1999a). These were studies based on maternal parasitaemia

and may not reflect the prevalence of low birth weight associated with placental infections in these settings. Nevertheless, a recent review showed that low birth weight associated with *P.falciparum* occurring in regions of Asia and S. America was comparable to the African settings of similar transmission intensity (Desai, *et al* 2007).

Type of low birth weight and risk: Past reports from Asia has suggested preterm births to be a more likely contributing factor of low birth weight in Asian setting where malaria transmission is generally low, seasonal or epidemic and with infections more likely to be associated with symptoms (Menon 1972, Wickramasuriya 1937). Recent studies in Thailand and Papua Indonesia estimated a prevalence of preterm birth at 11% and 16% respectively (Luxemburger, *et al* 2001, Poespoprodjo, *et al* 2008). The risk of low birth weight associated with placental malaria reported from Thailand was 1.8, and that in asymptomatic Papuan, Indonesian women was 1.9. Although these figures are comparable to that found in high transmission areas of Africa, the risk of preterm birth related to malaria preceding delivery is more frequent in Asia (Luxemburger, *et al* 2001). These studies suggest that lower acquired immunity results in symptomatic malaria leading to preterm delivery.

2.6.2.3 Impact of low birth weight

The mean reduction of birth weight observed in Thailand for *P.falciparum* infection was 151g in babies of primigravidae and 185g for second and third pregnancies (Nosten, *et al* 1991). Similarly a reduction of 192g was seen in Papua, Indonesia (Poespoprodjo, *et al* 2008). A more marked reduction was observed in India (350g) (Poespoprodjo, *et al* 2008, Singh, *et al* 1999a). However, the Indian study was based on a selected sample of symptomatic women all belonging to lower socioeconomic status with a control group of women with fever, both groups admitted in hospital. In addition there was no mention of the weighing scale and how and when babies were weighed. Furthermore, neither the mean birth weight of the study population nor the 95% confidence interval was stated, which makes it unclear where the true value of the mean difference in birth weight lies in the study population. Interestingly, early African studies reported comparable reductions of birth weight associated with placental malaria. Bruce-Chwatt found an average of 145g reduction, while Archibald reported a 170g decrease and Jelliffe observed 263g decrease in mean birth weight (Archibald 1958, Bruce-Chwatt 1952, Jelliffe 1968). Accordingly, despite differences in transmission, similar reductions of birth weight for *P.falciparum* was reported for the two settings in the range of 35-310g in a recent review (Desai, *et al* 2007, Guyatt and Snow 2004). However, in Thailand, unlike in Africa, birth weight reductions were observed in first, second and third pregnancies with less marked gravidity effect.

2.6.2.4 *P.vivax* and low birth weight

P.vivax associated low birth weight data is reported mainly from Asia. Although the impact is less severe than *P.falciparum*, a decrease in mean birth weight reductions of 107g and 108g was observed in Thailand and in Papua, Indonesia (Nosten, *et al* 1999, Poespoprodjo, *et al* 2008). Considerable reduction in mean weight was also observed in multigravidae. This contrasts to observations with *P.falciparum* and in high transmission settings, where low birth weight occurs primarily in primigravidae. A 1.6 fold increase in the odds of having a low birth weight when infected with *P.vivax* was reported in the Thailand study. In the Indonesian study, the risk of *P.vivax* associated preterm increased in the presence of fever while low birth weight associated with asymptomatic infections were also observed.

2.6.2.5 Fetal, Newborn and Infant Outcome

Abortions and Stillbirths: Severe malaria in pregnancy is associated with fetal loss resulting in abortions and stillbirths. Stillbirths ranging from 6%-13% and abortions between 2%-11% were reported from six different hospital based studies in India (Das 2000, Kaushik, *et al* 1992, Kochar DK, *et al* 1998, Nair, *et al* 1993, Singh, *et al* 1995). Similarly, studies from Myanmar, Thailand and Indonesia have accounted for stillbirths in women with severe malaria (Luxemburger C, *et al* 1997, Meek 1988, Naing, *et al* 1988, Poespoprodjo, *et al* 2008). The 1908 Punjab epidemic had documented 1100 cases in total for stillbirths, miscarriages and abortions among 4600 infected pregnant women (Christopher 1911). In the 1934-35 Ceylon (Sri Lanka) epidemic 13% fetal loss was reported. These suggest that severe malaria in pregnancy is associated with fetal loss and it is predominantly observed in epidemic prone low transmission areas. However, a recent review of twenty five malaria specific African studies reported a two-fold increase in the odds (2.19) for stillbirth with placental malaria (van Geertruyden, *et al* 2004). In this analysis, consisting of mainly hospital based cross-sectional studies, fetal mortality for Africa was reported to range from 2.3/1000 to 111/1000 live births. The same review included eleven studies from regions outside Africa and found fetal mortality ranged from 12/1000 to 27/1000. The overall fetal mortality in malaria-endemic countries was double that found in non-endemic countries. Additionally a systemic review of trials on malaria control has shown effective prevention resulted in 33% reduction of spontaneous abortions and stillbirths (Gamble, *et al* 2006). These findings highlight that even though severe malaria associated fetal loss is more frequent in low transmission setting it also occurs in high transmission settings.

Perinatal and neonatal mortality: Several studies have shown increase in mortality in malaria associated low birth weight babies (Greenwood, *et al* 1992, Guyatt and Snow 2004). In early 20th century, Blacklock (1925) reported 35% babies born to women with placental infections died within 7 days of life compared to 5% in the uninfected women (Blacklock, *et al* 1925). Another study from Nigeria showed early neonatal mortality doubled for babies born with infected placenta compared to women without malaria (Cannon 1958). Recently, studies have looked at the impact of placental malaria association specifically with low birth weight and infant survival. A study from Malawi found the risk of perinatal morbidity and mortality increased for low birth weight babies born to women with malaria infection (Nyirjesy, *et al* 1993). Another review from sub-Saharan Africa suggested that malaria in pregnancy could be an indirect cause accounting for 11 % neonatal and 5.7% infant deaths in Africa. In the same review, the estimates for infant deaths were 9.8% in primigravidae and neonatal mortality was nearly double (17.6) (Guyatt, *et al* 2001a). A later review of 117 studies found the mean perinatal-mortality in malaria endemic countries was 61/1000 whereas 25.8/1000 was in non-endemic countries (van Geertruyden, *et al* 2004). An earlier study estimated up to 200,000 infant deaths associated to malaria in pregnancy could occur in malaria endemic Africa per year (Steketee, *et al* 2001). While these studies indicated malaria to be an important determinant of neonatal mortality, effective malaria prevention have shown to reduce 27% of perinatal mortality (Gamble, *et al* 2006).

Several studies have implied the mechanism underlying neonatal mortality associated with placental malaria. Babies born to women with placental *P.falciparum* malaria have impaired immune responses which make them more susceptible to infections during infancy and the neonatal period (Brabin, *et al* 2004). Studies in Malawi, Papua New Guinea and the Gambia reported reductions in placental antibody transfer reflecting either interference or saturation of sysncytiotrophoblast receptors with non-specific immunoglobulins (Brair, *et al* 1994, de Moraes-Pinto, *et al* 1998, Okoko, *et al* 2001b). This is a factor suggested in babies born to malaria infected women showing increased susceptibility to streptococcal infections (Okoko, *et al* 2001a). In stable *P.falciparum* endemic Africa, placental malaria and anaemia are associated with early priming of malaria antigens partly explaining protection of neonates born to semi-immune from malaria. In contrast, in low transmission areas, as in much of Asia where women have low malaria immunity placental malaria could lead to neonatal and infant deaths(Luxemburger, *et al* 2001, Wickramasuriya 1937).

Infant morbidity and mortality: Malaria associated low birth weight contributes considerably to infant mortality in Africa. In one analysis, the fatality rate of malaria associated low birth weight was estimated at 37.5% accounting for 3-17 deaths per 1000 live births. These statistics estimated

that malaria associated low birth weight morbidity to be 167,000-976,000 cases and 62,000-363,000 deaths in <5 years children each year (Murphy, *et al* 2001). Based on these figures another review estimated 100,000 infant deaths could occur due to malaria in pregnancy associated low birth weight (Guyatt and Snow 2004). These figures indicate the high burden of low birth weight associated with malaria in pregnancy and that it is responsible for 35% of preventable low birth weight. However, with effective control either with IPTp or chloroquine prophylaxis there is a 43% reduction as shown by one review (Garner and Gulmezoglu 2006)(). An earlier study showed failure to use IPT, chemoprophylaxis or ITN in pregnancy could contribute to 26-82% of low birth weight and 3-8% infant mortality (Steketee, *et al* 2001). Importantly malaria related low birth weight is a measurable indicator of neonatal and infant outcome. In this regard, the birth weight nomogram developed for Africa is a useful epidemiological indicator of malaria control (Brabin, *et al* 1999).

Infant outcome in Asia: Far fewer studies from Asia have looked at the impact of malaria related low birth weight on infant outcome. Thailand where most of the Asian malaria in pregnancy information comes from found neonatal mortality increased in malaria related preterm LBW (Luxemburger, *et al* 2001). The risk of early infant death was also higher in this group when maternal fevers preceded delivery. One community study from Koraput in Orissa, India reported 17% neonatal deaths associated with maternal malaria (Das 2000). More studies are needed for understanding the consequences of malaria in pregnancy associated low birth weight in the Asian epidemiological setting.

Consequences to the child: Some recent studies have reported on malaria susceptibility in early childhood associated with the presence of placental malaria at delivery. Two studies, one from Gabon, and another from Tanzania both found infants born to women with *P.falciparum* placental infections were at higher risk of developing clinical malaria in early childhood compared to those born to women without malaria (Klein Klouwenberg, *et al* 2005, Schwarz, *et al* 2008). Interestingly both studies also found that infants born to multigravidae had a higher risk of infection than infants of primigravidae even after adjusting for HIV infections. An earlier study conducted in Cameroon that followed children for first 2 years of life found infants between ages 4-6 months were at higher risk of infection when born to women with placental infection (Le Hesran, *et al* 1997). They also observed between ages 17-26 weeks the survival rate decreased in the offspring's of placenta infected women while parasite prevalence was consistently high up to 18 months age. These findings suggested that offspring's of placenta-infected women are at higher risk of malaria in infancy. Additionally, the observations of multigravidae related infant

morbidity in the Gabon and Tanzania study suggest a shift in the impact of malaria in pregnancy from primigravidae to women of all gravidity in the African setting.

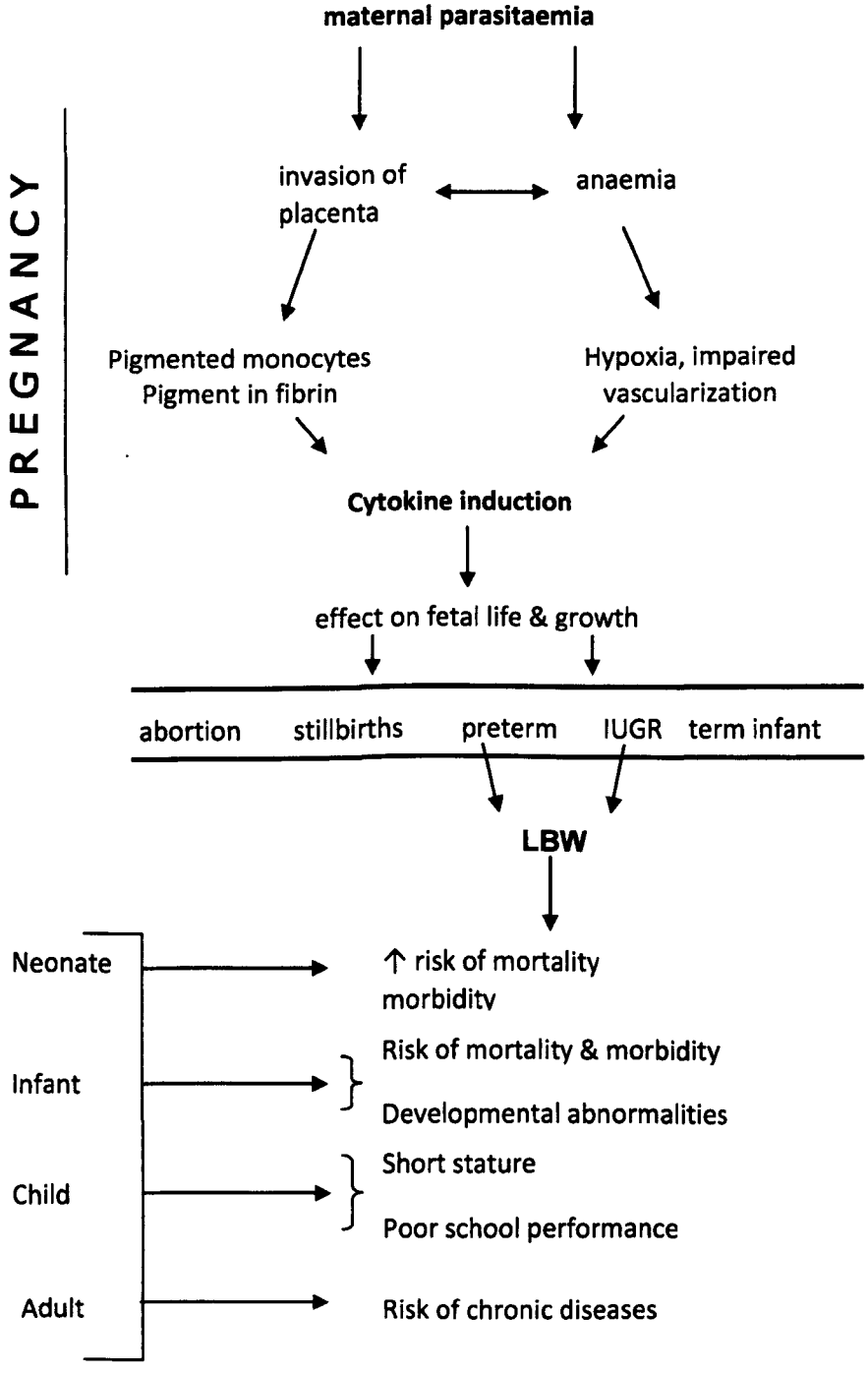
Congenital malaria: Although previously considered as uncommon, case reports of congenital malaria are increasing in current literature. Prevalence ranging from 0.3% to 33% has been reported for congenital malaria with more sensitive diagnostic tests (Desai, *et al* 2007, Vottier, *et al* 2008). Many studies have also described the presence of cord parasitaemia, although compared with the prevalence of placental infections, the corresponding level of cord parasitaemia and congenital malaria is low. These findings imply that the placenta serves as an effective barrier limiting transplacental transfer of malaria parasites. However, to explain the rare occasions of parasite reaching the fetus, the possibility of maternal, placental and fetal blood mixing during delivery or pathological damage to placental villi secondary to infection have been suggested. Interestingly, cases reports of congenital malaria from *P.vivax*, which is not known to pathologically damage the placenta, are more frequent (Valecha, *et al* 2007, Wiwanitkit 2006, Woods, *et al* 1974). To this effect a recent review by Vottier (2008) of congenital malaria in non-endemic areas found 58% of cases were due *P.vivax*, whereas 28% were due to *P.falciparum* and the rest due to *P.malariae* or *P.ovale* and few mixed species. These and the wide variation in prevalence could be due to differences in definitions of congenital malaria. Some investigators use the criteria of presence of parasites in the peripheral blood of the newborn on the first day of life. Other reports defined congenital malaria by detection of parasites in the first seven days of life while few case reports have accepted detection of parasites in first 28 days of life. In malaria endemic regions there is the possibility of exposure to mosquito bites in first few days of life of an infant. Hence, with the definitions in use, it is difficult to distinguish between congenital and neonatal exposure. While the mechanisms suggested for explaining congenital malaria is plausible it is also ambiguous. Similarly, the impact of cord blood parasitaemia on the newborn is unclear. Nevertheless increasing drug resistance, better testing and improved case detections could be adding to the increase in congenital malaria which in turn could add to the burden of malaria in pregnancy.

Long term consequences of low birth weight: The consequences differ for preterm-LBW and IUGR-LBW babies (Figure 2.5). The risk of immediate effects of mortality and morbidity is higher for preterm-LBW with disabilities being common in the long-term. Studies conducted in developed countries have documented the risk of cognitive impairment, poorer neuro-sensory defects, learning disabilities, behaviour problems and poor school performances for low birth weight children (Corbett, *et al* 2004, Davis 2003). On the other hand being IUGR-LBW the

effects manifest with poor growth and higher incidence of chronic diseases of adulthood, such as type 2 diabetes, cardiovascular and renal diseases (Barker 1995). There is less information from

FIGURE 2: 5: EFFECT OF MALARIA IN PREGNANCY IN FETAL LIFE, INFANCY AND ADULTHOOD

Effect of malaria in pregnancy on fetal life, infancy and in adulthood



developing countries about the long term sequelae of low birth weight infants although the prevalence of low birth weight in developing countries is higher than developed countries as shown earlier. One study in Mysore, India showed a higher prevalence of coronary heart diseases as adults in babies born with low birth weight to women with low weight in pregnancy independent of other known coronary risk factors (Stein, *et al* 1996). In developing countries being a female born with low birth weight adds to the cycle of disadvantages increasing the risk of having a low birth weight baby with resultant short stature particularly in adolescents who become pregnant before attaining full growth potential.

In developing countries, approximately 40% or more deliveries occur at home and those babies do not get weighted at birth (UNICEF 2004). For example, in the Indian DHS survey, 33% reported a weight record for babies (NFHS-3 2006). In many developing countries even when weighed the measurement maybe taken inaccurately or left unrecorded. For these reasons while birth weight measurement is a simple task, reliable data from developing countries are limited on this important determinant of child health. Because the impact and long-term effects of IUGR and preterm LBW are different, obtaining accurate gestational age becomes equally important as measuring birth weight. Such data would be necessary for prognostic studies on understanding the long-term consequences of malaria related low birth weight and mortality.

2.6.2.6 Maternal nutrition and low birth weight

Maternal anthropometric indices are used as indicators of maternal nutritional status and predictors of fetal outcome. The indices used for assessing pregnancy nutritional status include pregnancy weight gain, maternal height, MUAC and pre-pregnancy weight and body mass index (BMI) (WHO. 1997). Short stature which, in part indicates chronic under nutrition of childhood has been shown to have an association with increased risk of low birth weight. Values of MUAC <23 cm have also been associated with low birth weight and fetal and infant deaths. Likewise, women with lower pre-pregnancy weight or BMI were at greater risk of delivering low birth weight babies. However, in most malaria endemic setting women often lack a precise pre-pregnancy weight and therefore its use is limited in research.

Malaria transmission coinciding with food shortage season superimposes the effect of malaria in undernourished women (Rayco-Solon, *et al* 2005). Supporting this is evidence of increased birth weight with nutritional supplementation during food shortage season (Ceesay, *et al* 1997). However, to date a single study has assessed the parallel effects of maternal malaria and under-nutrition on the risk of *in utero* IUGR. Landis and colleagues using longitudinal fetal ultrasound observed in Congolese women one third of fetuses to be IUGR and the risk of IUGR was higher among under nourished women after 3 cumulative malaria infections (Landis, *et al*

2009). They also identified that peak prevalence of *in utero* IUGR occurred between 28-33 weeks gestation. An earlier study from Malawi that assessed fetal anthropometry reported fetal growth retardation in weight and length to be present from 30 weeks gestation (Kalanda, *et al* 2005). Another study that used antenatal ultrasound dating, although not specific to malaria-infected women reported a 20% prevalence of preterm births among rural women in southern Malawi (van den Broek, *et al* 2005). Although fetal ultrasonography is a better technique for assessing *in utero* growth, due to resource limitation malaria research has rarely used this method.

2.6.3 Maternal morbidity, mortality and symptomatic malaria

Clinically malaria diagnosis falls under two categories: uncomplicated or severe disease. The documentation of fever and chills and other non-specific symptoms in the presence of parasitaemia is diagnosed as uncomplicated malaria. The severity of symptoms depends on the infecting species and severe malaria characterised by signs of organ damage is commonly caused by *P.falciparum.* The lower level of acquired immunity in low transmission setting predisposes women to develop symptoms in the presence of parasitaemia. If left untreated they rapidly progress to severe malaria and are more susceptible to complications of cerebral malaria, hypoglycaemia and acute pulmonary oedema (WHO 2000). Thus, severe malaria has been more frequently reported from low, seasonal and unstable regions as in parts of Asia than from stable transmission settings (Nosten, *et al* 2004). Also, complications of severe malaria except for severe anaemia rarely occur in women in high transmission areas. However, recent studies in stable high transmission African setting suggest this may not be the case. A study in Mozambique found three out of four women attending a rural health clinic had symptoms suggestive of uncomplicated malaria (Bardaji, *et al* 2008). A large proportion of these women were also in their first trimester. Similarly, a study in Ghana found nearly half the women with parasitaemia of any density reported a history of illness in the previous 5 days or had a history of fever (Tagbor, *et al* 2008a). Other studies have also shown that a history of fever in the previous week was reported more frequently by parasitaemic women than aparasitaemic women. Therefore, malaria morbidity in highly endemic areas maybe more common than previously assumed and is likely to be contributing to the clinical burden of malaria in pregnancy.

Although symptomatic and severe malaria is considered to occur mainly in low transmission setting, estimates of malaria associated mortality in the both low and high transmission settings are comparable (Desai, *et al* 2007). This perhaps relates to difficulties in quantifying malaria attributed mortality. Many deliveries in malaria endemic regions occur at home and fatality either during pregnancy or during delivery may not be reported, in addition to lack of laboratory based malaria diagnosis. The validity of data obtained by verbal autopsies or

clinical records was shown by a recent study that assessed clinic-pathological data using necropsy (Ordi, *et al* 2009). Some studies in Africa have shown higher proportion of maternal deaths occurred during peak malaria season (Anya 2004, Romagosa, *et al* 2007). Another study in an unstable transmission area in Rwanda reported a fivefold increase in maternal deaths due to malaria during an epidemic year and a large proportion of maternal deaths in non-epidemic year (Hammerich, *et al* 2002). Analytical studies have shown that severe anaemia related to malaria is an important contributor to maternal deaths in stable transmission area. One retrospective analysis of maternal deaths in Kilifi found 23% was due to severe anaemia. Another analysis estimated that there would be 9 maternal deaths related to severe malarial anaemia per 100,000 live births in primigravidae when severe anaemia prevalence was 5% (Brabin, *et al* 2001a). Overall the percentage of malaria attributable deaths ranged from 0.5-23% in hospital studies and 2.9-17.6% in community studies in Africa (Brabin, *et al* 2002). The corresponding figures in low transmission areas are 0.6-12.5%. Some recent studies during malaria outbreaks in India and the case fatality rates of 6.5-24.2% reported during the past epidemic in Ceylon suggests that high mortality occur during malaria outbreaks in parts of Asia (Singh, *et al* 1999b, Singh, *et al* 1988, Wickramasuriya 1937). It is also likely that estimates for Asia could be higher if accounted for *P.vivax* associated anaemia and its clinical burden. In general, mortality rates from direct causes such as cerebral malaria are higher in low transmission areas, whereas in stable transmission areas contribution to mortality from indirect causes is greater.

2.7 Epidemiology of Malaria in Pregnancy

Malaria in pregnancy has a distinct epidemiology which largely depends on the intensity and stability of transmission and infecting species. On this basis, Africa and Asia present two separate epidemiological features of pregnancy malaria and is the focus of review in this section.

2.7.1 Falciparum endemic regions of Africa

Most regions of Africa, has high and stable transmission with *P.falciparum* as the predominant species which suggests that a large number of pregnant women are exposed to malaria. This was highlighted in a recent analysis that showed 32 million pregnancies are at risk of *P.falciparum* in the African region where transmission is stable (Dellicour, *et al* 2010). In these regions the prevalence of maternal malaria is also high. The estimated median prevalence of peripheral or placental infection identified by standard microscopy in a recent review was 27.8% and 26.0% for placental malaria (Desai, *et al* 2007). There are also parts of Africa that are prone to low or unstable transmission, but the population at risk of malaria is small (8.4 million) in comparison to the population at high risk of malaria. In these regions the median prevalence of

peripheral and placental malaria was 13.7% and 9.6%. In addition, *P.vivax* is prevalent in the countries around the horn of Africa and account for 11.9% transmission (Desai, *et al* 2007). Since, the highest burden is in areas of high transmission, this region has been the focus of research and has contributed to establish a control policy malaria for the at risk population in Africa.

2.7.1.1 Susceptible Groups

Gravidity: As stated earlier, although women in stable transmission regions have acquired partial immunity by the time they reach reproductive age, pregnancy increases their risk of malaria, particularly in primigravidae. As early as 1956, Archibald (Archibald 1956) showed a predilection of placental malaria in primigravidae (20.2% versus 12.5% in multigravidae). But it was Canon (1958)(Cannon 1958) who first highlighted the gravidity specific effect which is now a well recognised phenomenon in partially immune populations in Africa. Most present day studies show a two-fold increased risk of malaria in primigravidae compared to multigravidae.

Trimester: The parasite prevalence varies through the course of pregnancy. Peak prevalence has been observed between 13-16 weeks which is considered the time of highest susceptibility to infection (Brabin 1983). As the second half of pregnancy advances, the parasite rate decreases, reaching a pre-pregnant level after delivery (Brabin and Rogerson 2001, Menendez 1995). Some earlier investigators have suggested that the gestational variation is due to rate of recovery being faster than the rate of infection in the second half. However, more recent studies showed a continued increase in risk during the post partum period suggesting a possibility of recurrent and new *P.facliparum* infections in those being exposed (Diagne, *et al* 2000, Ramharter, *et al* 2005). In contrast, an earlier study showed that women cleared *P.falciparum* infection within 24-48 hours of delivery whereas *P.malariae* infections persisted after delivery (Nguyen-Dinh, *et al* 1988). The authors of this study suggest that a decrease in parasite load may occur with expulsion of placenta, which would have been harbouring sequestered parasites.

In malaria endemic countries most women by the time they make the first antenatal visit are well into their second trimester (Ouma, *et al* 2007, Rogerson, *et al* 2000, van Eijk, *et al* 2006). Perhaps this might be why data on first trimester infections are lacking. In one study that enrolled women in early pregnancy, high parasitaemia was observed (Brabin 1991). During early pregnancy, peripheral blood parasitaemia could be high because placental blood flow begins around 10-12 weeks and until then, parasite accumulation in the placenta may not start (Carter

1997, Jauniaux, *et al* 2003). Additionally, splenic changes have been observed in early pregnancy with decreasing spleen rates after 16 weeks (Brabin, *et al* 1988). This might also reflect changes corresponding to the timing of placental circulation and parasite sequestration. In a recent study, symptoms suggestive of malaria were observed in first trimester women attending antenatal clinic in rural Mozambique (Bardaji, *et al* 2008). Taken together these findings suggest significant malaria associated changes occur in first trimester and that it could be an important period for understanding the pathogenesis of pregnancy malaria before placental circulation is established.

Age: The increased risk of malaria with younger maternal age has been long recognised. A recent review identified pregnant adolescents are an important group particularly with adolescents making up more than half the primigravidae in malaria endemic countries (Lalloo, *et al* 2006). Several studies showed parasitaemia at first antenatal visit and at delivery were higher in women younger than 20 years (Rogerson, *et al* 2000, van Eijk, *et al* 2002, Zhou, *et al* 2002). Another study showed an association between dehydroepiandosterone sulphate and decreased parasite density among pubertal girls, even after adjusting for age (Leenstra, *et al* 2003). This suggests that age related immunity together with pubertal changes play a role in malaria infection. The adolescents are also at increased risk of morbidity and mortality as they are less likely to attend antenatal care or use prophylaxis appropriately (Granja, *et al* 2001, Lalloo, *et al* 2006).

ABO blood group and Duffy antigen: In stable *P.falciparum* transmission areas in the Gambia and Malawi an increased risk of placental malaria in primigravidae has been associated with blood group O phenotype with reduce risk in multigravidae (Loscertales, *et al* 2006, Senga, *et al* 2007).

The Duffy antigen has long been considered the antigen necessary for *P.vivax* to invade the red cells. This fact has been associated with absence or rare occurrence of *P.vivax* infections in central and West Africa and in parts of East Africa, where the majority of population are Duffy antigen negative. However, recent observations have demonstrated *P.vivax* transmission in Duffy negative populations in Western Kenya and Brazil (Cavasini, *et al* 2007, Ryan, *et al* 2006). In addition a study in children in Madagascar which looked at Duffy negative phenotype and genotype demonstrated that multiple strains of *P.vivax* infected Duffy negative individuals (Menard, *et al* 2010). These findings suggested a likely evolution of *P.vivax* strains and that the host susceptibility to *P.vivax* may not depend absolutely on the presence of Duffy antigen (Mercereau-Puijalon, *et al* 2010).

2.7.2 Malaria endemic regions of Asia

In contrast to the African region, most malaria endemic Asian countries have low or seasonal and unstable transmission with both *P.falciparum* and *P.vivax* being dominant. According to one analysis, overall 48 million pregnancies are at risk of malaria in south-east Asia. 16 million pregnancies are at risk in stable *P.falciparum* transmission areas, whereas 21 million pregnancies are at risk in unstable transmission regions of south-east Asia (Dellicour, *et al* 2010). The majority of pregnancies in this region are at risk of *P.vivax* (48 million). In an extensive review in 2007, Thailand and India were the only two Asian countries cited in an estimate of maternal and placental malaria prevalence in low transmission areas, which indicate the deficiency of data from the region (Desai, *et al* 2007). In Thailand the prevalence of maternal parasitaemia was 5.9% for any species and in India the overall maternal malaria prevalence was 6.1% and placental parasitaemia indentified by smear microscopy was 12.6% (Nosten, *et al* 1991, Singh, *et al* 2005). In a recent study in two eastern provinces of Indonesia, the overall prevalence of maternal malaria indentified by PCR was 12.3% and placental malaria was 4.8% (RA and Syafruddin, unpublished). Another study in southern Papua, Indonesia reported an overall prevalence of 16.8% maternal malaria at delivery (Poespoprodjo, *et al* 2008). The variations in these studies could be due to different diagnostic methods used. Nevertheless, these figures are comparable to low-transmission African setting estimates of a median prevalence of 13.7% and 6.7% for maternal and placental malaria respectively (Desai, *et al* 2007).

2.7.2.1 Susceptible groups

Gravidity: Most pregnant women in the Asian region are exposed to low or unstable transmission resulting in either low or no acquired immunity by the time they become pregnant. As a result susceptibility difference between primigravidae and multigravidae is modest and less marked unlike that observed in the African setting (Nosten, *et al* 2004, Poespoprodjo, *et al* 2008). In times of outbreaks or epidemics the gravidity effect is absent (Wickramasuriya 1937).

Age: Studies from the Asian region that have documented age as a risk factor are few and results vary. A study from Jharkhand, India reported peripheral parasitaemia was more common in women younger than 20years (aRR 2.6, 95%CI 1.03-6.98)(Hamer, *et al* 2009). Low transmission regions elsewhere that looked at age distribution found no association between age and malaria infection (Adam, *et al* 2009, Martinez-Espinosa, *et al* 2004). Little is known about adolescent pregnancies and malaria in Asia, although some studies from the region have reported higher

number of admission of adolescents (not specific to pregnant women) with severe disease (Luxemburger C, *et al* 1997, Singh, *et al* 1992).

ABO blood group and Duffy antigen: There are no studies showing blood group phenotype and host susceptibility to malaria in pregnant women in Asia. There are also very few studies in general population which shows varied results. In general malaria was more common in individuals with blood group A and less common in individuals with blood group O phenotype in India and Sri Lanka (Deepa, *et al* 2011, Gupta, *et al* 1980, Pathirana, *et al* 2005). The association of placental malaria and blood group phenotype would be of interest to explore in the Asian region.

As stated earlier the Duffy antigen serves as the receptor for *P.vivax* parasite to invade erythrocytes. *P.vivax* is the species widespread and common in Asia. Recent studies have shown polymorphism of the Duffy Antigen Receptor for Chemokines (DARC) and that natural acquisition of antibodies may vary with host genotype and level of DARC expression which may explain severe *P.vivax* infections occurring in some individuals (Langhi, *et al* 2006, Maestre, *et al* 2010).

2.7.3 Human Immunodeficiency virus-1 interaction

Both HIV-1 and malaria are highly prevalent in sub-Saharan Africa. An estimated 22.5 million people with HIV-1 and 33 million pregnant women at risk of *P.falciparum* live in this region (Dellicour, *et al* 2010, UNAIDS 2011). An increase of malaria in pregnancy in women of all parities has been associated with HIV-1 where both malaria and HIV-1 coexist (ter Kuile, *et al* 2004, Steketee, *et al* 1996a, Verhoeff, *et al* 1999). The increased susceptibility is explained by impairment of humoral immunity responses to placental VSA in HIV-1 infected women (Mount, *et al* 2004). The co-morbidity alters the gravidity specific immunity such that an HIV-1 infected multigravida may have a higher prevalence of malaria than an HIV-1 non-infected primigravida. One review showed an estimated 5.5% and 18.8% proportional increase in malaria in pregnancy attributed to HIV-1 in areas with corresponding HIV-1 prevalence of 10% - 40% (ter Kuile, *et al* 2004). The same review also showed that there is higher parasitaemia and placental malaria with the result that the adverse effects of malaria in pregnancy are higher in those with HIV-1 infections even in multigravidae.

In the absence of interventions to prevent mother to child transmission (MTCT) an estimated 15-30% of HIV-1 women transmit the virus to their infants *in utero* and or during intrapartum (Kourtis, *et al* 2006). Studies conducted during early 2000s to examine whether malaria and HIV-1 co-infection increased MTCT had shown variable results (Brahmbhatt, *et al*

2003, Inion, *et al* 2003, Mwapasa, *et al* 2004). These inconsistencies were thought to be either due to differences in epidemiology of maternal immune status due to different techniques used to diagnose placental malaria. Two recent studies, one that used placental histology with HRP-2 staining and serological ICT and another that used placental histology also found differing results (Brahmbhatt, *et al* 2008 {Msamanga, 2009 #1402). The current and earlier inconsistent results perhaps reflect the complexity of the problem both in terms of timing and transmission of HIV infection and immune response of malaria. In addition a study in rural Mozambique found malaria prevention with 2 doses IPTp combined with LLITN had no impact on the risk of MTCT of HIV-1(Lahuerta, *et al* 2006, Naniche, *et al* 2008). The effect of interactions of HIV-1 and malaria co-morbidity indicates that in sub-Saharan Africa the burden of malaria in pregnancy is no longer limited to paucigravidae.

2.7.4 Non-falciparum and mixed species

Historically, the first description of *P.malariae* was made by Golgi (1886) from blood taken from a severely ill pregnant woman (Duffy and Desowitz 2001). Other investigators in the past have described *P.malariae* in placental infections (Bruce-Chwatt 1952, Jelliffe 1967). However, present day focus has been on *P.falciparum* with attention to the effects of *P.vivax* increasing in the past decade. As already described in previous sections, *P.vivax* infections are associated with low birth weight and maternal anaemia; although the magnitude of the effect is lower than *P.falciparum.*

In areas where more than one species is endemic, infections of mixed species is more common than previously assumed. The effects and outcome of mixed species infections have been described mainly for concurrent infections of *P.falciparum* and *P.vivax* (Mayxay, *et al* 2004, Snounou, *et al* 2004). In such cases although *P.falciparum* is dominant, the complications of *P.falciparum* is decreased in the presence of *P.vivax* (Luxemburger C, *et al* 1997). In mixed infections, the focus of parasite detection by blood smear could be directed towards the dominant species thereby missing *P.vivax*, and could influence the antimalarial treatment. Thus, once *P.falciparum* infections are treated, reappearance of *P.vivax* has been observed. This suggests, with preventive programmes aimed at controlling *P.falciparum* infections, morbidity from *P.vivax* could increase with relapses of *P.vivax* hypnozoite that lie dormant in the liver for long periods. An increase in relapses among pregnant women has been observed in the Thai-Burmese border (Nosten, *et al* 1999). With *P.falciparum-vivax* interactions, in a smaller proportion of cases an increase in *P.falciparum* has been described when treated for *P.vivax* infections (Mayxay, *et al* 2004). These findings suggest that planning preventive programmes for areas where both *P.falciparum*

and *P.vivax* are co-endemic such as in India could be challenging, particularly in targeting the clearance of hypnozoites in pregnant women.

Although microscopic evidence of *P.ovale* in pregnancy has been rarely reported, recent PCR based studies in African countries have detected *P.ovale* in *P.malariae* in pregnant women (Walker-Abbey, *et al* 2005a). With more research using molecular approaches, it is likely new data on single non-falciparum species and mixed species infections would be available.

2.7.5 Sub-microscopic malaria

Submicroscopic infections and the associated impact in pregnancy is currently a little studied area. Few studies performed mainly for characterisation of *P.falciparum* have reported on the prevalence of sub-microscopic infections in some African countries. As expected, with the use of the more sensitive method of PCR, a high rate of infections below the microscopically detectable threshold was detected. A study in Cameroon found 54.9% submicroscopic infection, nearly double that detect by peripheral blood smear (27.5%) (Walker-Abbey, *et al* 2005b). Similarly, a study conducted among pregnant Gabonese women reported 44% infection in microscopically negative cases (Mayengue, *et al* 2004). Two other studies, one in Mozambique and the other in Ghana reported on sub-microscopic infection in pregnant women. These studies found an increase in sub-microscopic parasitaemia with increasing parity suggesting that pregnancy associated immune protection limited parasites to sub-microscopic level (Mockenhaupt, *et al* 2000, Saute, *et al* 2002) Saute, *et al* 2002). A decrease in haemoglobin level was also observed in women with sub-microscopic parasitaemia in Mozambique and Ghana. One study from low transmission area in Sudan also found a high prevalence of sub-microscopic infection, with no association with age or parity (Adam, *et al* 2005). The impact on birth weight was not assessed in these studies. Since, these were all cross-sectional studies, whether these infections later recrudesce is uncertain. There is no published data on sub-microscopic infection in pregnancy from Asia where the findings of a high prevalence could influence preventive strategies much more than in high transmission regions. The current study has looked at sub-microscopic infections and will be presented in the relevant result chapters.

2.8 Placental Malaria and Histopathology

2.8.1 The Placenta

The placenta is an organ unique to pregnancy that supports fetal growth and development. It receives its name from the Greek words *plakounta/plakous* meaning "flat disc-

like" with reference to its discoid shape. The placenta develops from the fertilized ovum that forms the fetus which makes it a feto-maternal organ. The importance of the placenta for the materno-fetal connection was recognised and revered from ancient times (Boyd, *et al* 1970). The belief that it is the 'soul of life' persists from the Pharaonic culture to many present day communities. For malariologists, this belief has shifted to knowledge that any insult to the placenta with the malaria parasite can harm the mother and her growing fetus. One method of detecting the presence of parasites and morphological changes of the placenta associated with malaria infections are by histological examination. Before describing histological lesions in malaria infected placenta it is helpful to appreciate the anatomy of placental structures relevant to placental malaria.

Development: On implantation of the blastocyst to the uterine epithelium, the trophoblast cells forming the outer layer of the blastocyst proliferate to form the placenta and fetal membranes (Boyd and Hamilton 1970). The chorionic plate represents the fetal aspect of the placenta and the basal plate the maternal region. In between these lie the intervillous spaces filled with projecting villi, the primary functioning unit of the placenta. The villi arise from the chorionic plate and continue to develop like a tree throughout gestation. It consists of the stem villi which supports the villous tree, the intermediate villi and the final branches the terminal villi. The terminal villi are where most of the fetal maternal exchange occurs and hence physiologically the most important units. The outer surface of the villus is lined with the multinucleated cell layer of syncytiotrophoblast which is formed by the fusion of the cytotrophoblast cells (Huppertz 2008). The syncytial layer thus lines the intervillous space and separates the maternal and fetal compartments. The receptors expressed by the syncytiotrophoblasts allow cytoadherence and thus sequestration of *falciparum* infected erythrocytes within the intervillous spaces. Hence, the syncytial layer and the intervillous spaces are the key anatomical structures associated with placental malaria pathology. The other structure that is important in placental malaria is fibrinoid, a homogenous material devoid of cells. It is derived either from clotting maternal blood and degenerating cells (fibrin type fibrinoid) or from secretory products of the extravillous trophoblasts (matrix-fibrinoid)(Kaufmann, *et al* 1996). The fibrinod is where haemozoin pigment stays entrapped for many weeks.

Circulation: The placental blood supply gets established from remodelling of uterine spiral arteries by the invading trophoblasts. Although still debated, investigators believe that arterial blood flow in the placenta becomes fully established after 10-12 weeks of gestation (Carter 1997, Jaffe, *et al* 1997). Until this time the placenta grows in a low oxygen tension during first trimester which evidence suggests is required for the success of pregnancy (Gude, *et al* 2004). Once

circulation is established maternal blood enters the intervillous spaces through the spiral arteries. The timing of malaria parasite sequestration is thought to correspond to the onset of maternal blood flow in placenta, although studies of placental histology on first trimester malaria infections are lacking (Brabin, *et al* 2004).

Protective function: The syncytiotrophoblasts ultimately becomes the layer of materno-fetal interface. This layer constitutes a protective barrier against infection from mother to the fetus. However some organisms, mainly viruses, few bacteria and protozoans like *T.gondii* manage to cross this barrier. The plasmodium species is known to pass into the placenta through the maternal circulation with a small number of reports on malaria parasites in cord blood seen by standard light microscopy. However parasitized fetal erythrocytes or parasites in the fetal structures have not been observed in histopathological studies (Ismail, *et al* 2000, McGready, *et al* 2004). In addition the presence of infected erythrocytes in the villous structure is rare, although few studies have reported occasional presence of pigment within villous stroma and in fetal vessels (Parekh, *et al* 2010, Walter, *et al* 1982). These histological findings implying that the syncytium where occasionally adherent parasites have been observed may protect the *Plasmodia* from reaching the fetus in immune women. However, syncytial damage has been reported in malaria infected placenta and cord blood parasitaemia suggesting the syncytial protection may not be effective in women non-immune to malaria (Crocker *et al* 2004, Parekh *et al* 2010).

2.8.2 Plasmodium species in the placenta

As described earlier, *P.falciparum* is the only species recognised to sequester in the placenta, although the presence of non-*falciparum* parasites in the peripheral blood of pregnant women is well recognised. A few studies have observed non-falciparum species in placental blood suggesting that the sluggish circulation within the placenta enables parasite passage through the organ. The series by Jelliffe *et al* reported cases of *P.malariae* in placental blood film, but not on histology (Jelliffe 1966, Jelliffe 1968). Likewise, more recent studies have shown the presence of *P.vivax* in placental blood. However in confirmed cases of peripheral blood *P.vivax* mono infection, only evidence of pigmented fibrin was found in the placenta (McGready, *et al* 2004).

Trophozoites and schizonts are the two commonly observed *Plasmodium* stages in the placenta. This is in keeping with the fact that cytoadhesion is a feature associated with mature-stages of infected erythrocytes (Yamada, *et al* 1989). Since haemozoin is found in the mature stage parasites, the observations of pigment in the placenta and within macrophages further support the presence of mature stage parasites (Romagosa, *et al* 2004). Although, the ring-stages of parasite are not known to cytoadhere, some studies have shown infrequent presence of ring-

stages in the placenta (Beeson, *et al* 2002). In this regard in an *in vitro* study Pouvelle demonstrated adherence of early rings to placental and other endothelial cells (Pouvelle, *et al* 2000). A recent histological case report showed the presence of ring-stage infected erythrocytes localised to one area in the placenta (Muehlenbachs, *et al* 2007a). This suggests the possibility that undetected by peripheral and placental smears ring-stage *P.falciparum* could remain dormant within the placenta.

2.8.3 Placental structures associated with malaria

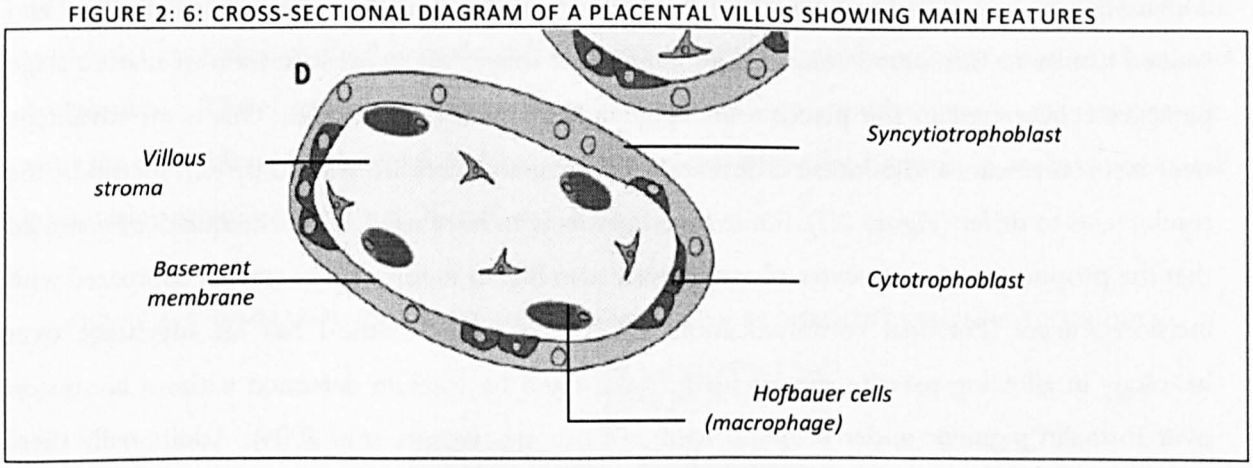
Several studies have described changes to placental structures associated with *P.falciparum* malaria infections. The significant features noted in the placenta are syncytial knotting, basal membrane thickening, necrosis of villi and intervillous fibrin deposition (Brabin, *et al* 2004). These structural changes were more commonly observed in cases of chronic malaria and severe parasitaemia. In cases with past infection the histological changes reported were not significantly different from uninfected placenta suggesting that changes to placental lesions resolve as infection subsides (Bulmer, *et al* 1993b, Galbraith, *et al* 1980, Ismail, *et al* 2000). The only feature associated with *P.vivax* infection was the presence of haemozoin pigment, which was less prominent than with *P.falciparum* infections (McGready, *et al* 2004). In the study on placental samples from Thai-Burmese border, the placental changes observed in few cases of mixed species (*P.vivax* plus *P.falciparum*) were comparable to *P.falciparum* related changes.

2.8.4 Inflammatory cells in Placental Malaria

Several studies have reported monocyte and macrophages occupied the intervillous spaces without an associated increase in the villi. In a large study of Tanzanian samples Ordi *et al* highlighted massive presence of mononuclear infiltrates in malarial placentae similar to massive chronic intervillositis first described by Labarrere et al (Ordi, *et al* 1998b). Previously massive chronic intervillositis was a condition associated with poor fetal outcome in patients with preeclampsia, hypertension, diabetes mellitus, heroine abuse and lupus (Labarrere, *et al* 1987).

Unlike the reports from *P.falciparum* endemic high transmission areas the phenomenon of massive chronic intervillositis was uncommon in low transmission areas. McGready *et al* in a study from Thailand found the rate of massive chronic intervillositis was low (1.7%). Additionally, polymorphonuclear cells were also scanty compared to reports from Africa, except in cases when malaria occurred close to delivery (McGready, *et al* 2004). Compared with African studies, the level of parasites found in the placenta in the Thailand series was also much lower.

FIGURE 2: 6: CROSS-SECTIONAL DIAGRAM OF A PLACENTAL VILLUS SHOWING MAIN FEATURES



Source: Huppertz, Journal of Clinical Pathology, 2008

2.8.5 Different sampling techniques and diagnostic methods

There are several different techniques for collecting placental tissue and blood samples for microscopic detection of placental parasitaemia and immuno-molecular studies. The sampling technique used in a study can influence results differently as each technique has strengths and weaknesses. The different sampling technique is possibly a major factor contributing to prevalence discrepancies between different studies.

Prick Smears: This method employs access to the placental space adjacent to the basal plate. A syringe with large bore needle is inserted directly into the placenta about 0.5 cm deep and blood is allowed to flow into the syringe by gently pulling and creating a vacuum. The blood collected can be used for preparing thick and thin smears and for other studies.

Incision Smears: This method of collection employs a shallow (0.5-1cm) cut into the intervillous space on the maternal surface of the placenta. The blood that seeps into the well is collected with blunt syringe or pipette for making thick and thin smears.

The staining and examination of prick and incision smears are similar to peripheral blood smears. In these methods, the compartment accessed is where mainly maternal blood is flowing and hence the parasites detected often correspond with parasitaemia observed in peripheral blood smears. A weakness of these methods is that mature stage parasites sequestering in the placenta can be indentified rarely. The strengths are it requires minimal equipment and the technique is simple and easy to perform.

Impression Smears: Impression smears are prepared with a small biopsy of the placenta tissue. The tissue is gently pressed on the slides to obtain imprints. Imprinted impressions are fixed and stained similar to thin blood smears. The impression smears allow identification of mature stage parasites sequestered in the placenta and pigments in mononuclear cells. This is an advantage over incision smears and because different blood compartments are studied by two methods, the results tend to differ (Figure 2.7). Some investigators who have used this technique demonstrated that the proportion of monocytes observed were also higher in impression smears compared with incision smears (Personal communication, Dr Taylor). This method has an advantage over histology in allowing parasite species identification and haemozoin detection without confusion over formalin pigment under standard light microscopy (Brabin, *et al* 2004). Additionally these smears are cheaper and quicker to prepare and examination easier.

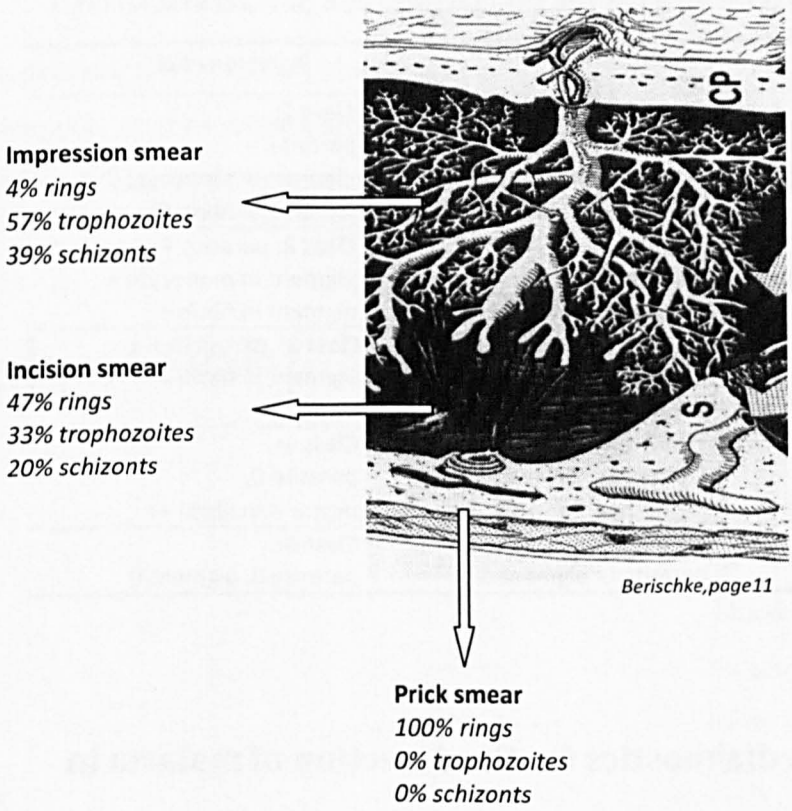
Histopathology: This method employs removal of a $2cm^2$ placental biopsy. Once collected the specimen is fixed in neutral buffered formalin to reduce formation of formalin pigments and sections prepared using standard histological technique. The formation of formalin pigment could be avoided with Streck tissue fixative; however it is no longer being produced by the manufacture (Streck Laboratories, Omaha, NE, USA). Histology is regarded as the 'gold standard' for diagnosing placental malaria due to its higher sensitivity compared with peripheral blood and incision smears (Rogerson, *et al* 2003a). Histology also has the advantage of tracking infection chronology and classifying accordingly into active and past infection. Besides malaria associated morphological changes occurring in the placenta can be studied with histology. Another advantage of the histology specimens is that it can be stored for long periods after processing into paraffin blocks and can later be used for histochemical, immunology and molecular studies if necessary.

Techniques for Immuno-molecular study: Blood samples for immunology studies can be collected through any of the above described techniques. Other techniques such as perfusion and tissue grinding have been used for immuno-molecular work. One study that compared several techniques showed perfusion and prick method were the two methods with least fetal cell contamination and therefore most suited for immunology studies (Othoro, *et al* 2006).

Laser Microdissection Microscopy: Laser capture microdissection is a new and evolving technique that is being widely applied to explore parasite molecular biology and host parasite interactions. The method allows precise extraction of individual or small cell populations from histological sections enabling subsequent analysis of the component cells (Chan, *et al* 2004). The

technique uses upright or inverted compound microscopes where tissues can be dissected through laser- assistance and pressure catapulting (Espina, *et al* 2006). The downstream applications of this method include different types of PCR analysis, microarray analysis and proteomics. This technique was recently applied to study gene expressions in syncytiotrophoblasts in placental malaria and the role of placental hypoxia in malaria associated intrauterine growth retardation (Boeuf, *et al* 2008).

FIGURE 2: 7: DIAGRAM ILLUSTRATING PARASITES IDENTIFIED BY DIFFERENT SAMPLING TECHNIQUES



Source: Taylor Presentation at Placental Histology Workshop Cameroon 2009 (modified)

2.8.6 Histopathological Classification

The different grading of histological classification is presented in table 2.3. Bulmer *et al* developed a systematic classification on the basis that the presence of parasite and pigments in the placenta indicated the progression of infection during pregnancy. Accordingly they graded infections into four classes: active, active chronic, past chronic and no infection. Later Ismail *et al* modified the classification to acute, chronic, past and no infection. Rogerson *et al* in his study demonstrating an association between low birth weight and pigmented monocytes altered the classification by sub-grading chronic infections into two classes. Based on these grading,

histology sections provide evidence of infection in pregnancy like a time tracker. In the acute stage only parasites can be detected and after phagocytosis of the parasites by inflammatory cells, malaria pigments (haemozoin) can be found in the monocytes within the intervillous spaces. Later fibrinoid covers the haemozoin containing monocytes and is recognised as pigments within the fibrinoid. Haemozoin is an inert substance and during the degradation process it can remain in the fibrin for weeks to months (McGready, *et al* 2002). Thus when parasites are present in the placenta it indicates active infection and pigments without parasite indicate past infection.

TABLE 2: 2: CRITERIA USED BY DIFFERENT AUTHORS FOR THE CLASSIFICATION OF PLACENTAL MALARIA

	Bulmer *et al*	Ismail *et al*	Rogerson *et al*
1	**Active Infection:** parasite + pigment in monocyte + pigment in fibrin = 0	**Acute Infection:** parasite +, pigment in monocyte + pigment in fibrin ±	**Class 1:** parasite + pigment in monocyte 0 pigment in fibrin 0
2	**Active-Chronic Infection:** parasite + pigment in monocytes + pigment in fibrin + +	**Chronic Infection:** parasite + pigment in monocytes + pigment in fibrin or cells++	**Class 2**: parasite + pigment in monocyte + pigment in fibrin ± **Class 3**: parasites + Pigment in fibrin ++
3	**Past-Chronic Infection:** parasite 0, pigment fibrin ++	**Past Infection:** parasite 0 pigment in fibrin ++	**Class 4:** parasite 0, pigment in fibrin ++
4	**No Infection** parasite 0, pigment 0	**No Infection** parasite 0, pigment 0	**Class 5:** parasite 0, pigment 0

2.9 The role of various diagnostics for the detection of malaria in pregnancy

The widely accepted method for malaria diagnosis remains blood film microscopy ever since Laveran (1889) first detected the malaria parasite. However microscopy is unavailable in many rural areas of malaria endemic countries and thus accurate parasitological diagnosis becomes a challenge. In many microscopy deficient facilities fever based presumptive treatment for malaria was used. Fever symptoms are not specific to malaria, nor can species be determined accurately, although treatment options differ for *P.falciparum* and *P.vivax* (Chandramohan, *et al* 2002, Mwangi, *et al* 2005). Moreover, with ACTs implemented as first line treatment, accurate diagnosis is recommended to avoid development of drug resistance as it happened in the chloroquine era (WHO 2010a). There are currently several newer diagnostic methods as potential alternatives to microscopy. These include antigen based rapid diagnostic tests (RDTs), molecular methods with different PCR techniques and other diagnostic modalities. This section describes some of the methods that are practical for the management and control of malaria in pregnancy.

The test techniques, their strengths and weaknesses and methods that are of research interest including initiatives for reporting diagnostic accuracy studies are reviewed.

2.9.1 Types of Diagnostics

2.9.1.1 Peripheral Blood film and Standard Light Microscopy

Thick and thin blood film microscopy is the most widely used method for malaria parasite detection. Microscopy involves three steps: preparation of thick and thin film, staining and examining for malaria parasites.

Thick blood film: Capillary blood taken either by finger prick, ear lobe or heal pricks (in babies) is commonly used to prepare blood films. This is because it is an easy procedure to perform and at the same time such samples provide a higher concentration of parasites in a smaller volume (Hommel 2002). Thick films contain many layers of blood spread over approximately $1cm^2$ area enabling examination of larger blood volume than thin films. The higher sensitivity for parasite detection at low parasitaemia is the main strength of thick films. The downside of it is due to red cell haemolysis during preparation, thick films are harder to examine particularly for inexperienced readers. Poor quality films may also contribute to false positivity with artefacts and platelets being mistaken for malaria parasites. In cases of *P.vivax,* morphology maybe less clear with fragments of cytoplasms. These qualities make thick films less suitable for identifying parasite species. However, they are better for quantifying blood parasitaemia using the standard formula for calculating parasite density counted against white cells (formula to calculate parasites/μl =number of parasites/ 200 leukocytes x 8000 white cells).

Thin blood films: Thin films are prepared by fixing with methanol which provides a single fixed layer of red blood cells. This allows clear view of species morphology which makes species identification with certainty easier. Thin films are useful to estimate the percentage of parasitaemia counted against red blood cells but are not suited in cases of low parasitaemia.

Blood film stains: The blood film staining which enhances contrast under microscope was developed by Romanowsky (Woronzoff-Dashkoff 2002). Giemsa enhanced the stability of the Romanowsky's methylene and eosin based stain by adding glycerol. Since then Giemsa stain has become the preferred technique for staining malaria slides. Other stains are available but are used less frequently. Field stain has an advantage with less preparation time, approximately 10 minutes compared to about 45-60 minutes to prepare a well-stained Giemsa slide. On the basis of shorter

preparatory time in India a modified version of the Field stain called JSB is commonly used (Gautam, *et al* 1992). Details of JSB stain is described in chapter 3.

Light Microscopy: Microscopy is still considered the 'gold standard' for malaria diagnosis. It has several strengths over many of the new generation diagnostic tools. This is evident by the fact that microscopy was the primary method in use during the historic malaria eradication programmes of the 50s and 60s (WHO 1957). Microscopy allows visualisation of parasite morphology, stages of maturity and estimation of parasite load. This has important implications for assessing disease severity, response to antimalarial therapy and management. In addition where health facility infrastructure is established microscopic examination becomes relatively inexpensive. When microscopy is performed by expert technicians with well prepared slides, the detection threshold stands around 20-50 parasites/μl (Moody 2002, Wongsrichanalai, *et al* 2007). The disadvantages of microscopic examination are that the method requires time, manpower, continuing skills supervision and quality control particularly in malaria endemic countries and expertise. Compared to expert microscopy, routine detection sensitivity is lower with thresholds of about 100-500parasites/μl and in rural field situations the detection sensitivity maybe even lower (Murray, *et al* 2008). Another weakness is that a single finger prick blood sample may not always pick circulating parasites. Furthermore, in pregnant women microscopy performed during antenatal care might miss parasites that remain sequestered in the placenta. The weaker qualities of microscopy make it less practical in areas where biological diagnosis is most needed. Hence other methods are sought and the newly emerging RDTs are becoming an acceptable alternative for microscopic examination.

2.9.1.2 Malaria Rapid Diagnostic Tests (RDT)

RDTs are new and evolving diagnostic tool being rolled out to improve malaria diagnosis and management. It is promoted to reduce presumptive fever treatment to maintain the effectiveness of ACTs. WHO now recommends parasite confirmation by microscopy or RDT before treatment is started. The number of RDTs distributed in Africa has increased to 11.5 million from near zero in 2005 and another 10.0 million distributed in Asia in 2008 (WHO 2008c). These figures suggest that the new generation RDTs are being accepted as an effective malaria diagnostic tool although challenges remain.

The first commercial RDTs were introduced in the late 1990s but had setbacks mainly due to cost and quality. Following it, WHO together with FIND put in place a quality assurance system to assist UN agencies and national governments with decisions of procurement of RDTs.

Through this system product sensitivity was assessed and RDTs short listed according to performance. In the first round of this program 42 products from 21 different manufacturers were tested (WHO 2008b). However current estimates show that there are approximately 60 brands and 200 commercially available tests (WHO-FIND 2009). The range of these RDTs are available in three formats; dipsticks, plastic cassettes or cards. The latter two devices, although more expensive are easier to use particularly in the field settings.

Principle of RDT: The RDTs are based on the lateral flow immunochromatography principle. The device contains a nitrocellulose membrane strip coated with two antibodies, one targeted to capture and the other to detect the plasmodium antigen (Moody 2002). The fixed capture antibody bind the parasite antigen when liquid passes across the membrane. For this purpose a buffer solution is used. The detection antibody usually consisting of gold particles bind to the captured antigen and appear as visible lines in the test kit when parasites are present in peripheral blood. The RDTs are devised to detect antigens on a small volume of blood, approximately 5µl and results produced within 15-20 mins. The test kits have a marked well to add the blood and buffer solution where a lysing agent is present.

Malaria Antigens used in Rapid Diagnostic Tests: The RDTs detect specific antigens produced by the *Plasmodium* parasites. Some tests can detect only *P.falciparum*, targeted to histidine rich protein-2 (HRP-2) which is a protein produced only by the *P.falciparum* species. HRP-2 is predominantly expressed by asexual parasites although young gametocytes also express it (Hayward, *et al* 2000). It is expressed on the RBC membrane and found on all phenotypes. The first RDTs were devised based on HRP-2 antigen only. However there are now many commercial 'Pan-species' RDTs available that can detect all of the four main species infecting humans.

The other target antigen in common use is *Plasmodium* lactate dehydrogenase (pLDH). This enzyme is produced during the glycolytic pathway of both sexual and asexual forms of the *Plasmodium* species (Hommel 2002, Makler, *et al* 1993). For this reason pLDH based tests are targeted to detect all the four common *Plasmodium* species. Some tests are devised to specifically detect *P.falciparum* and *P.vivax*, either with pLDH or in combination of HRP-2 and pLDH antigens. The other glycolytic pathway enzyme conserved in all four *Plasmodia* and used in RDT kits is aldolase.

Field evaluation of HRP-2 based RDTs has reported high sensitivity for *P.falciparum* with parasite densities > 100/µl (Hopkins, *et al* 2007). A disadvantage of HRP-2 tests is the persistence of detectable antigen up to several weeks after clearance of the initial infection resulting in potential false positive results (Bell, *et al* 2005, Laferi, *et al* 1997). Furthermore, since HRP-2 is

produced only by *P.falciparum*, such tests would miss non-falciparum species and are of no benefit in areas where non-falciparum species are endemic. In contrast, pLDH tests, although less sensitive than HRP-2 based RDTs in detecting *P.falciparum*, are more specific because pLDH rapidly declines to undetectable levels as parasites clear with initiation of antimalarial therapy (Hommel 2002).

Conditions Affecting RDT performance: Like other biological tests, malaria RDTs are prone to deterioration through exposure to heat and humidity. Manufacturers recommend storage between 4^0-30^0C, but, good control of storage and transport conditions are difficult to achieve in many field situations (Bell, *et al* 2006). Other factors that influence the RDT results are test preparation and reader interpretation. Additionally RDT performance is dependent on parasite densities and thus results of the same RDT kit could vary depending on the malaria epidemiology, transmission intensity and the endemic parasite species in a region.

Qualities expected of an RDT: The WHO evaluation system set recommendations both to manufacturers and users on the qualities expected of RDTs. To be selected for programmatic use the RDT results should parallel that of routine field microscopy performed by an average technician (WHO-TDR 2006). The minimum test sensitivity should be equal to or higher than 95% compared to microscopy at parasite densities of 100/μl. It should be stable during use and storage under routine field environmental conditions. Other qualities required of an RDT are the test procedure must be simple and easy to learn with minimum training while result interpretation should be straight forward. Most of the RDT trials show they have these qualities although there is wide variation in performance (sensitivity and specificity) among the different brands (WHO-FIND 2009).

2.9.1.3 Polymerase Chain Reaction (PCR) Testing

PCR is a more sensitive test for the detection of malaria species. However it is not suitable as an everyday diagnostic test for field use. It is expensive to perform, time consuming, requires special equipment and result quality maybe affected by technical errors. Although these qualities do not fit with the true definition of the 'gold standard' the low parasite detection threshold makes it a superior test than microscopy or RDT. PCR has ability to detect parasites at a level of 5 or less parasites per μl (Snounou, *et al* 1993). This low parasitaemia detection also makes the PCR capable of detecting sub-patent infections which the RDTs and microscopy are unable to detect.

There are different techniques for performing PCR analysis. The commonly used assay for malaria detection is a two step nested PCR based on the method first applied by Snounou (1993). In this method, the first step involves *Plasmodium* identification using a genus specific primer and the second step amplifies species with species specific primers. This method employs the subunit 18S rRNA for differentiation of *Plasmodium* species, with amplified targets analysed by gel electrophoresis with ethedium bromide and viewed under ultraviolet light.

Although PCR is an ideal assay for malaria diagnosis, in endemic countries its use is limited for research. PCR methods are employed in drug resistance studies, strain variation, species confirmation and in diagnostic accuracy studies. A disadvantage of PCR is that it detects nonviable parasites which could mislead treatment success in clinical cases (Murray, *et al* 2009).

2.9.1.4 Acridine Orange and Fluorescence Microscopy

Acridine orange dye and fluorescence microscopy was used by Shute and Sodeman in 1973 in a hospital based study to diagnose malaria (Shute, *et al* 1973). These investigators showed the method had no benefit over Giemsa stained slide microscopy. Following their conclusions the practical use of it for malaria diagnosis subsided. In the 1990s, in the search for alternatives to conventional blood film microscopy it resurfaced again. By this time some of its early disadvantages were overcome by the development of the interface filter which could be used with standard light microscopy (Moody 2002). Several studies were conducted using the new technique which showed high sensitivity over Giemsa stain microscopy (Baird, *et al* 1992, Wongsrichanalai, *et al* 1989). Nevertheless the technique still required special filters and technician training. The other disadvantage of the method is that it stains the nuclei of leukocytes and other cells such as Howell-Jolly bodies. Technicians reading such films may confuse these other cells and has to learn to accurately distinguish them. Although it could produce results rapidly which was sensitive, it was not a suitable method for field situations and thus is used for research purpose in laboratories.

2.9.2 Methods of Assessing Test Performance

2.9.2.1Test Accuracy Measures

The conventional approach for evaluating the accuracy of a new test is to assess the index test against a standard test often considered as the 'gold standard'. The levels the two tests agree are used to express the accuracy of the index test. The accuracy is reported by the sensitivity and specificity and predictive values including likelihood ratios and diagnostic odds. A test that has high sensitivity shows the capability to identify that the condition is truly present. A test with high

specificity shows that false positivity is low. However, often test accuracy studies are not so simple or straightforward due to either lack of perfect reference standards or absence of gold standards (Reitsma, *et al* 2009). A recent review has highlighted the difficulties encountered when assessing new tests and suggested ways to overcome it which is presented later (Bossuyt, *et al* 2003b, Rutjes, *et al* 2007).

2.9.2.2 Challenges with 'Gold Standard'

A test considered for gold standard is meant to provide an error free classification. Rutjes' extensive review draws attention to the unavailability of a test without some degree of uncertainty. They refer to these situations as 'no gold standard situation'. Example of the 'no gold standard situation' can be seen from reports on RDT trials. For example several RDT accuracy trials reviewed during this study, showed the majority of RDT evaluations compared it with microscopy. It is clear that the detection thresholds differ for RDT and microscopy. RDT performance become less accurate at parasite densities <100ul while an average microscopist detect parasites at a lower threshold (Murray, *et al* 2008, Wongsrichanalai, *et al* 2007). Several factors affect the microscopic detection which includes slide quality, technician skills and epidemiology of a region may influence the detection capabilities. Thus, microscopy by definition becomes an imperfect reference and gold standard (Ochola, *et al* 2006). On the same basis PCR which differs in detection threshold and is prone to technical errors do not fit as the perfect gold standard against RDTs. Thus RDT accuracy studies are hindered by the absence of 'gold standard'. In these types of situations the suggested approach is to apply the clinical test validation concept, which is to use the best available standard test (Rutjes, *et al* 2007).

2.9.2.3 Application of Resolver Test

Several studies showed the situation with imperfect gold standard as described above would lead to bias. The direction of bias would depend on the correlation between the index and reference test (Rutjes, *et al* 2006). To overcome this many investigators use a third test against the discordant results. This again is often seen with RDT accuracy studies. The discrepant results between microscopy and the RDT get tested with a third test, called the resolver test. PCR was the commonly used resolver test which is used to decide on the 'true false-positives' from apparent false-positives or apparent false-negatives. However several methodology papers showed evidence that this type of discrepant analysis lead to overestimates of sensitivity and specificity with potential for upward bias. Many expert statisticians and authors of diagnostic research critiqued the discrepant analysis approach and argued against such analysis (Hadgu 2001,

McAdam 2000). One approach put forward to correct the potential bias with discrepant analysis was to apply the resolver test to a random sample of concordant and discordant sample (Meier 1998). This approach however had limitations on estimates and standard error calculations when a sub-sample of concordant results had to be tested. Hawkins et al (2001) demonstrated a method where a sub-sample and an imperfect resolver test can be combined while deriving estimators and standard errors. WHO-TDR suggests to use the discrepant analysis with a sample of both concordant positive and negative results when evaluating RDTs (WHO-TDR 2006). However, RDT trials that have applied this approach to test with a third superior test are limited (Bell, *et al* 2005, Hopkins, *et al* 2007).

2.9.3 Reporting of Diagnostic Accuracy

Recently malaria literature has seen a surge in publications on malaria RDT trials. However studies vary in methodology, reference standards used and often batch differences of the same product affect the results besides storage and transport conditions. These shortcomings hamper comparison of results between studies and RDT performance between products and in different epidemiological settings. Study variations apply not only to RDTs but to all diagnostic studies. Bossyt *et al* after a review of diagnostic accuracy studies pointed out poor the methodological qualities and shortcomings on reporting key information on study design and analysis (Bossuyt, *et al* 2003b). To overcome it Standards for Reporting of Diagnostic Accuracy (STARD) was initiated similar to CONSORT (Consolidated Standards of Reporting Trials) by an expert committee of diagnostic researchers (Bossuyt, *et al* 2003a).

The objective of STARD was to improve the quality of reporting while enabling detection of potential bias and appraise study findings. To serve this purpose STARD consists of a 25 point checklist divided into five main topics and subtopics. Emphasis is given to important aspects such as study title, method, study design, participant selection, use of reference standards and analysis including other points. STARD is a useful tool to ensure the quality of a study during development phase and reporting.

Similar to STARD, the members of the group also developed a tool for quality assessment of systematic reviews of diagnostic accuracy studies. Known by the acronym QUADAS (Quality Assessment of Diagnostic Accuracy Studies) it consists of 14 items structured in a list of questions that is scored against yes, no or unclear (Whiting, *et al* 2003). The items fall under three broad categories which include 1) reporting of selection criteria, 2) description of index test execution and 3) description of reference standard execution. Both STARD and

QUADAS are useful tools in assessing study qualities which in turn is helpful to judge the quality of a test.

2.9.4 RDT use malaria in pregnancy

Numerous malaria RDTs have been evaluated in areas of varying transmissions in children and general population (Causer, *et al* 2005, Farcas, *et al* 2003, Ferro, *et al* 2002, Hopkins, *et al* 2008, Palmer, *et al* 1999, Tjitra, *et al* 2001, van den Broek, *et al* 2006). However few studies have evaluated these in pregnant women, in particular as a screening tool to detect asymptomatic infections. The studies that were done were mainly in pregnant women with clinical malaria or to diagnose malaria at delivery (Leke, *et al* 1999, Mockenhaupt, *et al* 2002, Singer, *et al* 2004). Average parasite densities in asymptomatic pregnant women would be much lower than in symptomatic patients with acute malaria. Thus with detection thresholds in currently available RDTs, it may not always detect parasites in asymptomatic women. Furthermore, most of the available information is focused on HRP-2 based tests' ability to detect *P.falciparum*. One study that used pLDH based RDTs reported sensitivities of 71% for peripheral blood and 38% for placental blood compared against microscopy in Malawian women (Mankhambo, *et al* 2002). A recent study in Nigeria performed with OptiMaL® dipsticks in antenatal women reported the test inferior compared with microscopy and PCR even though the values of accuracy indicators such sensitivity/specificity were not reported (VanderJagt, *et al* 2005). Another study that used OptiMaL® dipsticks in pregnant women in Ghana found high sensitivity and predictive values to detect malaria in antenatal women (Tagbor, *et al* 2008b). Far less is known about the role of combo RDTs in detecting *P.vivax* in pregnancy. It is clear that RDT accuracy studies are needed in pregnant women and to detect placental malaria and malaria in asymptomatic women. This tool has great potential for malaria control in pregnancy particularly with the growing interest in screening and treatment.

2.10 Treatment and Prevention of malaria in pregnancy

The focus of current global malaria control is on elimination. This change of strategy comes nearly 50 years after the historic Global Malaria Eradication Programme in 1955. Since then, new research and development has provided better and effective tools for the control of malaria. These include advances in drugs and treatment policy, vector control measures with insecticide treated nets and indoor residual spraying. In moving towards control it is important to reduce the disease burden by treating clinical disease and prevention of infection. However, in

areas with the highest burden of malaria in pregnancy, the majority of pregnant women remain asymptomatic. The asymptomatic infections are known to affect the pregnant woman and her infant adversely and therefore prevention in this group is essential. In contrast, in areas with low to moderate or unstable transmission, the risk of clinical disease in those infected is more frequent. Thus, the mainstay of control in high transmission regions as seen in much of sub-Saharan Africa is intermittent preventive treatment, whereas in low or unstable transmission regions as in much of Asia the focus is on case detection and treatment.

2.10.1 Anti-malarial drugs in pregnancy

Pregnant women are more likely to develop malaria infection with complications, particularly in non-immune women. Prompt treatment therefore is essential for pregnant women with uncomplicated malaria because of the potential to deteriorate with consequences to the mother and infant which could be fatal if left untreated. To treat malaria in pregnancy considerations should be given to the choice of drugs to treat uncomplicated malaria, severe malaria and drugs to use in first trimester. The choice of anti-malarial therapy will also depend on the local pattern of drug resistance in an area. Most anti-malarial drugs are considered safe and effective for oral therapy in the second and third trimester, although information on the safety and efficacy of these drugs in pregnancy is insufficient, particularly in first trimester (WHO 2010a). The treatment of malaria in pregnancy is also compounded by HIV infections which reduces the efficacy of anti-malarial (ter Kuile, *et al* 2004).

Drugs for uncomplicated P.falciparum malaria: In the past chloroquine which was a safe, effective and cheap drug was used. However, with widespread resistance the drug has been considered useless to treat *P.falciparum* infections in most parts of the world. WHO currently recommends an ACT in the second and third trimester and quinine plus clindamycin for seven days in the first trimester (WHO 2010a). If this treatment fails artesunate plus clindamycin could be used in first trimester.

Artemisinins in pregnancy: WHO has recommended ACT therapies as first line treatment for uncomplicated malaria in pregnancy in the second and third trimester since 2006. In the first trimester, ACTs should be used if it is the only effective treatment available for saving the life of the mother. Artemisinins has a rapid action and are eliminated within a few hours. It is therefore used in combination with long acting drugs to prevent recrudescence and the development of resistance. Several combination formulations with the more potent dihydroartmesinin and its derivatives are available for use in pregnancy (WHO 2006).

With the emergence of drug resistance to *P.falciparum,* early trials of artemisinin derivatives were conducted in Asia with safe and efficacious results (Mandal PK, *et al* 2004, McGready, *et al* 1999). However, animal studies in rats and rabbits have shown that the artemisinins are embryo toxic and potentially teratogenic in early pregnancy (i.e. early to mid-1st trimester) (Clark, *et al* 2004). Studies conducted later in humans including over 1100 documented pregnancies have shown no excess adverse effects on the mother or fetus when taken in first, second or third trimester (Dellicour, *et al* 2007, Ratcliff, *et al* 2007). Since, experience with ACTs in pregnancy are limited, it is recommended that countries set up pharmacovigilance system to keep track of potential adverse events to the fetus and pregnant women when using ACTs (WHO 2006).

South-east Asian countries were the first to start using artemisinin therapy in pregnancy. Among them, Indonesia is the first country to have used the fixed dose combination of dihydroartemisinin-piperaquine (DHP) in pregnancy. Since March 2006, DHP has been the first line treatment for both *P.falciparum* and *P.vivax* malaria, including for pregnant women in the second and third trimester, in southern Papua, Indonesia. Detailed safety information was collected on 1160 women, exposed to DHP during pregnancy and it was found to be safe and more effective than other antimalarials used in this area (Poespoprodjo, *et al* 2008). In 2010, DHP was made first-line treatment for malaria in the second and third trimester pregnancy in the rest of Indonesia.

Drugs for severe malaria: In the treatment of severe and life threatening malaria which is mainly caused by *P.falciparum*, the aim of treatment is to try and save the life of the mother. In such situations the choice of treatment should be considered on a risk versus benefit approach and on drug availability. Quinine is an effective choice, although hypoglycaemia is an associated adverse effect due to hyperinsulinaemia from hyperplastic pancreatic islets (Looareesuwan, *et al* 1985, White, *et al* 1983). WHO currently recommends intravenous artesunate for treatment of severe *P.falciparum* malaria, including in the first trimester.

Drugs to avoid in pregnancy and lactation: Antimalarials used in pregnancy are safe during lactation as well. Drugs not recommended for use in pregnancy and in lactating women are primaquine and tetracycline. Primaquine has a risk of intravascular haemolysis if the mother or baby is G6PD deficient and should not be used if G6PD status is unknown (Nosten, *et al* 2007). Tetracycline use is associated with disturbances of bone growth in the fetus and teeth discolouration. Other drugs to avoid in pregnancy are halofantrine, atovaquone-proguanil (Nosten, *et al* 2001).

Drugs for P.vivax: Chloroquine is the drug to treat *P.vivax* in areas where *P.vivax* is sensitive to the drug, whereas in regions where there is resistance, an ACT with a long acting partner drug is recommended (Nosten, *et al* 2007, WHO 2010a). In many parts of south-east Asia there is multidrug resistance strains. This includes resistance to sulfadoxine-pyrimethamine and chloroquine particularly observed in many areas of Indonesia and Papua New Guinea (Asih, *et al* 2009, Baird 2004).

2.10.2 WHO recommended control policy for sub-Saharan Africa

Malaria in pregnancy is entirely preventable and treatable with appropriate and timely interventions. Under the WHO Roll Back malaria initiative a Strategic Framework for Malaria Prevention and Control during Pregnancy for the African Region was developed (WHO 2004a). This framework was adapted by 44 African countries under the Abuja Declaration in 2000 (WHO-RBM 2003). Together with WHO recommendations a malaria preventive package was established to include IPTp with SP, vector control with ITNs and to ensure effective case management of malaria illness and anaemia.

Malaria case management and anaemia: WHO recommends provision of prompt and effective treatment of malaria illness to all pregnant women in endemic areas. Parasitological diagnosis with either standard microscopy or RDT is recommended for all cases with symptoms of malaria through antenatal service. In places this is not possible the decision to initiate treatment is based on the clinical symptoms (WHO 2010a). Interestingly at the Thai-Burmese border which is an area of low malaria transmission, weekly screening with prompt treatment demonstrated a reduction in maternal malaria illness resulting in reduced maternal mortality (Nosten, *et al* 1991). However, this study showed that women who attended weekly antenatal clinic still had anaemia and low weight babies suggesting that early detection and prompt treatment prevents severe disease but not all of the insidious consequences of malaria in pregnancy in these regions.

The package also includes as part of routine antenatal care the provision of iron and folate supplementation for anaemia (which is an important consequence of malaria) to all pregnant women. There is a long standing concern about the influence of iron supplementation in populations exposed to malaria. However, as discussed earlier, there are only a few studies that have assessed iron therapy in pregnant women with malaria and in these studies a harmful effect of iron have not been demonstrated. Since iron deficiency is common in pregnancy, weekly iron supplementation with adequate malaria protection is suggested for pregnant women. Similarly,

there has been conflicting reports about the benefit of folate supplementation in malarial anaemia with concurrent use of antifolate antimalarials. One study that explored the interaction between folate supplementation and sulfadoxine-pyrimethamine in pregnant women suggest the use of internationally recommended dose of 0.4mg/day and to avoid 5mg/day dose of folate in women using IPTp-SP as it decreases the efficacy of sulfadoxine-pyrimethamine (van Eijk, *et al* 2008).

Intermittent Preventive Treatment: Historically in most malaria endemic countries chemoprophylaxis defined as weekly or monthly administration of an antimalarial was carried out with weekly chloroquine as part of the malaria eradication program of 1950s. Through the years, the inability to sustain and poor compliance together with the development of resistance to chloroquine lost momentum for this policy. New investigations therefore were carried out for a safe, cost effective regime with easy patient compliance.

In the 1990s, several trials were conducted in different countries with different regimes of sulfadoxine-pyrimethamine (SP). A trial with intermittent sulfadoxine-pyrimethamine treatment revealed that severe malarial anaemia in pregnant women decreased by 39 % (Shulman, *et al* 1999a). Similarly, trials in Malawi and Kenya with either two or three dose sulfadoxine-pyrimethamine regimens found the drug was associated with reductions in low birth weight and peripheral and placental parasitaemia (Parise, *et al* 1998, Schultz, *et al* 1994, Verhoeff, *et al* 1998). These studies lead to the current WHO recommendation of IPTp-SP in the African region. A later review of nine trials of IPTp-SP in Africa found monthly IPT-SP in HIV positive women resulted in higher birth weight and less placenta. This regimen showed no additional benefit in HIV negative women, thus suggesting more frequent dosing for HIV positive women who are not using cotrimaxole prophylaxis (ter Kuile, *et al* 2007).

Intermittent preventive treatment in pregnancy aims at clearing asymptomatic peripheral and placental malaria and to provide protection against malaria in areas with moderate to high transmission where women have considerable protective immunity and are able to control the infections. IPTp is provided to pregnant women whether or not they are known to be infected. At least 2 doses of sulfadoxine-pyrimethamine (three tablets of 500mg sulfadoxine and 25mg pyrimethamine) is recommended for IPTp, starting in the second trimester or after quickening when women visit for scheduled antenatal care. The doses of sulfadoxine-pyrimethamine should be spaced at least 4 weeks apart. Sulfadoxine-pyrimethamine is the drug of choice as it is safe, effective and can be given in a single dose regimen under direct observation. The latter trials also showed that women infected with HIV-1 had poorer response as compared to HIV uninfected (ter Kuile, *et al* 2007).Thus in areas with high HIV prevalence women could benefit with a third dose of sulfadoxine-pyrimethamine.

Since the implementation of IPTp-SP policy, many African countries have seen increasing resistance to sulfadoxine-pyrimethamine (Alker, *et al* 2004, Harrington, *et al* 2009). Despite moderate loss of parasite sensitivity with treatment failure rates of up to 50% in symptomatic children <5 years at day 14, this review by ter Kuile *et al* (2007) showed IPTp with SP remains efficacious and is currently the only WHO recommended drug for IPTp. However, with growing concerns about increasing resistance, alternative drugs are being sought. In this regard, several trials are being conducted with different drug combinations with support from the Malaria in Pregnancy Consortium to indentify at least one safe and effective alternative to sulfadoxine-pyrimethamine for use in IPTp in the African region (MiPc 2010).

Insecticide-Treated Nets: WHO proposes that pregnant women should be supplied with an ITN early in pregnancy and be encouraged to sleep under it throughout the course of pregnancy and after delivery. The WHO Pesticide Evaluation Scheme recommends the new long lasting insecticide treated nets (LLINs) should maintain protection through 20 washings or for three years.

Use of bed nets in malaria endemic regions have been an age old habit. However, insecticide treated bed nets have been introduced more recently and still coverage has not taken off in all malaria endemic regions. Insecticide treated nets provide protection by either killing and or repelling the mosquito. Initial trials in several African countries in the 1990s showed conflicting results. A later review of ITN trials and curtains has demonstrated the beneficial effects of insecticide treated nets in malaria control and infant mortality (Lengeler 2000). In pregnant women a trial with insecticide treated nets as an alternative method for drugs was first carried out in Thailand by Dolan et al, (1993) where a decreased rate of anaemia was observed with the use of insecticide treated nets (Dolan G, *et al* 1993). The bed net trials in western Kenya which is an area of intense malaria transmission, ter Kuile *et al* (2003) demonstrated in women with gravidity less than five, reductions in parasitaemia, malarial anaemia and decrease in low birth weight by 28% (ter Kuile, *et al* 2003).These studies have lead to insecticide treated nets being promoted as an important intervention in reducing the impact of malaria in high transmission areas and incorporating it into prevention of malaria in pregnancy (Nahlen, *et al* 2003). A recent systemic review of 6 trials of insecticide treated nets showed it reduced the risk of low birth weight, stillbirth and abortions up to second to fourth pregnancy and reduced placental malaria in all gravidae (Gamble, *et al* 2007).

2.10.3 Coverage of interventions for pregnancy malaria

The interventions in the above described control package are aimed to be delivered as part of antenatal care and integrated into existing services. In Africa, approximately 70-77% of women attend antenatal care at least once (Crawley, *et al* 2007, UNICEF 2009). Thus, the new malaria interventions were implemented with anticipation to have high coverage and better compliance, unlike that of previous chemoprophylaxis era. Nevertheless, after nearly a decade of having a malaria preventive framework, a current analysis highlighted that coverage with ITNs and IPTp was inadequate (van Eijk, *et al* 2011). For example, in 39 of 47 countries that had a policy for IPTp, one in four women received at least one dose of treatment in 2007. Similarly, 17% had data for ITN although 45 of 47 countries had a policy for ITN distribution. Several factors have been identified to contribute to poor uptake (Crawley, *et al* 2007). Some countries such as Uganda have conducted community IPTp trials using health workers and drug distributers and found uptake reached 67.5% to 88.5% of pregnant women (Mbonye, *et al* 2007). A disadvantage of this approach is that it may affect antenatal attendance and this was observed in a community-based study of sulfadoxine-pyrimethamine distribution for IPTp in Malawi (Msyamboza, *et al* 2009).

2.10.4 Other methods of malaria control

2.10.4.1 Vector control

Indoor Residual Spraying: Indoor residual spraying with insecticides kills mosquitoes that come in contact with the sprayed surface. It also has a spatial repellency and an irritant effect on malaria vectors, which limit human –vector contact. There are 12 insecticides used for vector control and DDT is one, which has a long residual effect. Although IRS is better suited for focal endemic and epidemic prone areas, it is currently one the three main interventions of the Global Malaria Programme by WHO. Epidemiological data that evaluated toxicity of DDT including the presence of it in human milk and hormone modulating effects did not support a significant toxic effect (WHO 2007). However, the effect of indoor residual spraying on pregnant women has not been evaluated. Pyrethroids is the insecticide commonly used to treat bed nets and are found to be safe and well tolerated by pregnant women with no evidence of toxic effect to the fetus (Menendez, *et al* 2007).

Insect Repellent: The use of DEET (diethyltoluamide) in pregnant women was considered safe and a reduction of exposure to mosquito bites was observed. Another insect repellent, 20% diethylbenzamide showed a non-significant reduction of 28% in the incidence of *P.falciparum* in a

trial conducted in the Thai-Burmese border (McGready, *et al* 2001). There is no data from the African region on the use of insect repellents.

2.10.5 Control Policy for malaria endemic Asia

Control strategies for malaria in pregnancy in Asia have made little progress in recent times, despite a growing awareness of the importance of the burden of malaria in pregnancy in this region. Case management is the mainstay of treatment with ACTs for first line treatment in the second and third trimester according to WHO recommendations. On the other hand, prevention strategies are limited to inconsistent distribution of ITNs and passive detection of febrile cases. The lack a regional malaria preventive package perhaps reflects the challenge of developing a preventive policy due to the variability of transmission between and within countries as seen in India and Indonesia, multidrug resistance and existence of both *P.falciparum* and *P.vivax*. Thus unlike the African region a one-size fits all prevent package may not suit the Asian region.

Systemic reviews of treatment and prevention of malaria in pregnancy show an imbalance between research on treatment studies and studies on prevention in this region. The majority of treatment studies of malaria in pregnancy come from the Thai-Burmese border (Orton, *et al* 2008). In contrast, only one out of the 16 drug-based prevention trials was conducted in Asia (Garner and Gulmezoglu 2006). Similarly out of the five ITN in pregnancy trials only one was in Asia and again this was conducted in Thailand (Gamble, *et al* 2006). Interestingly there has been no IPTp study in this region, evident by the recent systemic review on IPTp trials (ter Kuile, *et al* 2007). Although the potential benefit from IPTp may be limited in the Asian region due to low transmission, IPTp trials are warranted for an evidence-based policy in the region.

Indonesia is one country in Asia that has adopted a national policy for prevention of malaria based on systematic screening and treatment in pregnancy, referred as single screening and treatment (SSTp) (Personal communication Drs Syaffrudin & Hawley). The concept of SSTp is to screen all pregnant women for malaria, with microscopy or RDT. Women who tests positive for malaria, are treated with diydroartemisinin-piperaquine (DHP) according to national policy, except in first trimester when they receive quinine. At first antenatal visit, all pregnant women also receive a LLITN. On subsequent antenatal visits, only symptomatic women are screened. A limitation of this policy is the lack of prevention of re-infection in later stage of pregnancy. The Thai-Burmese border has had a long practice of weekly screening and treatment, which has shown to reduce the burden of clinical disease. However, this approach may not suit routine practice where women are used to attending 3-4 times for antenatal care as scheduled visits.

2.10.6 New strategies for prevention and treatment

2.10.6.1 Intermittent screening and treatment

More recently, there has been a growing interest in 'intermittent screening and treatment in pregnancy' (ISTp). The concept of ISTp is to screen pregnant women with an RDT every 4-8 weeks using the same schedule recommended for focused antenatal care. The RDT positive women receive treatment with a long acting ACT to clear infections while providing additional post-treatment prophylaxis of up to 4-6 weeks, thereby minimizing the chance of new infection becoming symptomatic. The potential advantage of this method is that drug exposure is minimized and restricted to parasitaemic women, due to women with a positive result receive treatment. The potential disadvantage is that low-density infections could be missed and that it is more complex and likely to be more expensive to implement than IPTp. One trial conducted using this strategy in Ghana, in an area of moderately high transmission found that ISTp-SP or Amadioquine plus artesunate was a safe and effective strategy (Tagbor, *et al* 2010). This method of ISTp is likely to be suitable for the Asian region where transmission is low. Furthermore, this strategy would be a suitable alternative for the African countries where the incidence of malaria has decreased with improved control measures.

2.10.6.2 Seasonal IPT

The benefit of seasonal IPT to pregnant women has not been explored. Trials with IPT in Mali and Senegal conducted in older children during the malaria season showed to be effective in reducing the malaria burden (Dicko, *et al* 2008, Sokhna, *et al* 2008). This approach would be useful for the prevention of malaria in pregnancy in areas with highly seasonal transmission, as found in many parts of Asia such as in parts of India. The practicality of this approach and cost benefit should be assessed for formulating policy.

2.10.7 Control and Prevention of malaria in pregnancy in India

A national drug policy was first formulated in 1982 in India with periodic revisions. The most recent revision was in 2010 which includes a policy drafted in view of the increasing resistance to chloroquine. Chloroquine has been the drug used in India as first line treatment of all malaria cases for decades.

2.10.7.1 Treatment Policy

The current policy is developed with a focus on early detection and treatment. Accordingly, parasitological diagnosis with either microscopy or RDT is recommended for all clinically suspected cases of malaria. The recommendation for uncomplicated *P.vivax* cases in pregnancy is to treat with chloroquine for 3 days. In cases of *P.falciparum* the recommendation for women in second trimester onwards is treatment with artesunate for 3 days with single dose sulfadoxine-pyrimethamine on day 1. Quinine is the drug of choice for first trimester treatment (NVBDCP 2010).

Whenever parasitological diagnosis is not possible due to non-availability of prompt microscopy or RDT, all suspected malaria cases are to be started on chloroquine after smear is made. Once the results of microscopy are available, appropriate treatment as per the species, is to be administered. Anecdotal evidence suggest the use of parentral arteether, an oil based artemisinin derivative manufactured in India is commonly used in trimesters for uncomplicated malaria. Treatment of severe malaria cases is left to the discretion of the physician with guidelines to use any of the artemisinin derivatives or quinine as appropriate.

2.10.7.2 National Control Policy for malaria in pregnancy

There is no specific control policy for pregnant women drafted in the newly revised national drug policy. Chemoprophylaxis is advised in high *P.falciparum* endemic areas in the country (NVBDCP 2010). The use of personal protective measures with insecticide treated nets and insect repellent is encouraged. While a MiP control package as exists for Africa is lacking in the region, passive case detection, according to recommendation by SEARO/WHO is implemented. The lack of a specific control policy for malaria in pregnancy might be due to lack of awareness of the problem of MiP by policy makers or the limited MiP data from India has not captured sufficiently the scale of the MiP in the country. This suggests the need for evidence based data to formulate an appropriate policy in India. Furthermore, this is not a situation specific to India, but refers to the whole south-east Asian region (Greenwood, *et al* 2007, Menendez, *et al* 2007).

2.11 Malaria in India and Madhya Pradesh

India is a diverse country divided into 29 states, 7 union territories with 1.1 billion population living sub classed by tribes and castes, dominated by 18 different languages and 8 major religions. Of this diverse population 982 million live in malaria endemic regions mainly spread in the forested and foothill areas in north eastern and central India. The large susceptible

population and year round occurrence of malaria, make India the largest contributor of malaria in south-east Asia region (60%). According to SEARO-WHO in 2008 there were 1.5 million malaria confirmed cases and 1061 deaths accounted for malaria in India (SEARO 2008). 60% of these cases were from five states considered to have higher malaria prevalence and therefore higher burden: Orissa, Jharkhand, Chattisgarh, West Bengal and Madhya Pradesh. In contrast to the national and WHO figures of malaria mortality, a recent study which used verbal autopsy recorded that 3.6% of deaths in age less than 70 years were a result of malaria, and this figure scaled up to national level estimated 205,000 malaria attributed deaths per year (Dhingra, *et al* 2010, Hay, *et al* 2010). Although Dhingra and colleagues have attempted to include deaths that occur in the community, their findings might have overestimated mortality and have been critiqued due to their use of verbal autopsy. Several authors have commented on the uncertainty of Dhingra and colleagues estimates with suggestions for further validation, highlighting the limitation and bias of using verbal autopsy (Deonarine 2011, N. K. Shah, *et al* 2011, Valecha, *et al* 2011). Nonetheless the national malaria attributable mortality figures are also likely to be underestimates (Hay, *et al* 2010, Snow, *et al* 2005).

Malaria programmatic activities: The government unit responsible for malaria programmes both at central and the state level comes under the directorate of the National Vector Borne Disease Control Programme (NVBDCP). The NVBDCP is in charge of formulating treatment policy, provide technical support and carry out malaria monitoring and evaluation. To implement these activities District Malaria Offices headed by a District Malaria Officer have been established (NVBDCP 2008). At the district level the NVBDCP functions jointly with the district hospitals, primary health centres and their staff carry out passive case detection. In addition out-reach services are provided by health workers and community volunteers like malaria link workers and 'Accredited Social Health activists' (ASHAs) through fever treatment depots and drug distribution centres. This large decentralized surveillance system is supported by the Indian Council of Medical Research (ICMR) and NIMR and currently includes the target of reducing the 2000 malaria mortality and morbidity figures by 50% by 2010 (SEARO-WHO 2007).

The NVBDCP surveillance scheme which originated from the earlier National Malaria Eradication Programme mainly focuses on fortnightly passive case detection. They follow the 6 traditional malariometric indices of malaria monitoring which are percent *P.falciparum* (%Pf), annual blood examination rate (ABER), annual *falciparum* index (AFI), annual positivity index (API) slide positivity rate (SPR) slide *falciparum* rate (SFR) and deaths due to malaria (NVBDCP 2008). However, this extended surveillance system does not focus on pregnant women or under five children resulting in deficient data on these two high risk groups. In addition, the private

health sector which provides service to a large part of the population does not get covered under the NVBDCP scheme (SEARO-WHO 2007). Although the programme has many strengths it is often critiqued for the shortcomings and the gap between reported and estimated cases. For example, the NVBDCP reported 1.9-2.5 million cases in 2003 and 2007, whereas the estimates by others were between 15 million to 80 million cases (Sharma, *et al* 2006, Korenromp 2005). Furthermore, as reported by Dhingara et al, 86% of malaria deaths occurred outside formal health facilities, and thus may have not been recorded in programmes under NVBDCP. Although there is wide variation in these estimates it is clear that malaria poses a public health problem in the endemic states.

2.11.1 Epidemiology

2.11.1.1 Malaria Endemicity and Transmission Variation

Malaria epidemiology in India varies widely between states, driven by differences in the ecosystem, geography, climate and socio-ethnic conditions. Six *Anopheline* vectors transmit malaria in India, of which *An.culicifacies* and *An. fluviatilis* are the main vectors found in Madhya Pradesh (NIMR 2010c, Service 2002). Both vectors bite human and domestic animals mainly at dusk and dawn and rest indoors and outdoors after feeding. The two *Plasmodium* species present in Madhya Pradesh are *P.falciparum* and *P.vivax* with a seasonal transmission pattern and affecting all age groups (Singh, *et al* 2000).

India classifies malaria regions by the distribution of incidence using the annual parasite index (API) expressed as malaria cases per thousand population. Accordingly, most of malaria endemic areas fall under API <2 reflecting low transmission, while the states considered as highly malarious regions has an API >5. On this basis Madhya Pradesh is classed among the high endemic states contributing 6% of national malaria cases. However a recent estimate showed API =1.28 indicating that approximately 95,000 malaria cases occurred annually in Madhya Pradesh (Das Gupta 2007).

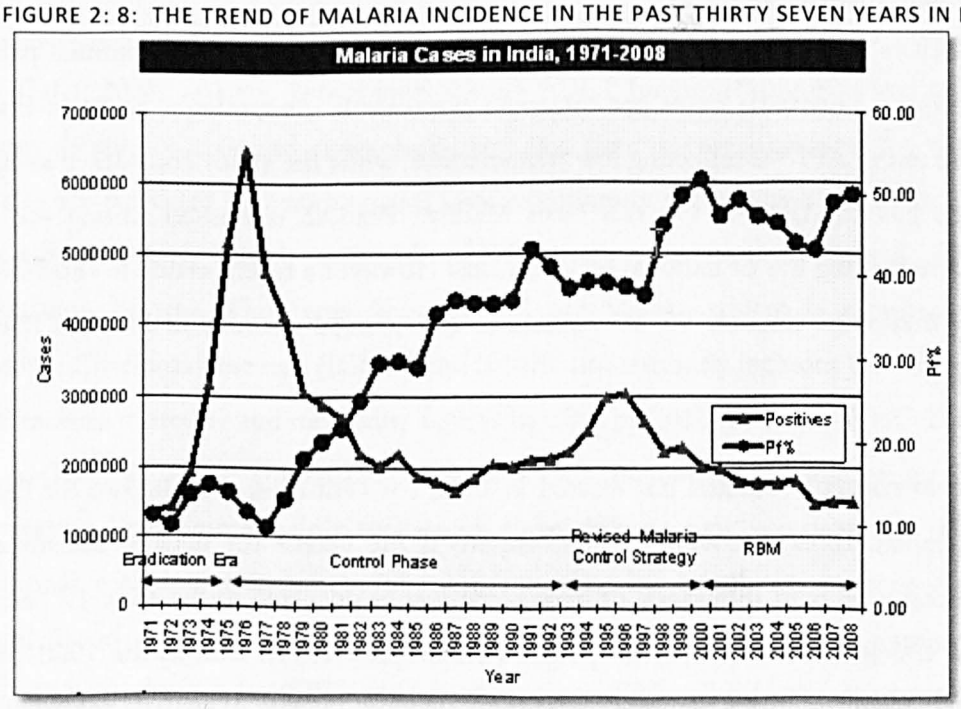
History of Malaria control: Malaria has existed in India for centuries, even before Sir Ronald Ross in Secunderabad, India discovered that mosquito is the vector for malaria transmission. Epidemics were common with thousands of lives being lost as occurred in the early 19th century Punjab epidemic which killed over 300,000 people (Christopher 1911). But, in the 1960s under the Global Malaria Eradication Programme, India saw a marked decline of malaria reaching near elimination with API 0.13 (Sharma, *et al* 1986). At this point there arose several setbacks to the

programme including interruption to spraying and increased resistance to DDT. With it although the incidence became lower than before there was a significant re-emergence in late 1970s with reports of several epidemic breakouts and reaching a static stage from mid 1990s onwards (Figure 2.6)(SEARO 2008)

2.11.1.2 Shift in Plasmodium Species

Over the past decade the relative prevalence of *Plasmodium* species has shifted with estimated *P.falciparum* cases reaching 50% in 2008 (Figure 2.9). Retrospective data from Madhya Pradesh collected over a 15 year period from 1986-2000 showed a relative decline in *P.vivax*, in favour of *P.falciparum* (N. Singh, *et al* 2004). Observations in Calcutta showed similar species shift from *P.vivax* to *P.falciparum* with *P.vivax* declining from 50% in 1984 to 20-30% in 1993-1997(Chattopadhyay and Sengupta 2000). While changes in ecological factors could contribute to the species shift, it is a likely consequence of the spread of chloroquine resistance of *P.falciparum*, while *P.vivax* remains sensitive to the drug. This hypothesis seems supported by an overlap in areas that show this shift to *P.falciparum* and those that show high levels of chloroquine resistance. While with this shift the burden related to *P.falciparum* can increase, there are reports from parts of India on cases of *P.vivax* with severe disease manifestations (Kochar, *et al* 2009, Kochar, *et al* 2005). Thus both species are likely to contribute significantly to malaria morbidity and to mortality.

FIGURE 2: 8: THE TREND OF MALARIA INCIDENCE IN THE PAST THIRTY SEVEN YEARS IN INDIA



Source: malaria situation in India SEARO-WHO

2.11.1.3 Season, environment and climate Change

Malaria is endemic in most parts of the country except in the areas above 1800m and low coastal regions. In areas where malaria exists the transmission intensity is variable between forested areas, desert and urban regions. For example a higher incidence of *P.falciparum* malaria cases were documented from forested villages of Chhindwara in Madhya Pradesh as well as in the forest areas in Orissa compared to the regions of hill plains and urban areas (Sharma, *et al* 2006, Singh, *et al* 2003). These variations indicate the epidemiological differences within the country related to environment and climatic conditions.

Malaria is closely linked to climate with temperature and rainfall influencing transmission. In many parts of India, like in Madhya Pradesh a seasonal transmission pattern is seen (Singh, *et al* 2000). The seasonality depends on the monsoon rainfall and varies between years, based on El Nino and La Nina patterns (NIMR 2010b). Studies have shown an association with weather oscillations and outbreaks and it is considered useful in predicting malaria epidemics (Bouma, *et al* 1994). The historic malaria epidemics that occurred in the Punjab were recently explained in relation to the El Nino effect (Bouma, *et al* 1996). Another study that included 123 years of weather data in Rajesthan found malaria outbreaks corresponded to the flood years (Akhtar 2010). Evidence from the outbreak patterns together with recent climate models predicting spread of malaria into new areas imply that more people would be affected by malaria in the coming years (Hay, *et al* 2004, Reitera, *et al* 2004). Furthermore with the current projections of global climate warming and changing weather patterns, changes to species distribution and transmission pattern could be expected in this region.

2.11.2 Effect of Economic Development

The rapid economic growth with large scale development projects in India has lead to changes in the ecosystem with clearing of forests and population migration. As a consequence, changes to prevailing vectors and increase in malaria incidence have occurred in parts of the country. These effects were further enhanced by population change with influx of migratory workers. A notable example is the large Narmada river hydro-project and the construction of the Bargi-dam in Jabalpur district which resulted in a 32 fold increase in SFR, an increase in the vector *An. fluviatilis* and a malaria epidemic outbreak in 1996 with 100s of fatalities (Singh, *et al* 1999b). Historical evidence also showed during the railway construction and development of the dry docklands in Bombay (Mumbai) in British India there was an increase in malaria in these areas related to labourers migrating from different parts of the country (Kerr 1983). Besides, an increased incidence of malaria with increase in population growth was observed among tribal populations in Betul region of Madhya Pradesh (Singh, *et al* 2006).These suggests that disruption

of ecosystem, human migration, population growth together with climate change could increase the burden of malaria in the future.

Economic burden: As early as 1930s Sinton in his treatise 'What malaria costs India' highlighted the economic burden of malaria in India. Taking the then average monthly earning of Rupees 7.5 by a productive age adult and 15 days of work lost per man year Sinton estimated rupee 1237 lacs ($27.5 million) of lost wages to the community in 1933 (Sinton 1935). A 1998 estimate based on a conservative approach calculated 1.86 million years of disability adjusted life years (DALY) lost to malaria in India (Yadav, *et al* 1991). A cost benefit assessment for control found net savings at US$ 1,431 million inferring each rupee invested in the malaria control program would provide a return of rupees 19.7(Kumar, *et al* 2007). Improved estimates of disease burden and malaria control can expect a higher aversion in the loss of DALYs.

2.11.3 Drugs and Insecticide Resistance

2.11.3.1 Antimalarial drug resistance

Chloroquine remained the drug of choice in India until policy changed recently to ACTs in chloroquine resistant areas. Chloroquine resistance was first reported in 1973 from field isolates in Assam (Sehgal, *et al* 1973). Since then other regions reported resistance of *P.falciparum* to chloroquine, indicating the problem was widespread. A 25% failure rate for chloroquine against *P.falciparum* was reported in a review on efficacy of anti-malarials throughout India (Biswas, *et al* 2003, Naman K. Shah, *et al* 2011). Two *in vivo* efficacy studies conducted in Madhya Pradesh, excluding pregnant women showed a 31% parasitological treatment failure rate by day 28 (ICMR 2005).

Although chloroquine resistance occurs mainly in *P.falciparum* there are reports of resistance in *P.vivax* from different regions in India. The initial chloroquine inefficacy studies in India were case reports (Garg, *et al* 1995). Development of resistance to chloroquine raises concern particularly with recent findings of severe disease manifestations with *P.vivax* when chloroquine remains the first line treatment for *P.vivax* malaria. Further systematic studies conducted in West Bengal and Orissa which are regions with chloroquine resistant, *P.falciparum* found 100% cure rate, but follow up time was insufficient and restricted to 7 days only (Nandy, *et al* 2003). A more recent study which included three *P.vivax* predominant states also observed 100% parasite clearance by day 28 verifying suggesting good chloroquine sensitivity of *P.vivax* (Valecha, *et al* 2006).

Chloroquine resistance to *P.falciparum* is associated with mutation in K76T codon in plasmodium falciparum chloroquine resistant transporter (*pfcrt*) gene. While the *pfcrt* K76T

mutation prevalence was high (96-100%), Indian field isolates were identified to have high genetic variations of the *pfcrt* gene (Vinayak, *et al* 2003). Additional studies have also found progressive increase in mutations in *pfcrt* and in multidrug resistance gene 1 (pfmdr1)(Das, *et al* 2007). These findings differ from the global finding of low genetic diversity found in the evolution of chloroquine resistance in *P.falciparum.* Indian investigators imply that the high diversity suggests that *pfcrt* in Indian parasites might be in a phase of 'genetic reconstruction' which is a concern as it may lead to faster development of resistance to new drugs.

2.11.3.2 Insecticide Resistance

Dichlorodiphenyltrichloroethane (DDT) was the insecticide used in the early eradication program, although as early as 1959 reports suggested resistance (Sharma and Mehrotra 1986). Subsequent programs relied on insecticides such as hexachlorocyclohexane (HCH)-deildrin and Malathion but vectors developed resistance. The two major vectors *An.culicifacies* and *An fluviatilis* responsible for malaria transmission in Madhya Pradesh are reported to be resistant to most insecticides including DDT and is proving to hamper malaria control through indoor spraying (NIMR 2010a).

2.11.4 Malaria in Pregnancy in India

A summary of 5 hospital based studies reviewed for adverse effects due to malaria in pregnancy in the mother and infant are presented in the Table 2.3. Although these studies were from different regions what is common to all the reports are significant infection with *P.falciparum*, maternal anaemia, and fetal consequences such as stillbirths, abortions and preterm deliveries. Four studies described complications in the pregnant woman with cerebral malaria and all the five studies reported maternal deaths. The study in Madhya Pradesh also reported that anaemia was significantly more common in pregnant women with either *P.falciparum* or *P.vivax* than in non-infected pregnant women or infected non-pregnant women and birth weights of infants born to infected mothers were lower than those of non-infected mothers. All the studies reported higher malaria frequency in primigravidae than in multigravidae. The gravidity effect observed in these studies might be related to the selection of only symptomatic study population and may not reflect malaria frequency in general pregnant population i.e. a combination of symptomatic and asymptomatic pregnant women.

Community based studies on malaria in pregnancy has been reported less frequently in India. One study from Orissa, a region highly endemic for malaria reported adverse birth

outcomes were more frequent in infected women than in non-infected women (Das 2000). Similarly, a community-based study of tribal population in Madhya Pradesh found that a higher proportion of *P.falciparum* malaria, abortion and stillbirths occurred in febrile primigravidae (ICMR 2004, Singh, *et al* 1998). Other community-based malaria in pregnancy studies is during outbreaks and suggests pregnant women have more severe *P.falciparum* associated adverse effects than non-pregnant women. These studies were all on febrile symptomatic women. There is a dearth of information from population based asymptomatic pregnant women. A study that included both symptomatic and asymptomatic women conducted recently in Jharkand state showed 1.8% prevalence in women attending antenatal clinics and 1.7% peripheral and 2.4% placental parasitaemia in women delivering in health facilities (Hamer, *et al* 2009). Although malaria prevalence was low, surprisingly the study also reported primigravidae and secundigravidae and women who reported a history of fever in the week prior to delivery had a higher risk of malaria. A study that modelled malaria in pregnancy burden based on prevalence reported from past studies estimated there could be 220,000 malaria in pregnancy cases per year with 1000 maternal deaths and 95,800 lost fetuses occurring in Madhya Pradesh (Diamond-Smith, *et al* 2009).

Studies on HIV-1 and malaria in pregnancy from India are deficient. Madhya Pradesh is considered a low HIV prevalent area in India with 1.7% prevalence reported in STD clinics and zero in antenatal clinics in 2007(HIV-Statistics 2007). There is no publications available onHIV-1 malaria interaction in pregnant women from Madhya Pradesh.

TABLE 2: 3: SUMMARY OF HOSPITAL BASED STUDIES IN PREGNANT WOMEN IN 5 DIFFERENT STATES OF INDIA

STATE	MADHYA PRADESH	RAJESTHAN	GUJRAT	GUJRAT	CHANDIGARH
Author/year	N Singh /1999*	Kochar DK /1999	Maitra N / 1993	Nair LS / 1993	Sholapurkar/ 1988*
Study period	1992-1995	---	1990-1991	1987-1988	1985
Sample (n)	2127	45	445	322	78
Predominant species	Pf (11%)	Pf	Pf (97%)	Pf (62%)	Pf (1.4 incidence)
Maternal anaemia (severe)/	++	20%	22%	7.9%	60%
Cerebral malaria	6.9%	75% (3/4)	1.1 (5)	--	7%
Maternal mortality from malaria	1.2%	41%	3.3% (15)	33% (4/12)	4%
Abortions	n=3	11%	31%	10.7%	5%
Stillbirths	n=2	---	---	10.7%	--
Preterm/prematurity	---	20%	--	--	9%
Low birth weight	++	--	--	++	--

Note: *studies reported on *P.vivax*, not shown in this table, ++ indicates parameter was mentioned, but values for proportions were not stated.

Chapter 3

Study Design and Methods

"But I was thinking of a way To multiply by ten, And always, in the answer, get The question back again"
-Lewis Caroll, Adeventures of Alice in Wonderland-

3.1 Introduction

The study aimed to assess the burden of MiP within a relatively short period of time and at relatively low cost. The US based CDC, Atlanta had compiled a Rapid Assessment Package for evaluating the burden of MiP in Africa. It was later adjusted for use in Asia and South America and developed into a WHO supported guideline for conducting MiP burden surveys. The rapid assessment methodology which is based on the principle of timely focused surveys aimed at gathering information required for needs analysis for policy makers, and together with the limited budget the methodology was chosen for this study.

This chapter provides an overview of the methodology. Thereafter it describes the study area and population, study design and details of the study procedures. Finally, the statistical methods used for the different analyses are outlined.

3.2 Rapid Assessment Package

The rapid assessment package consists of 10 modules and a set of tools containing both qualitative and quantitative questionnaires. They are as follows:

1. Antenatal clinic survey (ANC)
2. Delivery unit survey (DU)
3. Severe Malaria Disease Surveillance (DS)
4. Client exit survey
5. Antenatal Clinic Facility Assessment
6. Antenatal Clinic Health-Care Worker Observation
7. Focus Group/ Interviews with Health-Care Workers
8. Focus group/ Interviews with Trained Birth Attendants
9. Focus group/Interviews with recently/ currently pregnant Women
10. Focus group/ Interviews with Key Informants

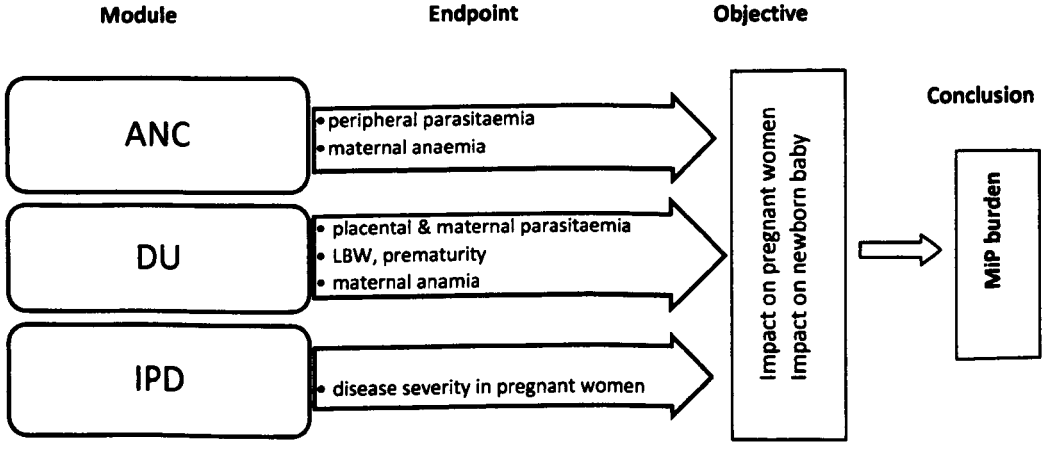
The overall package provides a standardised guidance to obtain data, allowing comparison of results between regions of varying malaria transmissions. The magnitude of the burden of MiP is assessed with the antenatal care (module 1), delivery unit (module 2) and disease surveillance tools (module 3). Coverage of interventions and sources of care are assessed with the facility (modules 4-7) and client dependent material, together with health care observation tools (modules 6-10). In conducting a rapid assessment survey, for each region or country, a minimum of 3-5 antenatal clinics and 2-3 delivery units are recommended to be included (Parise, *et al* 2003).

However, depending on the available resources, logistics and objectives, it is possible to vary the individual modules.

Selection of Modules for the Study

Three core modules are structured to obtain data on the impact of malaria on the mother and baby, which is essential to establish the burden (Figure 3:1). In this survey, the 3 recommended core modules and minimum number of sites (3 ANC and delivery units) were used because of limited funds. Before starting the survey, these tools were adjusted to incorporate questions to capture variations in the Indian socio-cultural and ethnic background, malaria treatment and control policy and transmission pattern. The modifications were adjusted after a pilot study of the rapid assessment methodology in Jabalpur in 2004 by the investigator (Ahmed 2004, MTrop dissertation).

FIGURE 3: 1: RELATIONSHIP BETWEEN STUDY MODULES & STUDY OBJECTIVES



3.3 Study Method (general)

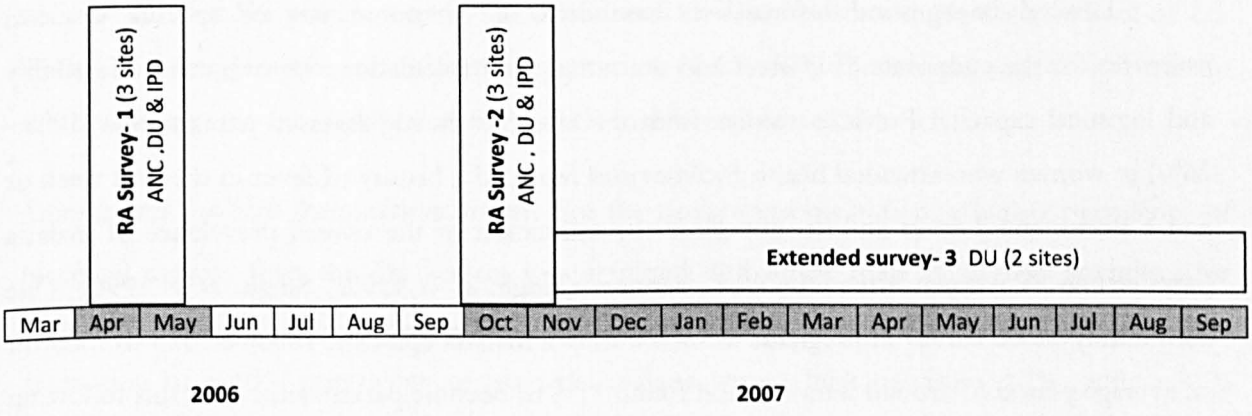
3.3.1 Study Design

The study was conducted in two phases (Figure 3:2):

1. The rapid assessment burden survey was conducted in the antenatal, delivery and inpatient units of three hospitals. It consisted of two 6-week cross-sectional surveys corresponding with the anticipated peak transmission season and low season.

2. After completion of the second rapid assessment survey, the study was continued to complete a full year of data collection. This was done in the delivery units in Katni and Maihar, the two rural sites for a further 46 weeks. Because of the limited budget, the ANC and IPD modules and Jabalpur, the site where almost no transmission was recorded in the peak season were excluded in the full year data collection.

FIGURE 3: 2: DIAGRAM SHOWING SURVEY PHASES



***Note**: shaded months shows when surveys were conducted*

3.3.2 Study outcomes

Antenatal Module

- *Primary outcome*: Prevalence of maternal peripheral parasitaemia by *plasmodium* species.
- *Secondary outcome*: Prevalence of mild/moderate maternal anaemia (Hb <11g/dL & Hb <9g/dL)

Delivery unit Module

- *Primary outcome*: Prevalence of placental malaria by *plasmodium* species.
- *Secondary outcome*: Prevalence of adverse pregnancy outcomes (low birth weight and preterm delivery) and prevalence of maternal parasitaemia and anaemia.

Inpatient Disease Surveillance Module

- A description of manifestation of malaria illness in pregnant.

3.4 Sample size selection for the overall survey

Sample size calculations were done using the StatCalc population survey module in EpiInfo 3.01version (2003). The following parameters were used for calculations: N baseline population = 1,000,000, α=0.05, Design effect = 1.5 to correct for cluster design (clinics). The primary consideration used to determine the sample size was the desired precision and the point estimate of the prevalence of malaria among pregnant women. However, an acceptable level of precision for other parameters of interest such as maternal anaemia, low birth weight and prematurity were also considered in calculating the sample size.

Limited background information hampered the determination of specific a priori estimates for the endpoints of interest and the sample size calculations were driven by feasibility and logistical capacity. Previous studies from the area has mostly assessed parasitaemia (18% - 26%) in women who attended health facilities and reported a history of fever in the past week or had a documented fever and do not allow the estimation of the overall prevalence of malaria parasitaemia in women attending ANC services (Singh, *et al* 1999a, Singh, *et al* 1996). One community-based survey in pregnant women during a malaria epidemic followed 325 women for an average period of around 2 month and found 11% to become parasitaemic over this follow up period (study conducted for 10 months between April 1995 and January 1996) (Singh, *et al* 1998).

The variable considered for the design effect was anaemia (Hb <11g/dL). Based on previous reports, it was assumed that a design effect of 1.5 was acceptable.

3.4.1 Rapid Assessment Surveys

3.4.1A: Sample size for Antenatal Clinics

Sample size for low transmission survey: A low prevalence was expected during the dry season survey (referred hereafter as survey 1). On this assumption a sample size of 800 women would allow estimation of a prevalence of 3%-15% maternal parasitaemia, (wide-range was assumed due to lack of specific data) with a precision of ±3% with a 95% confidence level and corrected for a design effect of 1.5. Taking into account the overall monthly antenatal attendance of 1050-1300 women at the antenatal facilities the sample size of 800 women from the three sites was considered an achievable target in six week period (Table 3:1).

Sample size for high transmission survey: A higher prevalence was expected in the post monsoon period (referred hereafter as survey 2). A sample size of 1100 women would allow estimation of a 20% prevalence of maternal parasitaemia with a precision of ±3% with a 95% confidence level

and corrected for a design effect of 1.5. Based on the monthly attendance figures, a total sample size of 1100 was an achievable target from the three sites in six weeks.

3.4.1B: Sample size for Delivery Unit

Sample size for low transmission survey: Similar to the antenatal survey, for the dry season the proportion of women with placental parasitaemia was assumed would be as low as 3%-5%. On this assumption a sample size of 400 would allow estimation of 3% prevalence of placental malaria with a ±3% precision and 95% confidence level and corrected for design effect of 1.5. Taking into account the monthly attendance rates of 550-675, the sample size of 400 was an achievable target in six week period from all three delivery units (Table 3:1).

Sample size for high transmission survey: For the post monsoon period, a higher prevalence of placental malaria than the dry season was assumed, but lower than in women attending for antenatal care in the same period. On this assumption, a sample size of 600 would allow estimation of a 10% prevalence of placental malaria with a high precision ±3%, with a 95% confidence level corrected for design effect of 1.5. Based on the total monthly attendance, 600 was an achievable target from all three units during the six week period.

TABLE 3: 1: POTENTIAL SAMPLE SIZE CAPACITY AT THE STUDY HEALTH FACILITIES

	Maihar	Katni	Jabalpur	Total
Antenatal Clinics:				
Attendance /month	150-200	400-500	500-600	1050-1300
Enrolment/mo (75%)	113-150	300-375	375-450	787-975
Enrolment 6weeks	170-225	450-563	563-675	1181-1463
Delivery Unit:				
Attendance/month	100-125	200-250	250-300	550-675
Enrolment/mo (75%)	75-94	150-188	188-225	413-507
Enrolment 6 weeks	113-141	225-253	282-338	620-732

Note: This shows the average monthly attendance at ANC and delivery unit at the 3 health facilities. The calculations were made on the assumption that 75% of women attending ANC and DU would meet the inclusion criteria and would agree to participate.

3.4.2 Year Round Survey

Sample size for enrolment at two delivery units

To calculate the sample size for the year round survey in the delivery units, findings of the rapid assessment surveys in combination with the anticipated number of deliveries were used. The wide variation in the anticipated maternal malaria prevalence from approximately 2% in the dry season to 6% in the post rainy season was taken into account. A sample size of 1500 would allow an estimation of 2-8% prevalence of placental malaria with precision ±2%. It would also allow estimation of other parameters of interest such as low birth weight, prematurity and maternal anaemia with a precision of ±3%. This sample size was considered an achievable target for the two sites, (Katni and Maihar) during the 46 week extended period.

3.5 Eligibility Criteria

All pregnant women ≥15 years were eligible for enrolment. The legal age for marriage in India is 18 years and above. However, in India some women younger than 18 years are married and run households. The aim was to include this vulnerable group of adolescents to enable generalization of results to the overall population of pregnant women. Because the community considers women less than 18 years old who are running independent households emancipated minors, they were allowed to sign their own consent.

Inclusion criteria for women at ANC

Pregnant women who met the following criteria on presentation to the antenatal clinic were enrolled:

- ≥15 years of age.
- Had felt quickening or gestational age was ≥14 weeks as determined by fundal height (14 week gestational cut off was selected for uniformity with previous rapid assessment studies in Africa and to avoid complications of drug use other than the national recommended policy in asymptomatic first trimester cases that detects positive results).
- Agreed to comply with study procedures.
- Able to provide informed consent.

Inclusion criteria for women at DU

Women who presented for delivery and met the following criteria were enrolled:

- ≥15 years of age.
- Agreed to comply with the study procedures.
- Able to provide informed consent.

Inclusion criteria for Inpatient surveillance

Women admitted to the inpatient wards during the 6 weeks of survey who met the following criteria were enrolled.

- Both pregnant and non-pregnant women aged 18-49 years (this study sample includes both pregnant and non-pregnant. Non-pregnant women below 18 years are not considered emancipated minors, and therefore 18years was chosen to keep uniformity of consenting age in both groups).
- Had a confirmed malaria diagnosis or at admission were being treated presumptively for malaria.
- Agreed to participate and comply with study protocol.
- Able to provide informed consent.
- For women who were delirious or unconscious on admission consent was obtained from the husband or accompanying adult.

General Exclusion criteria

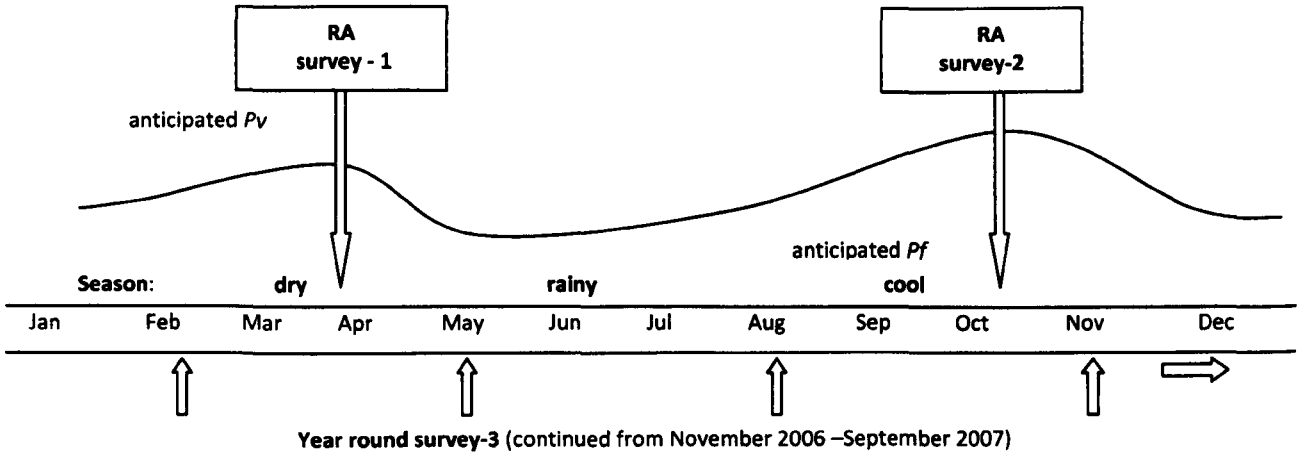
- Women with a mental disability unable to provide informed consent

3.6 Study Timing

Rapid Assessment Surveys: Women attending the antenatal clinics, delivery unit and inpatient wards were enrolled simultaneously from all the three health facilities. Survey 1 was conducted in the dry months of April-May 2006 for 6 weeks when *P.vivax* was expected to dominate (Figure 3:3). Survey 2 was conducted in October-November 2006 for 7 weeks shortly after the rainy season when *P.falciparum* was expected to dominate. These timings were chosen to capture the extremes of burden spectrum and the corresponding differences.

Year Round Survey: The survey was continued from survey 2 onwards until the end of September 2007. This was to determine the length of transmission period for the two species and to capture the species specific seasonal variation in the prevalence and burden throughout the year.

FIGURE 3: 3: DIAGRAM REPRESENTING ANTICIPATED MALARIA TRANSMISSION PEAKS & SURVEY TIMING



Pf= P.falciparum; Pv= P.vivax

3.7 Description of Study Area

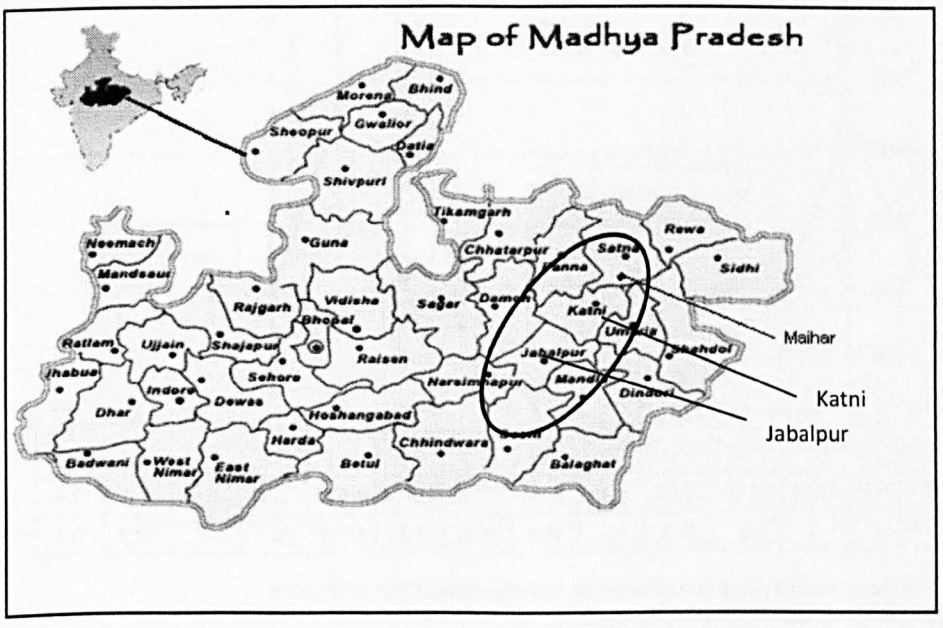
Madhya Pradesh, the name literally meaning 'middle province' is in central India (Figure 3:4). It is the second largest state in India with a population of over 60 million (60,348,023 Census 2001) covering an area of 308,245 sq km divided into 48 districts. The eastern districts where the study sites were located consist mostly of undulating rocky hills and forested areas with the river Narmada running across the area about 5 kms from Jabalpur city, the urban town selected for the study. The study was conducted at hospitals in three of the eastern districts, namely Jabalpur, Katni and Satna (Figure 3:4).

Climate: The climate is characterised by a hot dry "summer" from March through June with temperatures ranging from 30-45^{0}C and a cool "winter" in the months of October to February with temperatures of 15-25^{0}C. The rainy monsoon months extend from July to September with average temperatures of 19^0-30^{0}C. The eastern districts of the state gets more rainfall with an annual average of 112-137cm. while the rest of the state has an annual average ranging from 50-62.5 mm.

Rainfall in Study Districts: In Jabalpur district, the rainfall pattern of the past 38 years is shown in figure 3:5. The average monthly rainfall of the study year 2006 fell within the observed range

for the past years, except for March and May where the rainfall average was slightly higher. The rainfall pattern of Katni for the past 8 years and that of the past 15 years in Maihar are shown in

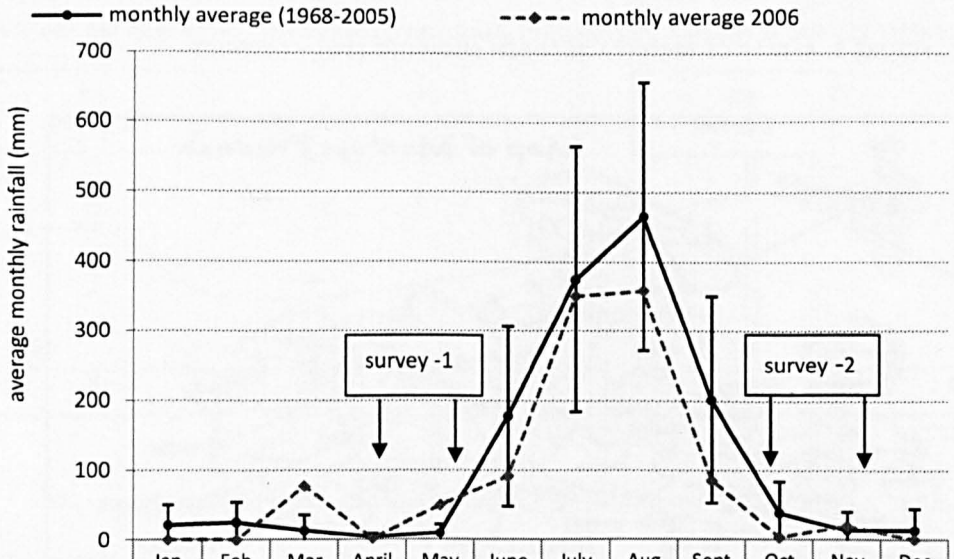
FIGURE 3: 4: MAP OF MADHYA PRADESH SHOWING STUDY DISTRICTS



(Figures 3:6 & 3:7). In Katni, the rainfall pattern for the study years 2006 and 2007 were comparable to the past years, although rainfall was at the lower range of the monthly deviation in the monsoon months of June, July and August in 2007. In Maihar, the rainfall pattern for the years 2006-2007 were within the range of past 14 years except for June which was drier compared to previous years while August–September rainfall was in the lower range.

Malaria transmission pattern: Both *P.falciparum* and *P.vivax* are endemic in this region of Madhya Pradesh with malaria transmission reported to be prevalent throughout the year. The transmission closely follows the rainfall pattern with *P.falciparum* dominating from September to November, following the rainy season. The prevalence of *P.vivax* varies, with higher prevalence reported between April-July when prevalence of *P.falciparum* starts to decline (N. Singh, *et al* 2004)(Figure 3:8). However, a change in the pattern of *P.vivax* epidemiology is seen in the years 1991-92. This report was based on passive surveillance of febrile cases between the years 1987-1992 among all age groups in 15 villages in Mandla, which is the district south east of Jabalpur.

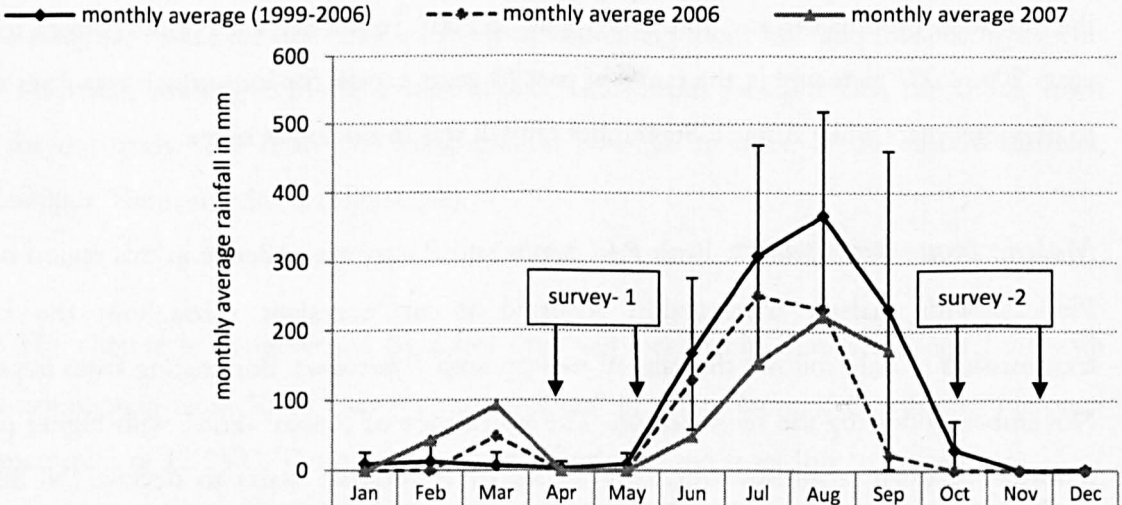
FIGURE 3: 5: MONTHLY RAINFALL IN JABALPUR DISTRICT IN 2006 AND 1968-2005



	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
monthly average (1968-2005)	21.63	25.29	13.59	4.23	9.54	177.98	375.08	466.06	201.83	38.49	13.29	13.47
monthly average 2006	0	0	77.2	1.6	49.5	91.8	350.7	358.6	85.5	3	19.8	0.3

Note: *vertical bars shows the standard deviation of average monthly rainfall for 1968-2006*
Data source: Jawaharlal Nehru Agriculture University, Jabalpur

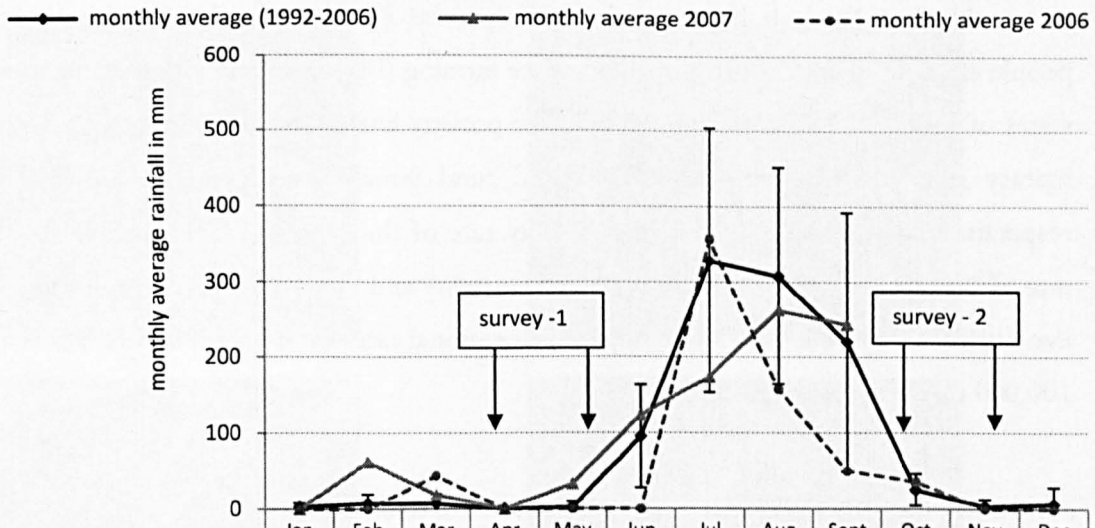
FIGURE 3: 6: MONTHLY RAINFALL IN KATNI REGION IN 2007 AND 1999-2006



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
monthly average (1999-2006)	10.67	13.44	7.74	4	9.97	169.08	309.7	367.66	231.49	30.16	0	1.1
monthly average 2006	0	0	49.2	0	0	131	251	231	23	0	0	0
monthly average 2007	0	43.5	93.5	0	0	49.7	153.6	220.6	172.5			

Note: *vertical bars shows the standard deviation of average monthly rainfall for 1999-2006*
Data source: District Collectorate Office, Katni

FIGURE 3: 7: MONTHLY RAINFALL IN MAIHAR IN 2007 AND 1992-2006

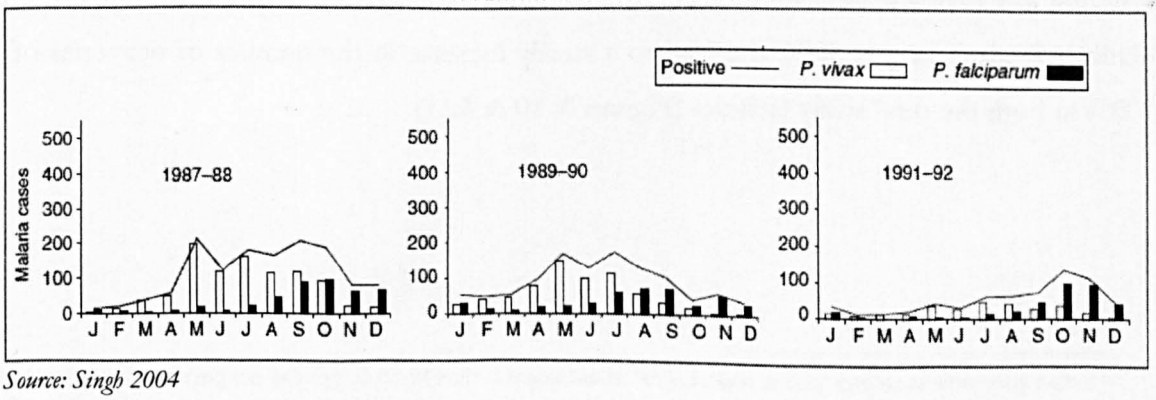


	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
monthly average (1992-2006)	2.85	7.31	7.15	0.38	2.08	95.61	328.57	308.07	220.86	25.5	3.43	6.5
monthly average 2007	0	62	19	0	33	124	174	263	244			
monthly average 2006	0	0	44	0	0	0	357	158	50	36	0	0

***Note**: vertical bars shows the standard deviation of average monthly rainfall for 1992-2006*
Data source: Water & Irrigation Office, Maihar

Malaria Vectors in the study region: The two dominant malaria vectors in Madhya Pradesh are *A.culicifacies* and *A.fluvitalis*. Of these, *A.culicifacies*, feeds on domestic animals but mainly bites humans and contributes about 95% of all malaria transmission in the region. Feeding and resting of this species occur both in and outdoors while breeding takes place in clean and polluted water habitats. The second vector *A. fluvitalis* behaves similar to *A. culcifacies* but breeds in water streams and contribute 1-2% of malaria transmission (Service 2002, Joshi 1998).

FIGURE 3: 8: MALARIA TRANSMISSION PATTERN IN MADHYA PRADESH



Source: Singh 2004

3.8 Study Population

Madhya Pradesh has a predominantly rural (73.3%) population with the majority of people engaged in agriculture and subsistence farming (Figure 3:9). It is one of the less developed states of India, with 37.43% living below the poverty line[2] (national estimate 26.1%). The overall literacy rate is 63.7% with an overall and rural female literacy rate of 50.28% and 43.0% respectively (Census 2001). The total fertility rate of the state is 3.1 comparable to the national rate of 2.7 (NFHS[3] -III 2005-2006). Infant mortality and maternal mortality rates are 72 per 1000 live births and 379 per 100,000, compared to national rates of 55 per 1000 live births and 301 per 100,000 respectively (SRS[4] 2007).

Antenatal Care: A national survey (NFHS-III) conducted in 2006 reported that 40% of women in Madhya Pradesh had 3 antenatal visits during their last pregnancy and that 29.7% delivered in health facilities. These reproductive health indices are changing with the recent introduction of a new rural reproductive health scheme, launched in 2005 by the Government of India as part of the National Rural Health Mission to improve the quality of rural health care (NRHM 2005-2012). Under this scheme a group of women designated as the Accredited Social Health Activists (ASHA) were selected at village level to improve the health care of women and children in rural areas, particularly those having difficulties in accessing health services. Included in their many tasks are motivating rural women to seek health care during pregnancy. To facilitate this, the ASHAs are responsible for arranging transport and accompanying pregnant women to the health facilities for seeking treatment, admission of risk pregnancies and deliveries. A monetary incentive of rupees 1000-1400 (US$ 25-35) is provided for the woman delivering at a health facility and additional rupees 200-400 is provided by the government to the ASHA to escort the pregnant woman. The ASHA scheme has shown to be very popular among rural women resulting in a dramatic increase in health facility attendance and deliveries. The scheme started in Madhya Pradesh in mid 2006 and lead to a steady increase in the number of deliveries of over 100% in both the rural study facilities (Figures 3: 10 & 3:11).

[2] Indian government defines 'below poverty line' as earning less than Rs 10.00 per day per person translating to Rs 276 monthly income in rural India, an amount that would buy food equivalent to 2200 calories per day, which is medically sufficient to prevent death.

[3] NFHS= National Family Health Survey, which in India is the equivalent of DHS elsewhere.

[4] SRS = Sample Registration System. This is a system used by the Office of the Registrar General & Census Commissioner, India for enumerating births and deaths and generating data on these vital events. More details can be found at :- http:censusindia.gov.in/Vital_Statistics/SRS/Sample_Registration_System.aspx

FIGURE 3: 9: FARMING AREAS ADJACENT TO THE RURAL STUDY SITES

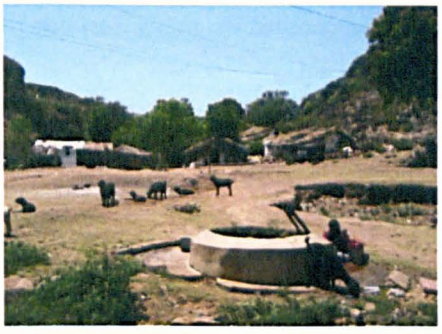
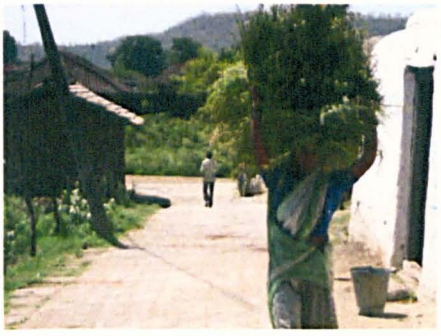
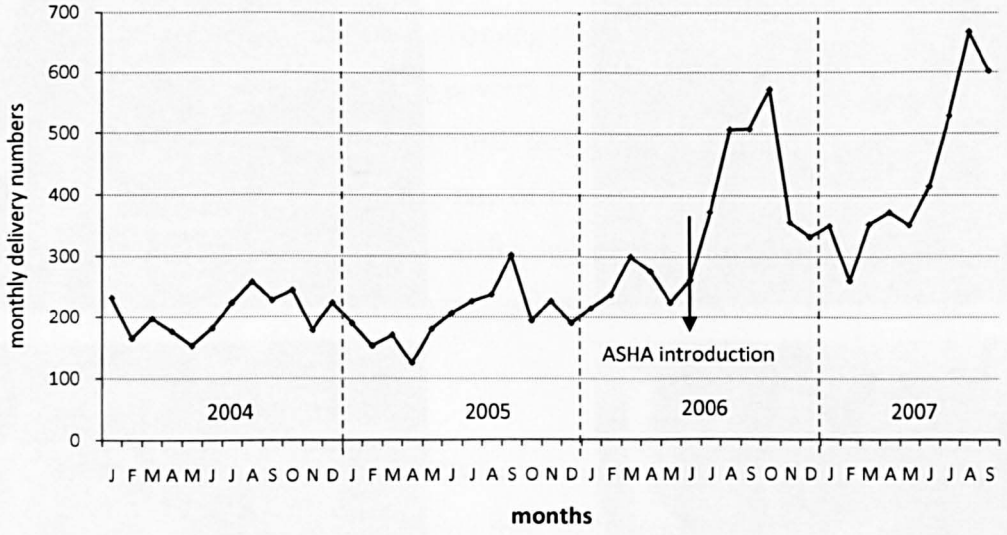
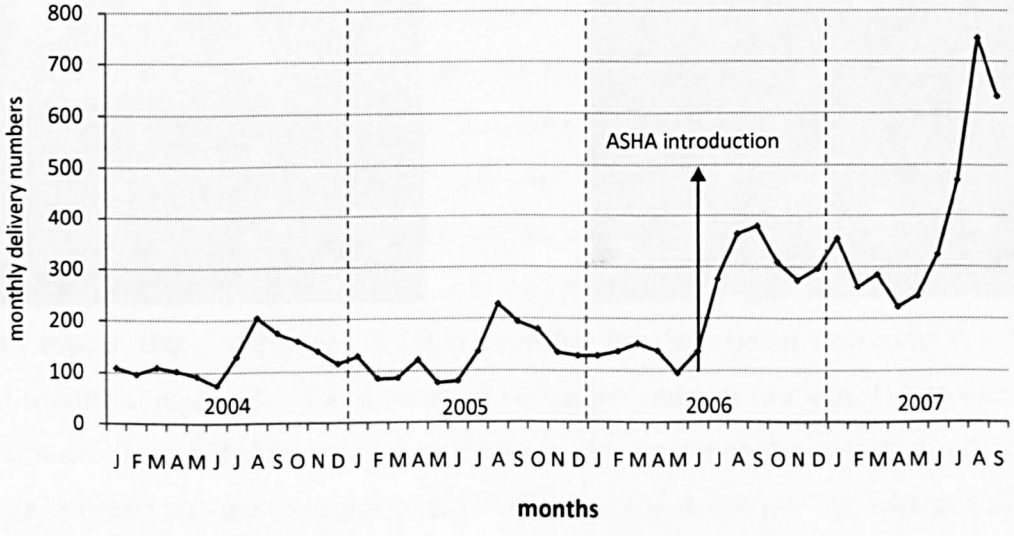





FIGURE 3: 10: MONTHLY DELIVERIES IN DISTRICT HOSPITAL KATNI FOR 2004-2007



Data source: Katni Hospital Registry

FIGURE 3: 11: MONTHLY DELIVERIES AT CIVIL HOSPITAL MAIHAR FOR 2004-2007



Data source: Delivery unit register

3.9 Selection of Study Sites

Three different levels of health facilities in the towns, Jabalpur, Katni and Maihar each approximately 100km apart in three adjacent districts were selected for the study. The 3 health facilities include a primary care civil hospital in Maihar, a secondary level district hospital at Katni and tertiary referral hospital in Jabalpur. The different levels of health facilities were selected to get a sample representative of the rural and urban population of pregnant women in the region. An additional factor was that the National Institute of Malaria Research (NIMR) had established

malaria clinics at Jabalpur and Maihar hospitals. Katni hospital, the new site chosen for the study, was located on the same route from Jabalpur to Maihar. The catchment of the three sites provided a suitable mix of urban and rural women and the site locations were near the main road which made logistics easier.

Site 1: Nethaji Subash Chandra Bose (NSCB) Medical College Hospital, Jabalpur

The Medical College Hospital is a tertiary referral facility situated in the city of Jabalpur. Jabalpur has a population of 1,098,000 (Census 2001) with approximately 0.8 million living in the surrounding peri-urban areas. The hospital catchment covers referrals from the adjacent 6 districts in addition to serving the population of Jabalpur municipality. The hospital has 2500 beds with a busy 100 bed Gynaecology/Maternity unit and a 4 bed delivery unit. The maternity department is equipped to manage high risk deliveries with facilities for obstetric and gynaecological surgery. The department is staffed with a number of doctors in training, nurse-midwives, nurse trainees and nine Obstetrics- Gynaecology Specialists. Antenatal clinics are run 6 days of the week from 9am-2pm with an average monthly attendance of 500 women.

Site 2: Katni District Hospital

The Katni district hospital is situated in central Katni municipality, a semi-rural town with a population of 186,738 (Census 2001). The hospital catchment serves an estimated population of 1,063,689 with greater than 50% of the population attending the hospital coming from the villages within Katni district. The Gynaecology and Obstetrics unit is staffed by three gynaecologists and 10 nurse-midwives. The hospital has a 32 bed maternity ward. The delivery unit has 3 beds with facilities to manage emergency obstetric surgeries. Routine ANC clinics are run 6 days of the week with an average monthly attendance of 400 women.

Site 3: Civil Hospital & Private Antenatal Clinic in Maihar

The Civil Hospital is a level one primary care facility situated in Maihar, which is a town in Satna district that has a population of 229,307(Census 2001). The Civil Hospital, serves the population from the villages adjacent to Maihar together with Maihar municipality which has a population of 34,342. The hospital has 51 beds in total and is staffed with five Specialist physicians including one Gynaecologist. The maternity unit has 5 trained nurse-midwives who manage non-risk pregnancies amounting to a monthly average of 100-150 deliveries. These delivery figures have changed considerably with the introduction of the AHSA program. The nurse-midwives manage the hospital ANC together with the Gynaecologist. The same Gynaecologist runs a private antenatal service that charges a fee, but is attended by the same catchment population. The two ANCs combined have an average monthly attendance of 150-200

pregnant women. The women attending ANCs in both the hospital and the private clinic were recruited for the study.

3.10 Ethical Considerations & Clearance

Ethical clearance

This study was conducted as part of a collaborative project between NIMR/ICMR India, LSTM and CDC Atlanta, USA. The ethical committee and the review boards of each of the three institutions approved the study protocol. Annual reports were submitted while the study was running and ethical approval was renewed yearly. Any changes made to the study were also submitted to the institutions and approval sought as amendments of the initial protocol.

Informed consent

Written informed consent was obtained from each participant. The consent forms were translated first to Hindi then translated back to English for an accuracy check and printed finally in Hindi. The consent included information about the purpose of the study, the study procedures, risk and benefits, confidentiality and voluntary participation. The participants had their right to refuse enrolment explained and it was emphasised that any refusal would not affect their access to routine care provided in the antenatal clinic, delivery unit or inpatient services. For the participants who were unable to read the consent form was read out by a team member and asked questions to assess their understanding of the content. Women unable to sign were asked to provide a thumbprint while a witness attested the consent procedure. Minors below 18 years who were married and running independent households were allowed to sign their own consent. A waiver for parental consenting of minors was obtained from the Ethical Committees during protocol approval.

Confidentiality

Each participant was assigned a unique identification number which was used in the questionnaires, logbooks, blood slides and blood samples. The names of participants and identification information were documented in the questionnaires and consent forms. After data cleaning was completed the names were removed from the final data set. The data forms were filed and kept in locked cabinets during the study and after data entry was completed. Access to the locked cabinets and data set was permitted only to authorized study personnel. The identification of participants was not used in any analysis or reports.

3.11 General Study Procedures

The study was designed for enrolling pregnant women attending the antenatal clinics (ANC), hospital delivery units (DU) and patients admitted for suspected malaria illness in these health facilities (Table 3:3).

TABLE 3: 2: STUDY UNITS INCLUDED FOR THE TWO PHASES OF THE 3 SURVEYS

	Rapid Assessment Surveys			Year Round Survey
Study Facility	Antenatal Clinic	Delivery Unit	Inpatient Ward	Delivery Unit
Jabalpur	√	√	√	-
Katni	√	√	√	√
Maihar	√	√	√	√

3.11.1 Consent & Enrolment

General: Women attending the antenatal clinic or delivery unit were first screened for study eligibility. If they fulfilled the study criteria they were approached for consent and enrolled. Antenatal enrolment was not restricted to first ANC visits. Since IPTp was not implemented in the region, whether women were enrolled at first ANC visit or at a subsequent visit during the 6 week study period was unlikely to affect the study objectives. A structured questionnaire printed in bilingual format (English & Hindi) was administered in Hindi by a study staff, after obtaining informed written consent (Annexe 1). A logbook entry of important information such as the identification number, age and test results were kept for all the participants. Any refusals to enrol were noted and their age and gravidity recorded to allow comparison of baseline characteristics between the enrolled women and refusals.

Consent in Delivery Unit: The consenting procedure in the delivery unit was based on the stage of labour on arrival in the unit. Women presented to the delivery unit at various stages of labour. Study staff were trained to judge whether a woman was able to provide consent prior to delivery or after delivery. This was based on whether a woman was able to converse clearly without being interrupted by labour pain. Staff was instructed to observe a woman for several minutes to assess her labour pain status and then approach for consenting. If a woman was in a comfortable phase of labour, consent was obtained and measurements and finger prick blood samples were taken. Because parasites have been shown to clear soon after delivery (Nguyen-Dinh 1988 quoted in (Brabin and Rogerson 2001), the aim was to get the blood smear and haemoglobin prior to

delivery. The completion of the questionnaire and the rest of study procedures, if needed was completed post delivery when the woman was more comfortable. If a woman was in an advanced stage of labour consent was delayed until after delivery, and the finger prick for haemoglobin and malaria status was deferred until consent was obtained after delivery. The proportion of samples taken after delivery was small: in survey 1 there was 5 cases in Jabalpur, 3 in Katni and 0 in Maihar (overall 1.9%); in survey 2 there was 9 cases in Jabalpur, 6 in Katni and 2 Maihar (2.7%). Although this was noted in the log book in the three surveys, it was not entered as separate data.

3.11.2 Questionnaires

Antenatal Clinic Questionnaire

The ANC questionnaire consisted of five main sections (Annexe 2). The first section included questions on demographics including age, marital status, schooling and socioeconomic status of participants. The next sections covered questions on reproductive history. The third section was on the history of current pregnancy regarding malaria or fever related symptoms, health care seeking behaviour, malaria preventive measures together with the use of antimalarial drugs for treatment or prevention during pregnancy. The last part consists of a section to record the measurements, test result and drugs given to the women.

Delivery Unit Questionnaire

The delivery unit questionnaire was structured similar to the ANC questionnaire covering general identification and demographic parameters, reproductive and malaria morbidity and prevention history during pregnancy (Annexe 3). In addition a section was included which covered maternal and birth outcomes and baby measurements. An additional form was used for Ballard scoring to determine the gestational age of the baby (Annexe 4).

Inpatient Data Form

Inpatient surveillance forms consisted of questions related to identity and demographic data (Annexe 5). Information was extracted from the patient's hospital records while any missing data was obtained directly from the participant. The data obtained included symptoms patient presented with at the time of admission, results of any investigations done during the hospital stay, history of antimalarial drug use together with treatment in hospital and admission and final discharge diagnosis documented in the patients file.

3.11.3 Study Tasks

A: Antenatal Clinic

After completion of the questionnaire the axillary temperature, blood pressure and mid-upper arm circumference (MUAC) were measured (Figure 3:12). Two measurements were taken for accuracy assurance and their average used for data analysis. Whenever there was a $>0.5^0$C discrepancy between the two temperature measurements, both recordings were repeated after approximately ten minutes time interval. Similarly, for blood pressure if the difference between readings was >10 mmHg for either the systolic or diastolic pressures, a new set of recording was taken. For the assessment of MUAC, new measurements were taken when a discrepancy >5mm was found between two readings. Blood was drawn by finger prick for assessment of the haemoglobin level, malaria RDT (pLDH First Response Pf/Pv®) and blood smear. In addition, a blood sample was collected in an EDTA tube for PCR.

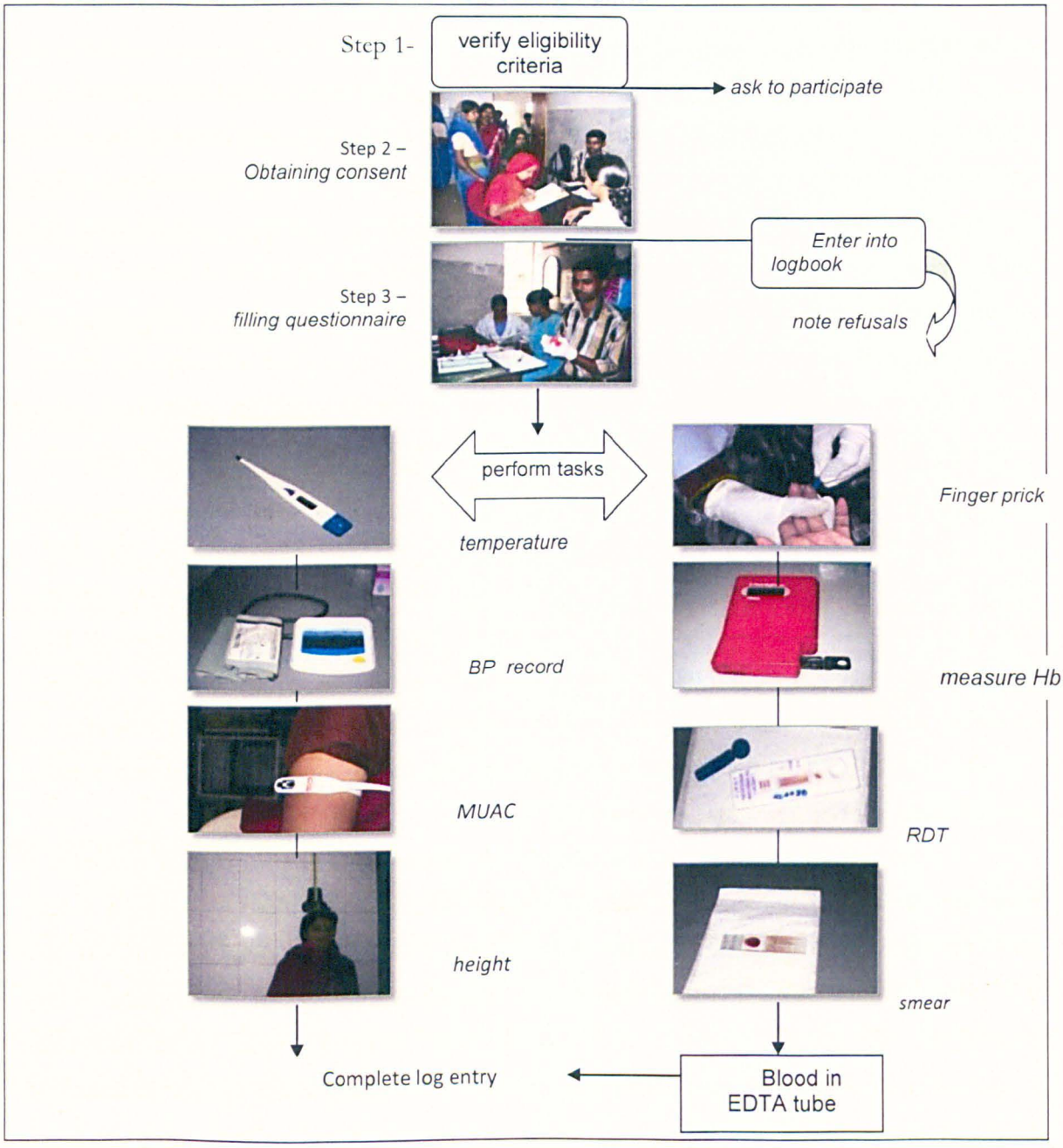
For women who reported a history of fever within the past week or who had a documented fever (temp $\geq 37.5^0$C) but a negative RDT result, their blood slide was examined while they waited in the clinic (Figure 3:13). Any women found to be positive for malaria by RDT or microscopy were referred to the hospital ANC staff to inform the gynaecologist for treatment according to the national guidelines. For all other women, malaria slides were read daily after the closure of ANC. If any of these slides were found positive, the responsible hospital staff member was informed in order to contact the woman.

At the time of the survey conduction, the recommended drugs for malaria treatment in pregnancy were chloroquine as first line (chloroquine 600mg stat followed by 300mg x 3 days) and sulfadoxine-pyrimethamine (3 tablets of 500/25mg) as second line drug. Quinine was used for severe malaria. The recommendation for chemoprophylaxis was chloroquine 300mg base/week. All women found to be anaemic (Hb <11g/dL) were given haematinic supplementation (the preparation used commonly was Fefol tablets which has a combination of 150mg ferrous sulphate and 0.5mg folic acid). Any woman found to have moderate-severe anaemia (Hb <9g/dL) was referred back to the ANC staff for appropriate management.

B: Delivery Unit

Similar procedures to those used in the antenatal clinics were carried out for delivery unit participants. In addition a measurement of maternal height was taken from these women. Again two measurements were taken and if there was a discrepancy of ≥5 mm between two recordings, it was repeated. Placental sampling was done approximately within 3 hours from delivery for all births that occurred before 11pm daily. Because of budget constraints, deliveries occurring between 11pm and 7am were not included.

FIGURE 3: 12: WORK SCHEME AT ANTENATAL & DELIVERY UNITS

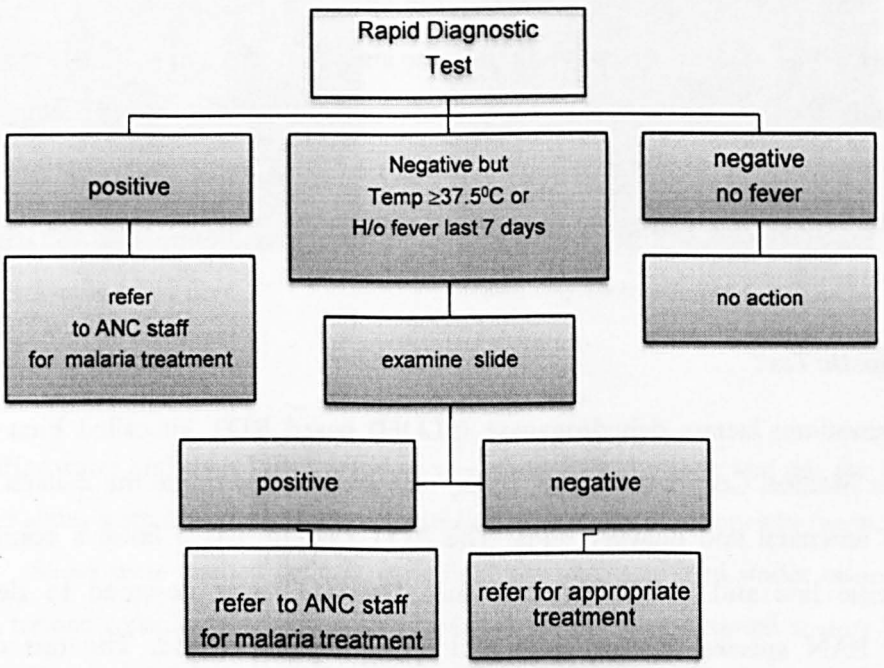


***NOTE**: HEIGHT RECORDED ONLY IN DU.*

Maternal weight was not recorded because this was a cross-sectional study and pre- pregnancy weight is unknown in these women. Instead MUAC, which is an indicator of maternal nutritional status and remains stable through pregnancy, was taken.

Placental blood was collected from the maternal side for incision and impression smears. A biopsy was also taken for histopathology examination (details of the procedure are described in the chapter on placental histology). An additional blood sample was collected in EDTA tubes for PCR. Cord blood smears were only introduced during the second survey and the extended year round survey. All singleton live babies were examined for physical abnormalities and weight recorded. Babies were weighed within 2 hours of delivery time. Babies delivered by Caesarean section were weighed within 24 hours of delivery. Gestational maturity was assessed by a staff member trained to perform the Ballard score (Figure 3:14). If a baby appeared unwell at delivery, Ballard assessment was delayed until condition stabilized and if illness persisted beyond 24 hours

FIGURE 3: 13: ALGORITHM FOR MALARIA DIAGNOSIS & TREATMENT



examination was deferred. For quality assurance, examination of babies and performance of all study tasks was supervised by the investigator (RA, a paediatrician) on a weekly basis at each study site.

C: Inpatient Disease Surveillance

A study staff member approached all female patients between the age of 18-49 years admitted with malaria, or cases with fever suspected of malaria, to request participation in the study. After obtaining informed consent, data was extracted from the hospital records. Consent was obtained from the husband or attending adult care-giver for women with altered

consciousness. Information missing in the records was obtained directly from the patient. Data collected included presenting symptoms of illness, results of investigations performed while in hospital, treatment taken before admission and in the hospital, admission and discharge diagnosis. This component did not include performing extra tests other than those done by the hospital physicians.

3.11.4 Diagnostic Procedures

A: Haemoglobin Assessment

Haemoglobin was assessed using the HemoCue machine (HemoCue® B-Hemoglobin, Angelholm Sweden). After wiping off the first drop of blood, the microcuvette was filled with capillary blood. The cuvette sides were cleaned for any excess blood before placing in the HemoCue machine. The machines were verified at the start of each day for quality control using the standard quality control kit supplied by the manufacturer. The verification reading was noted in the logbook to ensure haemoglobin readings did not deviate more than 0.3g/dL of the haemoglobin standard.

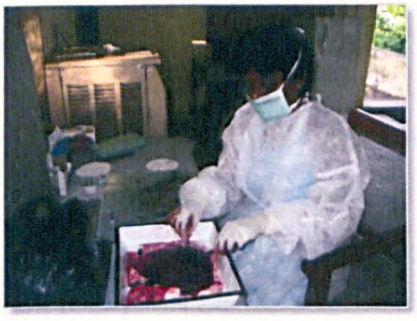
B: Rapid Diagnostic Test

The plasmodium lactate dehydrogenase (pLDH) based RDT kit called First Response Pf/Pv®, (Premier Medical Corporation, Ltd, India) was used to determine the malaria status for women in both antenatal and delivery units. The RDT cassette has 3 lines: a control line, a *P.falciparum* specific line and a PAN species line. The RDT was designed to detect both *P.falciparum* and PAN species *(P.vivax)* with a drop of capillary blood. The test details are described later in the chapter 7.

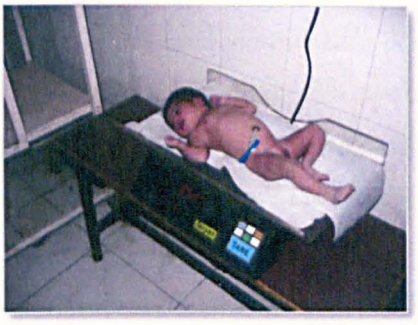
C: Blood Smears

Peripheral Slides: A thick and thin blood smear was made from each participant on the same slide. The thin smear was first fixed with methanol. The top part of the thin smear was not fixed if it was too close to the thick smear. All blood smears were stained with JSB stain (Jaswant Singh & Bhattacharjee 1944). The JSB stain is an Indian preparation used for rapid staining similar to Field stain. It consists of two solutions: JSB-1 which is methylene blue based and JSB-2 which is eosin based (red). It is advantageous over Field stain because parasites stain clearer and it can be used for both thick and thin smears. The slides were first dipped in JSB-2 solution, washed with

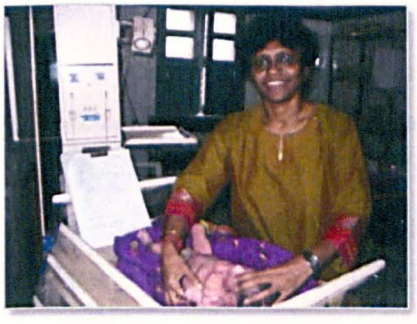
FIGURE 3: 14: DELIVERY UNIT PROCEDURES



1-Placenta sampling



2-Baby weighing



3-Performing Ballard score

4-measuring maternal height

sterile buffer water and then JSB-1 solution was poured on the slide and left for 40-50 seconds. Thereafter slides were rinsed gently in tap water and left to dry in an upright position.

Slides were stained daily at each field site and examined under oil immersion 100x lens by a trained technician. Parasites in peripheral smears were counted against 300 leukocytes and densities calculated based on the assumed leukocyte count of 8000 per μL of blood. The standard formula, (parasite μL= no. asexual parasites x 8000 /no. of WBC) was used. A blood smear was considered negative when no parasites were detected after examining 200 high-power fields. Two hundred instead of the more commonly used 100 fields were read to enhance the sensitivity to detect lower density infections. The number of asexual parasites and gametocytes were counted and recorded separately in the laboratory result forms. Thin smears were used for species identification.

Placental Slides: The placental slides were also stained with JSB solution as described above. Thick and thin placental incision smears were examined similar to the peripheral blood films. In addition schizonts and malaria pigment were counted separately per 300 WBC. The number of

pigmented WBC were counted and expressed as a percentage. E.g. if 20 pigmented WBCs were counted among 300 total WBC then the pigment percentage was 6.6%. (20/300 x100). Impression smear results were expressed as percent parasitaemia by counting the number of infected red blood cells and dividing this by the total number of red cells in 50 high power fields. The number of parasites per micro litre cannot be used for these smears because the average number of WBCs in the intervillous space is not known. The methods used for placental histopathology examination is detailed in chapter 5, describing the histopathology findings.

Quality Control Mechanism: The first microscopic examination was performed at each field site by a technician unaware of the RDT result. After the first reading, the slides were transported weekly to the NIMR field station in Jabalpur, the central research facility of the study. Here all the slides were re-examined by a senior technician. The second examiner was blind to the results of the first reader and the RDT results. The discrepant results between first and second reader were re-examined by the senior microscopist at the central field station and the investigator (RA) and findings taken as final. Discrepancy was defined by parasite positive and negative detections and any RDT and slide discrepancies or *vice-versa.* The positive slides and 10% of the negative slides were also re-examined by the investigator (RA) for quality control.

D: Blood for Molecular studies

Finger prick blood (250-500uL) from women attending the antenatal clinic and placental and cord blood from were collected in an EDTA microtainer tubes. These samples were spun at 1500 rpm for 10 minutes and the plasma and red blood cell pellets were separated and stored in the freezer. The samples were transported from the field sites twice weekly in thermo-cool boxes with ice-packs to the central location in Jabalpur and stored at -70^{0}C. PCR was performed to confirm species detected by microscopy and RDT on the red blood cell pellets from the malaria positive samples collected in antenatal clinics. In addition PCR was performed for detection of sub-microscopic infections in a randomly selected sub-sample of the malaria negative pellets as described in more detail in chapter 7. PCR analysis was carried out at the NIMR field station laboratory in Jabalpur.

PCR Amplification of Plasmodium genus: DNA was extracted from the red cell pellet using the BS 484 EZ 10Spin Column Genomic DNA Kit (Bio Basic Inc, Ontario, Canada). The primary reactions were conducted in volume of 25µl total consisting of 5µl of DNA solution in 20µl of reaction mixture containing the reagents Taq buffer 1X, $MgCl_2$ 1mM, dNTP 200µM, forward and

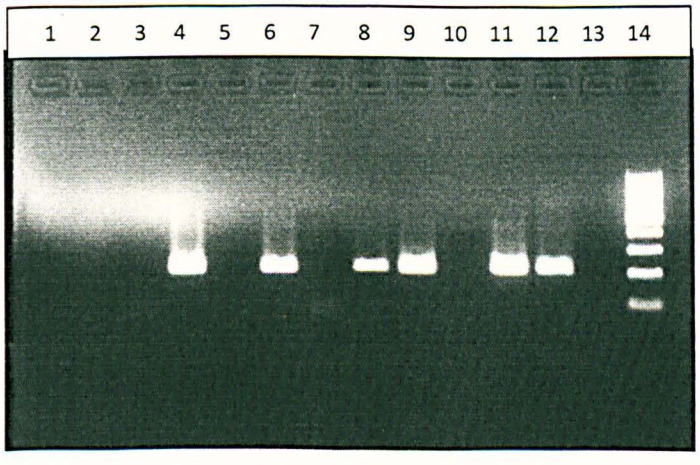
reverse primer 240nM and 1U of Taq DNA polymerase EP0402 (Fermentas International Inc, Ontario, Canada).

Amplification of P.falciparum: An aliquot of 2µl in 1:10 dilution of the first reaction mixture and reagent concentration of dNTP 160µl, forward and reverse primer 200nM, Taq buffer 1X, $MgCl_2$ 1mM and 1U Taq DNA polymerase was used for the second round of *P.falciparum* nested PCR. The species specific primers encompassed 18S ribosome RNA. The amplified DNA was electrophoresed on 1.5% agarose gel and DNA bands visualized with ethidium bromide in the gel documentation system (Figure 3:15). Of the 314 samples selected for PCR analysis, 122 samples had blood volume ≤30µl. The second reaction for the low blood volume samples were performed on a 1:50 dilution. The PCR analysis was based on the principle described by Snounou *et al* (1993)

Amplification of P.vivax: Using a similar technique and reagent mixture as for *P.falciparum* the amplification of *P.vivax* was performed with a species specific primer. The annealing temperature was different to that used for the *P.falciparum* analysis. After DNA amplification, electrophoresis on agarose gel was done and DNA bands were viewed in the gel documentation system.

The staff that performed PCR was unaware of the microscopy and the RDT results. The identification numbers on the PCR list were anonymised and data analysis was performed only after all the PCR results had been entered.

FIGURE 3: 15: AMPLIFICATION OF *P.FALICPARUM* FOR DIFFERENT DNA STRAINS



Explanation : **Lane 1-11**: DNA isolates ; **Lane 12** : positive control ; **Lane 13**: negative control; **Lane 14**: 100 bp DNA ladder

3.12 Study Definitions

1. *Symptomatic malaria*: An axillary temperature ≥37.5^{0}C, or a history of fever in the past seven days, in the presence of asexual forms of any *plasmodium* species detected on thick or thin smear or a positive RDT.

2. *Asymptomatic malaria*: Presence of any *plasmodium* species detected on thick or thin blood smears or by a positive RDT in the absence of a documented fever or a history of fever in the previous 7 days or at visit.

3. *Severe malaria*: A confirmed *P.falciparum* or *P.vivax* malaria by RDT or microscopy and the presence of any of the following: cerebral malaria, severe anaemia, renal failure, pulmonary oedema, hypoglycaemia, shock, bleeding or repeated convulsions. (Selected from the WHO guidelines using those symptoms that were used in the day-to-day routine record keeping of clinical inpatients).

4. *Fever*: A documented axillary temperature ≥37.5^{0}C

5. *Anaemia*: Haemoglobin <11.0g/dL

6. *Moderate-severe anaemia*: Haemoglobin < 9.0 g/dL

7. *Severe anaemia*: Haemoglobin <7.0g/dL

8. *Placental malaria*: Presence of asexual parasites of any *plasmodium* species on an impression smear made from the maternal side of placenta.

9. *Low birth weight (LBW)*: A birth weight <2500g taken within 24 hours of delivery.

10. *SGA*: Small for gestational age is defined as a birth weight below the 10th percentile on birth weight for sex specific gestational age percentile chart (Williams reference curves)(Williams 1975).

11. *Intrauterine growth retardation (IUGR)*: Term baby (≥37 weeks by Ballard score) with birth weight <2500g

12. *Preterm baby*: Delivery before 37 weeks of gestation as assessed by Ballard score

13. *Abortion*: Expulsion of products of conception prior to 20[5] weeks completed gestation (estimated by LMP or fundal height)

14. *Stillbirth*: Delivery of a dead fetus where death had occurred after 20 weeks completed gestation estimated either by last menstrual period or fundal height measurement (if LMP unknown).

[5] In India abortion is legal under the Medical Termination of Pregnancy (MTP) Act 1971. Through the Act, the MTP Rules, 2003 permit abortions up to 20 week gestation, hence a 20 week cut off was used for defining abortion for the study. For details of MTP Act refer to http://www.mohfw.nic.in/MTP%20ACT%201971.htm

3.13 Preparation for conducting the surveys

Study Team

The study teams were set up with locally hired staff working at each site. Each team consisted of 4 staff members and included 1 interviewer in the antenatal clinic, 2 nurse/midwifes for the delivery unit and 1 technician. A member of the team assigned as the team leader was responsible for keeping stock of supplies at each site and overseeing the work. The data entry clerk and second laboratory technician worked at the central location at NIMR, Jabalpur. Additional assistance for data management, microscopy and logistics management were provided by the respective staff at NIMR, Jabalpur.

Staff Training

Prior to commencing the surveys, a one week workshop was held for training the study team in obtaining consent, filling the questionnaires and the specific tasks and procedures related to handling of blood and biohazard material according to universal precautionary measures. During the workshop the team members were taught how to perform finger pricks, make blood smears, use HemoCue machine and to perform and read the RDT results. Their skills were updated on measuring temperatures, blood pressure with a digital machine, MUAC, and maternal height. Staff was also trained to obtain placental samples, measuring birth weight and the use of Ballard score for assessing the newborn gestational age. At the end of the workshop a pilot study was run at each site for two days to familiarise the staff with all the procedures in conducting the survey. Regular enrolment was commenced after assessing the performance of the staff and adjusting for problems encountered during the pilot period.

Safety Precautions

Universal safety precautions were followed when collecting peripheral and placental blood samples with the use of gloves, gown and mask. Each site was provided with sharps and biohazard containers for safe disposal of sharps and infectious waste. When sharps and bio hazardous waste containers were full they were sent for incineration. Any blood spills were cleaned immediately with 10% chlorhexidine. The members of study team who had not had Hepatitis B vaccination were vaccinated at the time of training workshop. All team members were instructed on the procedures to be followed for needle stick and accidental bio hazardous fluid exposure. Additionally, safety guidelines covering accidental injury was included in the study handbook ensuring HIV testing and availability of post exposure prophylactic treatment with anti-retroviral.

3.14 Data Management

Data entry screens were developed using Microsoft Access (2003) and Visual Basic software packages. The data screens incorporated a range of logical entry restrictions and skip patterns. The data entry clerk was trained in using the data screen and reading the study questionnaires. Two different individuals (one being the investigator) carried out data entry and entered the forms in duplication (double entry). The two data sets were compared using the EpiInfo (version 3.4.3) data compare utility. All inconsistent errors identified were checked against the hard copies and discrepancies corrected. In addition, the performance of the data entry clerk was assessed regularly using random checks for a selected number of questionnaires. Data files were backed up daily. Data were cleaned using range and consistency checks. In the cleaned data set, personal identifier information was removed. The hard copies of the questionnaires are archived at NIMR field station in Jabalpur.

3.15 Data analysis and statistical methods (Overview)

The data analysis was done using the statistical programs SAS 9.1, (SAS Institute, Cary, NC). The data from the two rapid assessment surveys were used for comparison of the results between urban and rural sites and for differences between the two transmission peaks. The data from the extended survey, together with the second survey were used as the year round survey data. The inpatient data provides descriptive information of malaria illness severity in pregnant women. Peripheral parasitaemia often referred to in this thesis as maternal malaria were expressed as a proportion (%) based on the results of peripheral blood microscopy. Placental parasitaemia estimates were based on the impression smear results, unless stated otherwise. Anaemia is categorised as mild (Hb= 10-11g/dL), moderate (Hb = 7-9g/dL) and severe (Hb <7g/dL). The relative contributions of parasitaemia and associations between maternal anaemia, low birth weight and other parameters are reported separately for *P.falciparum* and *P.vivax*. Differences in maternal haemoglobin, birth weight and prematurity were assessed as continuous variables.

Different types of statistical methods were used to analyze the diverse data collected to meet the different study objectives described in chapter 1. The main statistical methods applied to obtain the results relating to each objective are briefly outlined below. The details of the statistical methods used for data analyses are described in the respective result chapters.

1. *Epidemiology and burden of MiP*: Log-binomial regression was used for the analysis of dichotomous outcome variables. GENMOD and the COPY-method, which provides

better approximate maximum likelihood estimate (MLE) was used for the multivariate analysis. The COPY-method functions on the principle of the log binomial method and involves a process of data simulation on an expanded data set of the original data using MLEs when data does not converge on log binomial model. These same statistical methods were used for the analysis of the determinants of adverse birth outcomes.

2. *Studies on Placental Histology*: General linear models (GLM) were applied to estimate the association between histological features and placental infection on birth outcome. Birth weight, gestational age and haemoglobin were taken as continuous variables in this analysis.
3. *Accuracy of the Rapid Diagnostic Test:* This was assessed comparing the results of sensitivity, specificity, positive and negative predictive values with reference to microscopy and PCR. A modified discrepant analysis approach was used where the concordant and discordant samples between microscopy and the RDT were selected for analysis and compared with PCR as the resolver test.
4. *Comparison of different diagnostic tests*: The sensitivity/specificity and positive /negative predictive value pairs were used to determine the diagnostic accuracy of the test methods for detecting placental malaria (placental histopathology was considered as the disease status). The likelihood ratios were used to express the ability of the tests to accurately detect placental infection using placental histopathology as the gold standard.

Chapter 4

The Epidemiology and Burden *of* Malaria in Pregnancy

"To understand God's thoughts we must study statistics, for these are the measures of HIS purpose"
Florence Nightingale, 1820-1910.

4.1 Introduction

Development of optimal evidenced based malaria in pregnancy control policy for India has been challenging because of lack of data on the magnitude of the problem. Previous studies from India reported on the susceptibility of pregnant women to the harmful effects of malaria in pregnancy. These studies focused on the effects of disease severity among symptomatic women presenting to urban health facilities, or on community surveys conducted during malaria outbreaks (Chattopadhyay and Sengupta 2000, Das 2000, Singh, *et al* 1995). There is little known about the prevalence and the role of asymptomatic malaria in pregnancy and further systematic quantitation of the burden of malaria in pregnancy is needed to determine the public health impact of malaria in pregnancy in India.

The primary objective of the study as stated in chapter 1 was to determine the burden of malaria and the main determinants of risk in women attending the antenatal and delivery units in an urban and rural setting of Madhya Pradesh. This chapter discusses the malariometric findings, including a description of the seasonality of peripheral and placental malaria and, the relative contributions of symptomatic and asymptomatic *P.falciparum* and *P.vivax* infections. The association between birth outcome and placental malaria are discussed in the next chapter (5).

4.2 Methods

4.2.1 Enrolment Procedures

The details of the general study design and methods were described in chapter 3. In brief, the study recruited women in three health facilities from the antenatal clinics, the delivery units and inpatient wards, during two 6-week periods; one during the dry season and one during the post-monsoon malaria transmission season using the rapid assessment methodology. In addition, the delivery unit component was extended after the second survey for a further 46 weeks ('survey 3') to complete a full "year round survey" (October 2006-September 2007) to capture the length of transmission season and the seasonal variation in species. For survey-3, the delivery units of the rural sites, Katni and Maihar were selected, because of the near zero malaria prevalence detected in Jabalpur, the urban site, during the rapid assessment surveys. Due to limited funding, enrolment from the antenatal clinics was excluded. During the survey-3, a random selection of one in three and one in four eligible deliveries were enrolled at Maihar and Katni, respectively, to allow spacing out the total sample over the duration of the study. Women could enrol only once in the

antenatal module and once in the delivery module. If a woman previously enrolled in the antenatal clinic attended for delivery, the study recruited her as a new participant in the delivery unit module.

4.2.2 Statistical Methods

The analyses presented in this chapter relate to the burden and risk identifier models associated with malaria. Since the rapid assessment surveys were designed to use pooled data from the 3 sites, analysis of the antenatal module was performed on site specific and combined data. The pooled data from the three sites were used for the univariate and multivariate models. The delivery unit analyses also include site specific and combined data of the year round surveys consisting of only the rural and semi-rural sites for outcomes of interest includes.

All analyses were performed using SAS® version 9.1 (Statistical Application Software, SAS Institute Inc. Cary NC). Graphs were produced using Microsoft Excel 2007. Chi-square (χ^2) and Fisher's exact test (for cells with an expected frequency ≤ 5) were used to compare proportions for categorical variables. PROC GENMOD was used to compute the unadjusted (univariate) and adjusted (multivariate) prevalence ratio (PR) and 95% confidence intervals for binary and categorised variables. Results were considered to be statistically significant when the 2-sided *P*-value was <0.05.

4.2.2.1 Determinants Analysis

Log binomial regression: Log binomial regression was used to calculate prevalence ratios and to measure the strength of associations between risk factors (as independent predictor/exposure variables) and malaria (as outcome variable) in univariate analyses. Examinations for malaria outcome were performed separately for a) any malaria (any species), b) *P.falciparum* and c) *P.vivax,* for peripheral and placental parasitaemia. The association between malaria (as exposure variable) and anaemia and fever as outcome variables were also assessed by species, for the antenatal clinic and delivery unit data.

Log binomial regression was preferred over logistic regression because it has the benefit of directly estimating prevalence ratios (risk), whereas logistic regression estimates odds ratios, which are more difficult to interpret (Lee, *et al* 1994, Skov, *et al* 1998). Furthermore, (Deddens, *et al* 2008) and colleagues showed that the log binomial method has advantages over robust Poisson regression, in that it results in less bias, smaller standard errors and did not result in modelled prevalence estimates below zero or exceeding one.

One limitation of the log binomial model is a greater possibility of models failing to converge, particularly in situations where the outcome is relatively rare. To overcome this problem, Deddens (2008) and colleagues had developed a process called the COPY-method.

The Copy-method: The method comprises of an expanded large data-set of the original copies (minimum 100, recommended 1000 or more, up to 100,000 copies) minus one copy of the original data-set and one copy with the dependent variable transposed (1s and 0s are reversed). With the COPY-method, the correct estimate of the standard error of the prevalence ratio is estimated after adjusting the standard error of the prevalence ratio on the large modified data-set. Some investigators have recommended using the 'robust Poisson method' when the log binomial method fails to converge (Spiegelman, *et al* 2005). However, as mentioned earlier, the 'robust Poisson method' gives larger standard errors compared to the COPY-method and may produce probabilities greater than 1. The shortcomings of the COPY-method are that it involves some data manipulation and maybe more sensitive to outliers and model misspecification, while rarely it may produce invalid Wald confidence intervals. On the other hand, the advantage of COPY-method is that it produces correct approximation of the maximum likelihood estimates (MLE) and can be run using the existing PROC GENMOD procedure that had failed on the original data. The group that developed the COPY-method also provides a SAS MACRO for executing the method. The MACRO first runs the PROC GENMOD procedure on the original dataset. If no convergence occurs, it automatically switches to the COPY-method and MLE set to 1000 copies.

4.2.2.2 Multivariate Modelling

The univariate analyses were performed with malaria as the response/ dependent variable. All the variables with *P-value* <0.1 were considered for inclusion in the full (initial) model. In addition, other known predictor variables such as gravidity and maternal education were also included in the full model, even if the *P-value* was above the selected threshold of 0.1. Firstly, collinearity between all the independent variables was assessed using the Pearsons correlation to produce a correlation coefficient matrix (Annexe 6 & 7). When pairs of variables with values of Rho greater than 0.7 were used and the model was found to be unstable, one variable out of the multi-collinear pairs was selected for inclusion. For example, fever in pregnancy and drug use in pregnancy were two variables highly correlated (Annexe 6). When they were both in the model, the model became unstable, so fever was selected. The next step included assessing for interactions. There was no evidence of

interactions and therefore interaction terms were not included in subsequent modelling steps.

The study site variable was assessed initially in the multivariate model for antenatal data which included thirteen other variables of interest. Using sequential backward elimination, non-significant variables were deleted manually to obtain the final model. The order proceeded with the variable with the weakest association leaving the model first. A *P-value* <0.05 based on the Wald type 3 statistic was used as the significance threshold. In this initial model, site proved to be a strong predictor in the univariate analysis, but dropped out in the last step. A second model was run including only the rural population who are likely to have more malaria and moderate-severe anaemia. Site was not assessed as a variable in this second model. Surprisingly moderate-severe anaemia was not a determinant in the rural model. Because of the known association between anaemia and malaria we assessed a third model which excluded site, but including the variable residence which consisted of women residing in both rural and urban areas from all 3 sites (full population). The third predictor model was initiated with thirteen variables and the results of this model are presented.

Gravidity was not a statistically significant parameter, but was forced into the models. In the multivariate models, multigravidae was considered ≥ 3 pregnancies, to strengthen the reference group due to the small number of women in gravidae 4 and 5. The seasons when included as a 3 category variable resulted in unstable models and was therefore used as a *bi*-variate variable representing the high transmission season (cool period) and the low transmission season (dry and monsoon rain). A similar model was set up for the determinants of placental malaria using the delivery unit data. Eleven variables fitted into the initial delivery module model.

4.2.2.3 Attributable Fractions

The population attributable fraction (PAF) was calculated using the following formula: PAF = prevalence $_{(E)}$ (RR -1) / 1+ prevalence $_{(E)}$ (RR -1), where RR= relative risk and (E) = exposure. The formula (RR-1) / RR was used to calculate the attributable fraction exposed (AF_E) in the exposed cases.

4.3 Results

4.3A: Rapid assessment surveys in antenatal clinics

4.3A.1 Characteristics of enrolled women

During the two 6-week rapid assessment surveys, 1817 women were enrolled from the antenatal clinics in the three sites: 817 in dry season (survey-1) and 1000 in post-monsoon season (survey-2). Refusal to participate was rare: only 10, 6 and 0 women (0.9%) refused in Jabalpur, Katni and Maihar, respectively. The differences in age or gravidity between the enrolled women and women who refused were not significant. The main reason quoted for refusal was a lack of time among the women, many of whom had already waited for a long time for their routine antenatal check up.

The characteristics of women who participated in the two surveys are provided by site in Table 4:1. The mean (SD) age was 23.7 (3.9) years; 80.9% were aged between 20 and 29 years. Nearly all the participants reported to be married (99.9%). As expected, the women enrolled in Jabalpur were predominantly from urban areas (70-79%) while in Maihar, the population was predominantly rural (76-81%). The population in Katni was from both urban and rural settings. The average levels of education and the socio-economical status were lower in Katni and Maihar, the semi-rural and rural sites, than among the women attending the antenatal clinic in Jabalpur. Overall, 36.7% of the women were primigravidae while 4.3% were grand multigravidae (≥5 pregnancies). The median gestation at first antenatal visit was 24 weeks (range 18-32).

Self reported history of malaria control measures

Bed net use was moderate with 41% women reporting to use it at anytime during pregnancy, while 34% reported using a bed net in the previous night (Table 4:1). Only 11 women (0.6%) reported having an insecticide treated bed net. Twenty women (1%) reported using drugs for malaria prevention in pregnancy (chloroquine). More than half the women (55%) reported taking iron and folic acid provided as a fixed combination supplement (commonly used combination is Fefol capsules/tablets containing 150mg ferrous sulphate and 0.5mg folic acid).

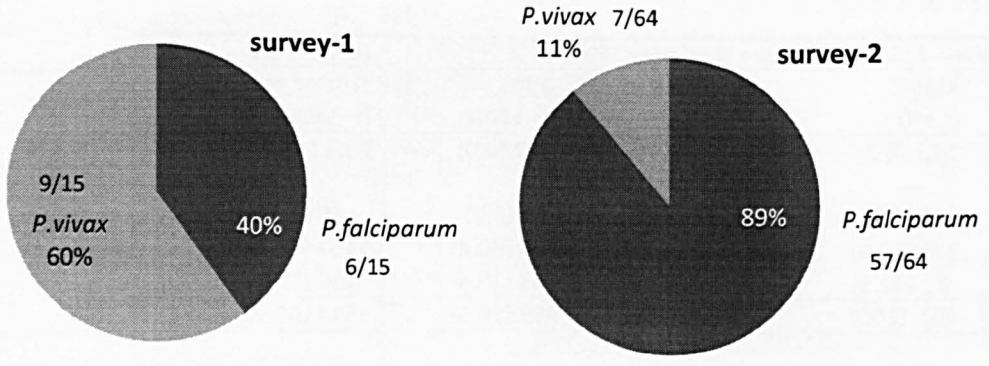
TABLE 4: 1: CHARACTERISTICS OF 1817 WOMEN ENROLLED FROM 3 ANTENATAL CLINICS IN 3 SITES (SURVEY 1&2)

Dry season survey-1 (6 weeks April-May 2006)				
Survey sites:	**Jabalpur**	**Katni**	**Maihar**	**Total**
Characteristics	(n=286)	(n=307)	(n=224)	(n=817)
Age in years, mean (SD)	23.4 (3.5)	24.3 (3.9)	22.9 (4.3)	23.6 (3.9)
Age Group				
<20 yr, n (%)	24 (8.4)	19 (6.2)	32 (14.3)	75 (9.2)
20-29 yr, n (%)	238 (83.5)	249 (81.4)	176 (78.6)	663 (81.4)
>30 yr, n (%)	23 (8.1)	38 (12.4)	16 (7.1)	77 (9.4)
Married, n (%)	285 (100)	306 (100)	224 (100)	815 (100)
Residence				
Urban, n (%)	200 (70.2)	199 (65.0)	52 (23.2)	451 (55.3)
Rural, n (%)	85 (29.8)	107 (35.0)	172 (76.8)	364 (44.7)
Caste Category[♣]				
OBC, n (%)	138 (48.9)	97 (32.0)	101 (45.1)	336 (41.1)
GEN, n (%)	68 (24.1)	108 (35.7)	61 (27.2)	237 (29.0)
SC, n (%)	*44 (15.6)*	*61 (20.1)*	*49 (21.9)*	*154 (18.8)*
ST, n (%)	32 (11.4)	37 (12.2)	13 (5.8)	82 (10.1)
Education				
No schooling, n (%)	44 (15.4)	98 (32.0)	63 (28.1)	205 (25.2)
Primary, n (%)	35 (12.3)	107 (35.0)	108 (48.2)	250 (30.7)
Secondary[**], n (%)	206 (72.3)	101 (33.0)	53 (23.7)	360 (44.1)
Higher, n (%)				
SES rank in quintiles, n (%)				
0=Poorest	33 (11.5)	40 (13.1)	76 (34.0)	149 (18.3)
1=Second	64 (22.4)	61 (19.9)	52 (23.2)	177 (21.7)
2=Third	53 (18.5)	77 (25.2)	48 (21.4)	178 (21.8)
3=Fourth	63 (22.1)	58 (19.0)	29 (12.9)	150 (18.4)
4=Richest	73 (25.5)	70 (22.8)	19 (8.5)	162 (19.8)
Gravidity				
Primigravidae, n (%)	112 (39.2)	111 (36.3)	89 (39.7)	312 (38.2)
Secundigravidae, n (%)	104 (36.4)	89 (29.1)	54 (24.1)	247 (30.3)
Gravidae-3, n (%)	53 (18.5)	66 (21.5)	43 (19.2)	162 (19.8)
Gravidae-4, n (%)	10 (3.5)	30 (9.4)	21 (9.4)	61 (7.5)
Multigravidae, (≥5)[1] n (%)	7 (2.4)	10 (3.3)	17 (7.6)	34 (4.2)
Gestation 1st ANC visit, median (IQR)*	24 (20-28)	24 (18-30)	20 (17-26)	24 (18-28)
MUAC, cm, mean (SD)	24.1 (2.7)	24.5 (2.8)	23.2 (2.1)	24.0 (2.7)
BP Systolic, ≥ 130mm Hg, n (%)	Not measured in this survey			
BP Diastolic, ≥ 90mm Hg, n (%)				
Bednet use (any), n (%)	195 (68.6)	104 (33.9)	79 (35.2)	378 (46.4)
Bednet use previous night, n (%)	181 (63.7)	94 (31.1)	46 (20.5)	321 (39.6)
ITN (any), n (%)	2 (0.7)	0	0	2 (0.3)
Used any drug to prevent malaria, n (%)	3 (1.1)	1 (0.3)	6 (2.7)	10 (1.2)
Reported using haematinics, n (%)	179 (63.0)	263 (85.9)	85 (37.9)	527 (64.7)

[♣] OBC= Other Backward Caste, GEN= General Caste, SC=Scheduled Caste, ST=Scheduled tribe; [**] = secondary & higher combined in survey 1; [1]= pregnancy number; SES=socioeconomic status;*IQR=Interquartile range (25th -75th percentiles);

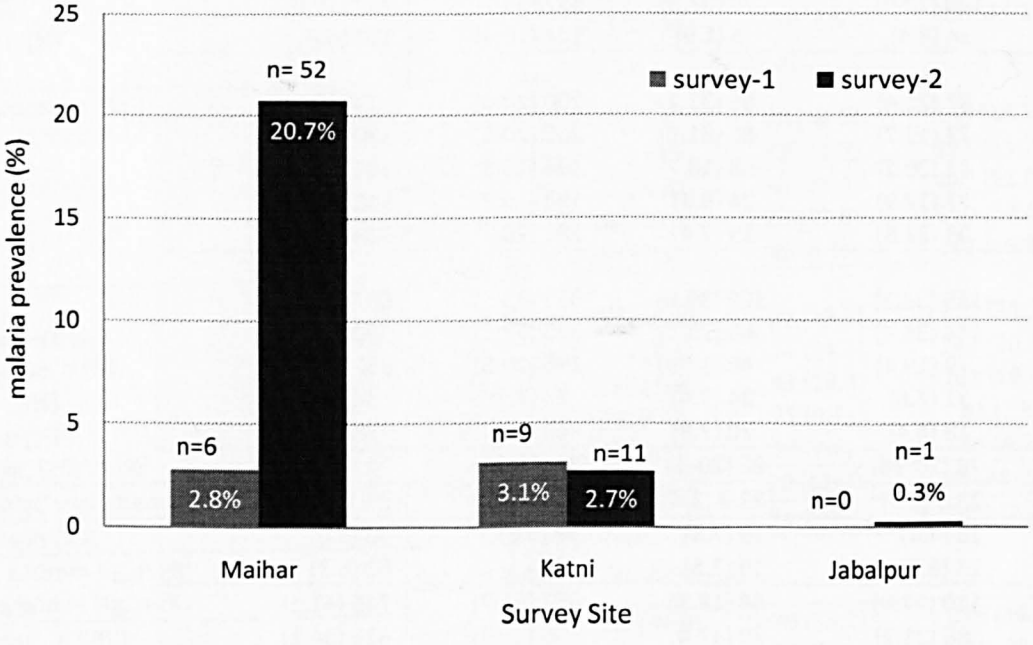
Post-monsoon survey -2 (6 weeks October-November 2006)				Survey 1 & 2
Jabalpur (n=337)	Katni (n=407)	Maihar (n=256)	Total (n=1000)	Total (n=1817)
23.4 (3.5)	24.1 (4.2)	23.2 (3.9)	23.7 (3.9)	23.7 (3.9)
30 (8.9)	27 (6.7)	34 (13.5)	91 (9.2)	166 (9.2)
284 (84.5)	325 (80.5)	189 (75.0)	798 (80.4)	1461 (80.9)
22 (6.6)	52 (12.9)	29 (11.5)	103 (10.4)	180 (10.0)
337 (100)	407 (100)	255 (99.6)	999 (99.9)	1814 (99.9)
267 (79.2)	256 (62.9)	48 (18.8)	571 (57.1)	1022 (56.3)
70 (20.7)	151 (37.0)	208 (81.3)	429 (42.9)	793 (43.7)
174 (51.6)	166 (40.8)	135 (52.7)	475 (47.5)	811 (44.8)
49 (14.5)	107 (26.3)	53 (20.7)	209 (20.9)	446 (24.7)
53 (15.7)	65 (16.0)	48 (18.8)	166 (16.6)	320 (17.7)
61 (18.1)	69 (16.9)	20 (7.8)	150 (15.0)	232 (12.8)
48 (14.2)	113 (27.8)	80 (31.3)	241 (24.1)	446 (24.6)
57 (16.9)	140 (34.4)	101 (39.5)	298 (29.8)	548 (30.2)
167 (49.6)	120 (29.4)	70 (27.3)	357 (35.7)	707 (39.0)
65 (19.3)	34 (8.4)	5 (1.9)	104 (10.4)	114 (6.3)
28 (8.3)	87 (21.4)	85 (33.2)	200 (20.0)	349 (19.2)
53 (15.7)	72 (17.7)	80 (31.3)	205 (20.5)	370 (20.4)
66 (19.6)	82 (20.1)	48 (18.7)	196 (19.6)	386 (21.3)
101 (29.9)	73 (17.9)	24 (9.4)	198 (19.8)	348 (19.2)
89 (26.4)	93 (22.8)	19 (7.4)	201 (20.1)	363 (20.0)
120 (35.6)	135 (33.2)	100 (39.0)	355 (35.5)	667 (36.7)
123 (36.5)	144 (35.4)	66 (25.8)	333 (33.3)	580 (31.9)
70 (20.8)	79 (19.4)	46 (17.9)	195 (19.5)	357 (19.7)
18 (5.3)	31 (7.6)	24 (9.4)	73 (7.3)	134 (7.4)
6 (1.8)	18 (4.4)	20 (7.8)	44 (4.4)	78 (4.3)
20 (16-28	28 (20-36)	28 (20-34)	24 (18-32)	24 (18-32)
23.7 (2.3)	23.5 (2.7)	22.9 (2.9)	23.4 (2.6)	23.7 (2.6)
13 (3.9)	18 (4.4)	19 (7.5)	50 (5.0)	50 (5.0)
20 (6.0)	23 (5.7)	19 (7.5)	62 (6.3)	62 (6.3)
209 (62.0)	110 (27.0)	48 (18.8)	367 (36.7)	745 (41.1)
180 (53.6)	86 (21.2)	29 (11.4)	295 (29.6)	616 (34.1)
0	9 (2.2)	0	9 (0.9)	11 (0.6)
1 (0.3)	7 (1.7)	2 (0.8)	10 (1.0)	20 (1.1)
168 (50.1)	211 (51.9)	95 (37.1)	474 (47.5)	1001 (55.0)

FIGURE 4: 1: PREVALENCE OF PLASMODIUM SPECIES IN ANTENATAL WOMEN IN SURVEYS 1&2



***Note**: prevalence estimated on microscopy results*

FIGURE 4: 2: MALARIA (ANY) PREVALENCE BY SITE & SURVEY IN WOMEN AT 3 ANTENATAL CLINICS



***Note:** the figures on top of the bars are the number of malaria positive cases by microscopy*

4.3A.2 Malaria, anaemia and malaria morbidity among women attending antenatal clinics

Malaria infection

The overall prevalence of malaria detected by microscopy, defined as the presence of asexual stages of *Plasmodium* species, was 4.5%. Of these 79.8% were mono-infections with *P.falciparum* and 20.2% were mono-infections with *P.vivax*. There were no mixed infections

detected by microscopy (Table 4: 2). In survey-1, conducted during the dry season months of April and May 2006, the prevalence of malaria infection among the antenatal women was 1.9%, of which 60% were *P.vivax* (1.1%) and 40% *P.falciparum* (0.7%) (Table 4: 2), (Figure 4: 1). During survey-2, conducted in October and November 2006 (post-monsoon transmission season), the overall prevalence of parasitaemia was 6.5% with 89% caused by *P.falciparum* (5.7%) and 11% by *P.vivax* (0.7%) (Figure 4: 1). Thus, *P.vivax* was the predominant species in the dry season and *P.falciparum* in the post-monsoon transmission season. Malaria prevalence varied remarkably between the three study sites ranging from a low 0.3% in Jabalpur, the urban site, to 20.7% in Maihar, the rural site (Figure 4: 2). The proportion of parasitaemia increased rather than decrease with increase in gravidity, however the differences were small and there was no statistically significant difference between gravidity groups (P=0.6) an in measures of trend (Figure 4: 3).

Anaemia

Nearly two thirds of women had mild anaemia (Hb <11g/dL) with similar prevalence in the two surveys (61.7% and 63.9%) (Table 4: 2). The mean (SD) haemoglobin level was 10.2g/dl (1.9). Moderate to severe anaemia (Hb<9g/dl) was present in 22% of the antenatal women. The proportion of moderate to severe anaemia was higher in the post-monsoon transmission season survey (26%) than in the dry season (17%) and higher in the rural sites than in the urban site. The presence of moderate to severe anaemia increased with gravidity and was 17.7% in primigravidae and 34.7% multigravidae (Figure 4: 4).

Morbidity

Self reported history of fever at any time during pregnancy was higher in the post-monsoon survey (42%) than in the dry season survey (25%) (Table 4: 2). Similarly, fevers in the week prior to enrolment were higher in the post-monsoon survey (23% versus 10.7%) but documented fever at visit was comparable between the two surveys (5% and 4%). The overall drug use to treat fevers any time during pregnancy was 23% and 7% in the week prior to the antenatal visit respectively, while the reported use of antimalarials was low in both surveys (2.7% and 0.9%).

Sub-Microscopic Parasitaemia (PCR)

Blood samples of 314 women had PCR assays performed (see chapter 7). This included 262 samples that were parasite negative by microscopy and RDT examination. The negative samples were selected randomly out of the 1000 women enrolled from the antenatal clinics in survey 2 to perform PCR to assess RDT sensitivity (details described in chapter 7). Sub-

TABLE 4: 2: MALARIA, ANAEMIA & MALARIA MORBIDITY IN 1817 WOMEN AT 3 ANTENATAL CLINICS IN 3 SITES (SURVEY 1&2)

Dry season survey 1 (6 weeks April - May 2006)				
Survey Site:	**Jabalpur** (n=286)	**Katni** (n=307)	**Maihar** (n=224)	**Total** (n=817)
Overall Parasitaemia‡, n (%)	0.0	9 (3.1)	6 (2.8)	15 (1.9)
P.falciparum, n (%)[1]	0.0	5 (55.6)	1 (16.7)	6 (40.0)
P.vivax, n (%)[1]	0.0	4 (44.4)	5 (83.3)	9 (60.0)
Mixed, n (%)[1]	0.0	0.0	0.0	0.0
Parasitaemia				
Primigravidae, n (%)	-	4 (3.7)	1 (1.1)	5 (1.6)
Secundigravidae, n (%)	-	3 (3.7)	3 (6.0)	6 (2.6)
Gravidae-3, n (%)	-	0	1 (2.4)	1 (0.7)
Gravidae-4, n (%)	-	2 (7.1)	1 (5.0)	3 (5.3)
Multigravidae (≥5)[2], n (%)	-	0	0.0	0.0
Haemoglobin (g/dL), mean (SD)	10.7 (1.6)	10.0 (1.6)	10.5 (1.9)	10.4 (1.7)
Mild anaemia (Hb <11g/dL), n (%)	159 (56.6)	216 (71.3)	100 (44.6)	499 (61.7)
Mod-severe anaemia (Hb <9g/dL), n (%)	34 (12.1)	65 (21.4)	41 (18.3)	140 (17.3)
Severe anaemia (Hb <7g/dL), n (%)	9 (3.2)	10 (3.3)	9 (4.0)	28 (3.5)
Moderate-severe anaemia (Hb <9g/dL)				
Primigravidae, n (%)	12 (10.8)	26 (23.4)	11 (12.4)	49 (15.8)
Secundigravidae, n (%)	11 (10.8)	21 (23.8)	11 (20.4)	43 (17.6)
Gravidae-3, n (%)	8 (15.4)	12 (18.1)	12 (27.9)	32 (19.9)
Gravidae-4, n (%)	3 (30.0)	4 (13.3)	5 (23.8)	12 (19.7)
Multigravidae (≥5)[2], n (%)	0	2 (25.0)	2 (11.8)	4 (12.9)
Fever[3] documented at visit, n (%)	11 (3.9)	14 (4.6)	17 (7.6)	420 (5.2)
Reported fever current pregnancy, n (%)	35 (12.4)	74 (24.5)	100 (44.6)	209 (25.8)
Used antimalarials for fever, n (%)	3 (1.0)	4 (1.3)	15 (6.7)	22 (2.7)
Used any drug for fever, n (%)	15 (5.4)	49 (16.2)	76 (33.9)	140 (17.4)
Reported fever previous week, n (%)	19 (6.7)	27 (8.9)	41 (18.3)	87 (10.7)
Used antimalarial for fever, n (%)	0	2 (0.65)	7 (3.1)	9 (1.1)
Used any drug for fever, n (%)	6 (2.1)	8 (2.7)	21(9.4)	35 (4.3)

‡=estimates by microscopy; [1]= Column percentages, presence of asexual parasitaemia; [2]= pregnancy number; [3]= axillary temperature ≥37.5°C

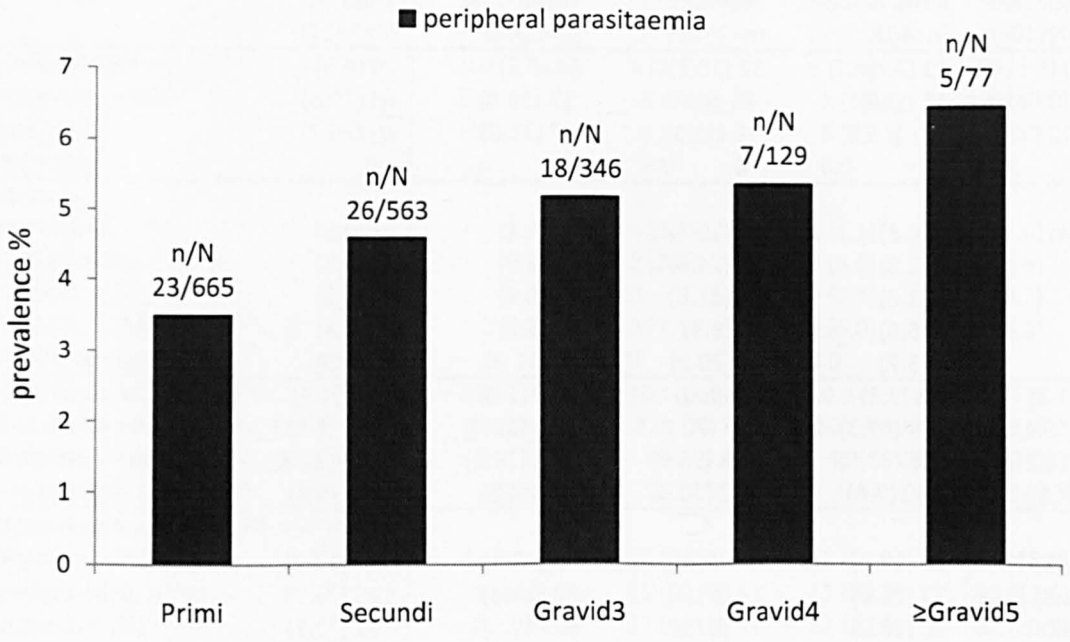
microscopic parasitaemia was detected in 64 out of the 262 negative samples: 42/64 were *P.falciparum* mono-infections, 17/64 *P.vivax* mono-infections and 5/64 mixed species. Sub-microscopic parasitaemia was prevalent in all the 3 sites and the detection rate in the 314 samples were: 9% *P.falciparum* (9/99) was detected in Jabalpur, where the prevalence was near zero by peripheral smear. In Katni and Maihar this was 12.9% (15/116) and 18.1% (18/99) *P.falciparum* mono-infections respectively. *P.vivax* mono-infection rates were 6.0%, (7/116) at Katni and 10.1%, (10/99) at Maihar, with 2 and 3 mixed species respectively

Post monsoon survey 2 (6 weeks Oct-November 2006)				Survey 1&2
Jabalpur (n=337)	Katni (n=407)	Maihar (n=256)	Total (n=1000)	Total (n=1817)
1(0.3)	11 (2.7)	52 (20.7)	64 (6.5)	79 (4.5)
0	11 (100)	46 (88.5)	57 (89.0)	63 (79.8)
1 (1)	0	6 (11.5)	7 (11.0)	16 (20.2)
0	0	0	0	0
1 (0.84)	1 (0.8)	16 (16.3)	18 (5.1)	23 (3.5)
0	4 (2.8)	16 (24.6)	20 (6.0)	26 (4.6)
0	3 (3.6)	14 (31.8)	17 (8.9)	18 (5.2)
0	2 (6.5)	2 (8.3)	4 (5.7)	7 (5.4)
0	1 (5.7)	4 (20.0)	5 (11.4)	5 (6.5)
10.6 (1.7)	9.8 (1.8)	9.5 (2.3)	10.0 (1.9)	10.2 (1.9)
186 (55.4)	274 (67.3)	179 (70.0)	639 (63.9)	1138 (63.0)
51 (15.2)	113 (27.8)	98 (38.3)	262 (26.2)	402 (22.3)
7 (2.1)	30 (7.4)	32 (12.5)	69 (6.9)	97 (5.4)
16 (13.5)	26 (19.3)	27 (27.0)	69 (19.5)	118 (17.7)
17 (13.8)	37 (25.6)	28 (42.4)	82 (24.6)	125 (21.7)
14 (20.0	30 (38.0)	22 (47.8)	66 (33.9)	98 (27.5)
1 (5.7)	11 (35.5)	11 (45.8)	23 (31.5)	35 (26.1)
3 (50.0)	9 (50.0)	10 (50.0)	22 (50.0)	26 (34.7)
3 (0.9)	9 (2.2)	31 (12.3)	43 (4.3)	85 (4.7)
157 (46.8)	99 (24.4)	165 (64.7)	421 (42.1)	630 (34.9)
18 (5.3)	0	9 (3.5)	27 (2.7)	49 (2.7)
124 (37.2)	83 (20.4)	66 (26.2)	273 (27.3)	413 (23.0)
30 (8.9)	118 (29.3)	81 (31.7)	229 (23.0)	316 (17.5)
1 (0.3)	4 (1.0)	2 (0.8)	7 (0.7)	16 (0.9)
11 (3.3)	57 (14.1)	25 (9.8)	93 (9.3)	128 (7.1)

4.3A.3 Malaria associated anaemia and morbidity in women attending ANC

P.falciparum: Women with *P.falciparum* infection had an increased risk of being anaemic (Hb <11g/dL) compared to aparasitaemic women (Table 4: 3). *P.falciparum* infected women were more likely to have moderate to severe anaemia (RR 2.6, 95%CI 2.1-3.4) compared to aparasitaemic women. The mean haemoglobin levels were 1.6g/dL lower in women with *P.falciparum* infection. There was no statistical difference in the risk of anaemia between

FIGURE 4: 3: MALARIA (ANY) PREVALENCE BY GRAVIDITY IN WOMEN AT 3 ANTENATAL CLINICS (SURVEY 1&2)



Note: Cochran-Armitage trend test $P = 0.09$; Somer's D R|C trend test with malaria as response and gravidity as predictor variable, $P = 0.09$

FIGURE 4: 4: ANAEMIA BY GRAVIDITY IN WOMEN AT 3 ANTENATAL CLINICS (SURVEYS 1&2)

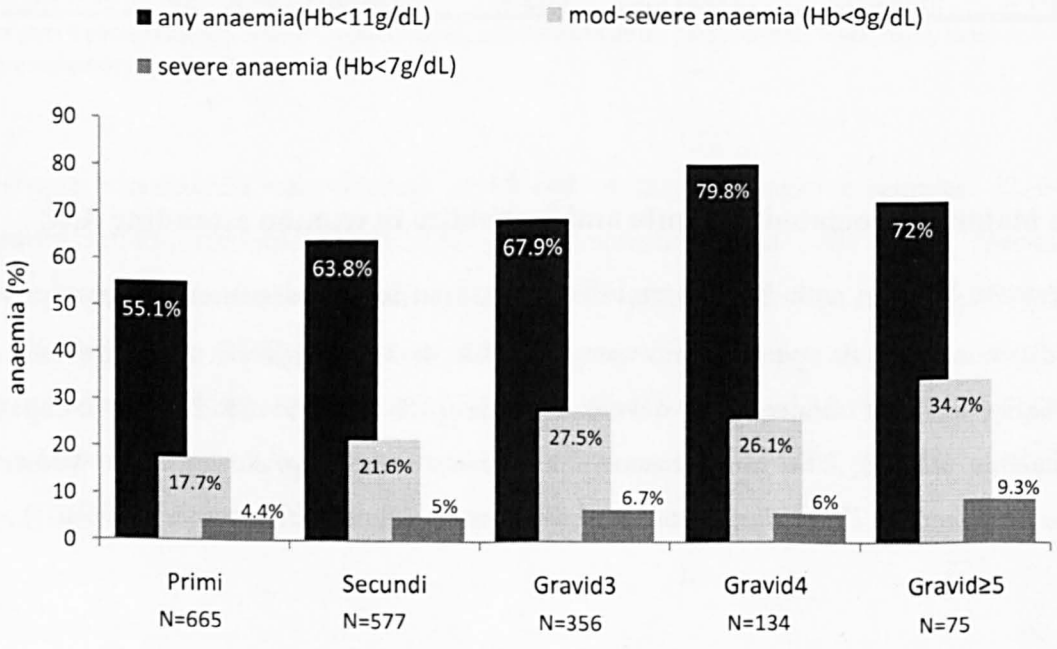
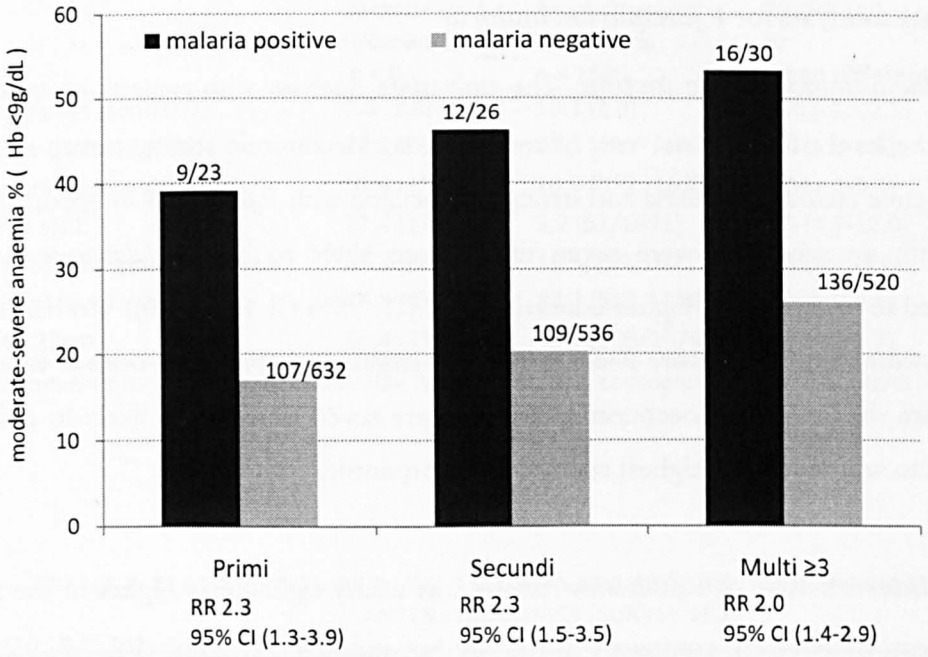


FIGURE 4: 5: ASSOCIATION BETWEEN MALARIA & MODERATE-SEVERE ANAEMIA



parasitaemic women who were asymptomatic (48.3%) and symptomatic (46.0%, documented fever or history of fever previous week) (RR 1.0, 95% CI 0.6-1.6). There was also no statistical difference in the association between any malaria and moderate to severe anaemia (<9g/dL) in primi, secundi and multigravidae (Figure 4: 5). Women with *P.falciparum* infection were at greater risk of a documented fever than aparasitaemic women (RR 7.5, 95% CI 4.7-12.0) (Table 4: 3). Women with a *P.falciparum* infection were 4 times more likely to report a history of fever in the past week, or twice as likely at any time during pregnancy. A MUAC <23cm was observed in 56.4% women with *P.falciparum* compared with 42.3% in aparasitaemic women. Overall 63.3% of parasitaemic women were symptomatic with either a history of fever in the previous week or with documented fever (not shown in the table).

P.vivax: The associations between *P.vivax* malaria and anaemia were not statistically significant (RR 1.1, 95%CI 0.8-1.6) although the proportion with anaemia among the *P.vivax* infected women was high (11/16, 68.8%, Table 4: 4). Women with *P.vivax* infection were 7 times more likely to have a documented fever than women without infection (RR 6.8, 95% CI 2.8-16.6), and were three times more likely to have a history of fever in the past week than aparasitaemic women.

4.3A.4 Risk factors of malaria in women at antenatal clinics

4.3A.4.1Univariate analysis for *P.falciparum malaria*

Education and Socio-demographic factors: The univariate analysis with malaria as outcome variable showed the level of education, area of residence, socioeconomic status, survey site and season were predictive factors of malaria and strongly associated with *P.falciparum* infection (Table 4: 5). Women with no schooling were seven times more likely to have *P.falciparum* malaria infection compared to women with higher education (PR 7.1, 95% CI 1.98-52.0). Women living in the rural areas were nine times more likely to develop malaria compared to women in urban areas. Women from the lowest socioeconomic quintile were seven times more likely to develop malaria compared to women in the highest socioeconomic quintile.

Other factors of interest: Risk of *P.falciparum* malaria was nearly eight times higher in the post-monsoon "cool season" (winter), compared to the dry hot months (summer) (PR 7.7, 95% CI 3.3-17.2). There was no significant association between age (categorized either into 2 age groups of ≤ 20 and ≥ 21 years, ≤30 and ≥31 years, or in 3 age groups as shown in the Table 4: 5), gravidity or caste and *P.falciparum* infection among women attending antenatal clinics. The risk of *P.facliparum* was 1.7 times (95%CI 1.06-2.84) higher in women with MUAC <23cm compared with women MUAC >23cm.

Morbidity factors: The presence of anaemia (Hb <11g/dL) and moderate-severe anaemia (Hb <9g/dL) was strongly predictive of the presence of *P.falciparum* malaria (PR of 4.1 and 4.4 respectively) (Table 4: 6). A history of drug use for fever, anytime in pregnancy and the use of antimalarial in the past week also indicated an association with *P.falciparum* infection.

Bednet use: Among women who reported using a bed net anytime in pregnancy, the risk of *P.falciparum* malaria was lower compared with women who did not use bed nets (PR 0.4, 95% CI 0.2 - 0.68). Similarly, women who reported sleeping under a bed net in the previous night had a lower risk of having *P.falciparum* infection (Table 4: 6).

Vivax malaria

Women living in the rural area and rural sites were at a higher risk of having *P.vivax* malaria compared to those living in urban areas (Table 4: 5). Fever at antenatal visit and history of fever in the previous week were predictive of *P.vivax* infection ([PR 8.4, 95% CI 2.7-25.2] [PR

TABLE 4: 3 ASSOCIATIONS OF ***P.FALCIPARUM*** MALARIA AND ANAEMIA, FEVER & MUAC IN WOMEN AT 3 ANTENATAL CLINICS (SURVEYS 1&2)

	Parasitaemic Women, % n = 63	**Aparasitaemic[♣] Women, %** n = 1680	**RR (95%CI) *or* mean difference**	***P-value***
Haemoglobin mean (SD)	8.6 (1.8)	10.2 (1.8)	-1.6 (-1.1,2.1)	<.0001
Anaemia (Hob<11g/dL)	87.3 (55/63)	61.8 (1037/1679)	1.4 (1.3-1.6)	<.0001
Moderate-severe anaemia[*]	55.6 (35/63)	20.9 (352 /1679)	2.6 (2.1-3.4)	<.0001
Fever at visit[††]	27.4 (17/62)	3.7 (61/1671)	7.5 (4.7-12.0)	<.0001
History of fever previous week	60.3 (38/63)	15.5(259/1671)	3.9 (3.1- 4.9)	<.0001
History of fever in pregnancy	74.6 (47/63)	33.3 (559/1680)	2.2 (1.9-2.6)	<.0001
MUAC <23cm	56.4 (35/62)	42.3 (709/1679)	1.3 (1.1-1.7)	0.02

[♣]= aparasitaemic for any malaria species; RR= Relative Risk; CI = confidence Interval; [*]Hb <9g/dL; [††]=axillary temperature ≥37.5^{0}C

TABLE 4: 4: ASSOCIATION OF ***P.VIVAX*** MALARIA AND ANAEMIA, FEVER & MUAC IN WOMEN AT 3 ANTENATAL CLINICS (SURVEY 1&2)

	Parasitaemic Women, % n = 16	**Aparasitaemic[♣] Women, %** n = 1680	**RR (95%CI) *or* mean difference**	***P-value***
Haemoglobin mean (SD)	10.2 (1.8)	10.2 (1.8)	0.0	0.9
Anaemia (Hb <11g/dL)	68.8 (11/16)	61.8 (1037/1679)	1.1 (0.8-1.6)	0.6
Moderate-severe anaemia[*]	12.5 (2/16)	20.9 (352/1679)	0.6 (0.2-2.1)	0.5[†]
Fever at visit[††]	25.0 (4/16)	3.7 (61/1671)	6.8 (2.8-16.6)	0.0026[†]
History of fever previous week	50.0 (8/16)	15.5 (259/1671)	3.2 (2.0-5.3)	0.0002
History of fever in pregnancy	50.0 (8/16)	33.3 (559/1680)	1.4 (0.8-2.4)	0.2
MUAC <23cm	37.5 (6/16)	42.3 (709/1679)	0.8 (0.5-1.6)	0.7

[♣]=aparasitaemic for any malaria species; RR= Relative Risk; CI =Confidence Interval; [*]Hb <9g/dL; [††]=axillary temperature ≥37.5^{0}C; [†] =Fisher's Exact test;

5.3, 95% CI 2.0-14.0]). None of the other factors assessed in the univariate analysis showed a statistically significant association with *P.vivax* malaria (Table 4: 6).

Sub-microscopic infections: A comparison of the demographic and morbidity parameters for sub-patent, patent and negative infections are shown in Tables 4: 7 & 4: 8. Sub-patent infections were significantly associated with education, area of residence and in the rural study sites. Compared with women without malaria, the risk of sub-patent infection was greater in women with a documented fever (temp≥37.5^{0}C).

TABLE 4: 5: UNIVARIATE ANALYSIS OF DEMOGRAPHIC FACTORS ASSOCIATED WITH MALARIA INFECTIONS IN WOMEN AT 3 ANTENATAL CLINICS (SURVEY 1&2)

	any malaria infection		
	Parasitaemia |n (%) n=79 (positive)	**PR (95% CI)**	***P-value***
Age group			
<20 yr	7 | 165 (4.2)	0.7 (0.28-1.87)	0.67
20-29 yr	62 | 1424 (4.4)	0.7 (0.39-1.43)	
>30 yr	10 | 172 (5.8)	reference	
Caste Category[†]			
OBC	38 | 789 (4.8)	1.4 (0.78-2.51)	
SC	17 | 306 (5.6)	1.6 (0.82-3.20)	0.51
ST	9 | 228 (3.9)	1.2 (0.51-2.60)	
GEN	15 | 440 (3.4)	reference	
Education			
No Schooling	32 | 432 (7.4)	8.4 (1.15-60.59)	
Primary	29 | 533 (5.4)	6.1 (0.84-44.66)	0.0002
Secondary	17 | 691 (2.5)	2.7 (0.37-20.68)	
Higher	1 | 113 (0.9)	reference	
Residence			
rural	66 | 772 (8.6)	6.6 (3.72-12.5)	<0.0001
urban	13 | 997 (1.3)	reference	
SES			
Poorest	27 | 342 (7.9)	5.5 (2.14-14.14)	
Second	24 | 356 (6.7)	4.7 (1.81-12.19)	<0.0001
Third	15 | 379 (3.9)	2.7 (1.01-7.52)	
Fourth	8 | 344 (2.3)	1.6 (0.53- 4.91)	
Richest	5 | 349 (1.4)	reference	
Gravidity			
Primigravidae	23 | 655 (3.5)	0.5 (0.21-1.38)	
Secundigravidae	26 | 563 (4.6)	0.7 (0.28-1.79)	0.57
Gravidae-3	18 | 346 (5.2)	0.8 (0.31-2.10)	
Gravidae-4	7 | 129 (5.4)	0.8 (0.30-2.51)	
Multigravidae (≥5)[*]	5 | 77 (6.5)	reference	
Survey Site			
Maihar	58 | 467 (12.4)	75.6 (10.5-544.05)	<0.0001
Katni	20 | 694 (2.9)	17.6 (2.36-130.38)	
Jabalpur	1 | 609 (0.2)	reference	
Season			
Cool humid months	64 | 989 (6.5)	3.36 (1.93-5.85)	<0.0001
Hot dry months	15 | 780 (1.9)	reference	

PR=Prevalence Ratio, CI= Confidence Interval; [†]=OBC=other backward caste, SC=scheduled caste, ST=scheduled tribe, GEN=general caste; [*]=pregnancy number; SES=socioeconomic status;

P.falciparum infection			*P.vivax infection*		
Parasitaemia |n (%) n=63 (positive)	**PR (95% CI)**	***P-value***	**Parasitaemia |n (%)** n=16 (positive)	**PR (95% CI)**	***P-value***
6 | 164 (3.7)	0.7 (0.27-2.19)		1 | 159 (0.6)	0.5 (0.04-5.63)	0.85
49 | 1411 (3.5)	0.7 (0.33-1.53)	0.71	13 | 1375 (0.9)	0.8 (0.17-3.41)	
8 | 170 (4.7)	reference		2 | 164 (1.2)	reference	
29 | 780 (3.7)	1.3 (0.65-2.30)		9 | 760 (1.2)	2.5 (0.54-11.64)	
13 | 302 (4.3)	1.5 (0.68-3.08)	0.82	4 | 293 (1.4)	2.9 (0.53-15.80)	0.41
8 | 227 (3.5)	1.2 (0.49-2.81)		1 | 220 (0.5)	0.9 (0.08-10.60)	
13 | 438 (2.9)	reference		2 | 427 (0.5)	reference	
27 | 427 (6.3)	7.1 (1.98-52.00)		5 | 405 (1.2)	-	
23 | 527 (4.4)	4.9 (0.67-36.11)	0.0002	6 | 510 (1.2)	-	0.56
12 | 686 (1.8)	1.9 (0.25-15.10)		5 | 679 (0.7)	-	
1 | 113 (0.9)	reference		0 | 112	reference	
55 | 761 (7.3)	9.0 (4.34-20.00)	<.0001	11 | 717 (1.5)	3.0 (1.06-9.09)	.04
8 | 992 (0.8)	reference		5 | 989 (0.5)	reference	
20 | 335 (5.9)	6.9 (2.07-23.02)		7 | 322 (2.2)	3.7 (0.78-17.97)	
22 | 354 (6.2)	7.1 (2.17-23.79)		2 | 334 (0.6)	1.0 (0.14-7.30)	
12 | 376 (3.2)	3.7 (1.05-12.97)	0.0001	3 | 367 (0.8)	1.4 (0.23-8.41)	0.15
6 | 342 (1.8)	2.0 (0.51-8.04)		2 | 338 (0.6)	1.0 (0.14-7.22)	
3 | 347 (0.7)	reference		2 | 346 (0.6)	reference	
18 | 659 (2.8)	0.4 (0.16-1.11)		5 | 637 (0.8)	-	
21 | 558 (3.8)	0.5 (0.22-1.49)		5 | 542 (0.9)	-	
16 | 344 (4.6)	0.7 (0.27-1.89)	0.30	2 | 330 (0.6)	-	0.09
3 | 125 (2.4)	0.4 (0.09-1.50)		4 | 126 (3.2)	-	
5 | 77 (6.5)	reference		0 | 172	reference	
47 | 456 (10.3)	---	<0.0001	11 | 420 (2.6)	16.4 (2.06-123.4)	0.0002
16 | 690 (2.3)			4 | 678 (0.6)	3.5 (0.4-32.05)	
0 | 608	reference		1 | 609 (0.2)	reference	
57 | 982 (5.8)	7.7 (3.33-17.2)	<0.0001	7 | 932 (0.8)	0.6 (0.24-1.72)	0.37
6 | 771 (0.78)	reference		9 | 774 (1.2)	reference	

TABLE 4: 6: UNIVARIATE ANALYSIS OF MALARIA MORBIDITY AND CONTROL MEASURES ASSOCIATED WITH MALARIA INFECTIONS IN WOMEN AT 3 ANTENATAL CLINICS (SURVEY 1&2)

	Any malaria infection		
	Parasitaemia | n (%) n=79 (positive)	**PR (95% CI)**	***P-value***
Anaemia (Hb <11g/dL)			
Yes	66 | 1108 (5.9)	3.0 (1.67-5.43)	<0.0001
No	13 | 659 (1.9)	reference	
Mod-severe anaemia (Hb <9g/dL)			
Yes	37 | 389 (9.5)	3.1 (2.03-4.78)	<0.0001
No	42 | 1378 (3.1)	reference	
Fever at visit††			
Yes	21 | 82 (25.6)	7.5 (4.8-11.79)	<0.0001
No	57 | 1676 (3.4)	reference	
History of fever past week			
Yes	46 | 306 (15.0)	6.6 (4.3-10.16)	<0.0001
No	33 | 1452 (2.3)	reference	
Used antimalarials past week			
Yes	5 | 16 (31.3)	7.3 (3.45-15.81)	<0.0001
No	74 | 1751 (4.2)	reference	
Used any drug past week			
Yes	21 | 128 (16.4)	4.6 (2.88-7.31)	<0.0001
No	58 | 1624 (3.6)	reference	
History of fever in pregnancy			
Yes	55 | 614 (8.9)	4.3 (2.67-6.83)	<0.0001
No	24 | 1145 (2.0)	reference	
Used antimalarials in pregnancy			
Yes	4 | 49 (8.2)	1.8 (0.71-4.91)	<0.27†
No	75 | 1719 (4.4)	reference	
Used any drug in pregnancy			
Yes	26 | 405 (6.4)	1.6 (1.03-2.57)	0.04
No	53 | 1345 (3.9)	reference	
Used any drug to prevent malaria			
Yes	2 | 20 (10.0)	2.2 (0.59-8.49)	0.22†
No	77 | 1726 (4.5)	reference	
Used haematinics			
Yes	34 | 971 (3.5)	0.6 (0.4-0.95)	0.03
No	45 | 1726 (5.7)	reference	
Used bednet in pregnancy			
Yes	18 | 723 (2.5)	0.4 (0.25-0.71)	0.0008
No	61 | 1044 (5.8)	reference	
Used bednet previous night			
Yes	13 | 598 (2.2)	0.4 (0.21-0.68)	0.0008
No	66 | 1162 (5.7)	reference	
MUAC (<23cm)			
Yes	41 | 750 (5.5)	1.5 (0.96-2.29)	0.07
No	37 | 1007 (3.7)	reference	
BP Systolic (≥130mm Hg)			
Yes	1 | 46 (2.2)	0.3 (0.04-2.31)	0.35†
No	62 | 936 (6.6)	reference	
BP Diastolic (≥90mm Hg)			
Yes	3 | 59 (5.1)	0.8 (0.25-2.41)	1.00†
No	60 | 923(6.5)	reference	

PR= Prevalence Ratio; CI= Confidence Interval; † = Fisher's Exact test; ††=axillary temperature ≥37.5^{0}C;

P.falciparum infection			*P.vivax infection*		
Parasitaemia | n (%) n=63 (positive)	**PR (95%CI)**	***P-value***	**Parasitaemia | n (%)** n=16 (positive)	**PR (95% CI)**	***P-value***
55 | 1097 (5.0) 8 | 654 (1.2)	4.1 (1.96-8.55) reference	<0.0001	11 | 1053 (1.0) 5 | 651 (0.8)	1.3 (0.47-3.89) reference	0.56
35 | 387 (9.0) 28 | 1364 (2.1)	4.4 (2.71-7.14) reference	<0.0001	2 | 354 (0.6) 14 | 1350 (1.0)	0.5 (0.12-2.38) reference	0.54†
17 | 78 (21.8) 45 | 1664 (2.7)	8.1 (4.84-13.41) reference	<0.0001	4 | 65 (6.2) 12 | 1631 (0.7)	8.4 (2.77-25.23) reference	<0.003†
38 | 298 (12.8) 25 | 1444 (1.7)	7.4 (4.51-12.01) reference	<0.0001	8 | 268 (2.9) 8 | 1427 (0.6)	5.3 (2.01-14.06) reference	0.0002
5 | 16 (31.3) 58 | 1735 (3.3)	9.3 (4.33-20.18) reference	<0.0001	0 | 11 16 | 1693 (0.9)	---	
19 | 126 (15.1) 44 | 1610 (2.7)	5.5 (3.32-9.15) reference	<0.0001	2 | 109 (1.8) 14 | 1580 (0.9)	2.1 (0.47-8.99) reference	0.27†
47 | 606 (7.8) 16 | 1137 (1.4)	5.5 (3.15-9.63) reference	<0.0001	8 | 567 (1.4) 8 | 1129 (0.7)	1.9 (0.75-5.27) reference	0.15
3 | 48 (6.3) 60 | 1704 (3.5)	1.8 (0.57-5.45) reference	0.24†	1 | 46 (2.2) 15 | 1659 (0.9)	2.4 (0.32-17.81) reference	0.35†
22 | 401 (5.5) 41 | 1333 (3.1)	1.8(1.07-2.95) reference	0.02	4 | 383 (1.0) 12 | 1304 (0.9)	1.1 (0.36-3.49) reference	0.76†
2 | 20 (10.0) 61 | 1710 (3.6)	2.8 (0.73-10.68) reference	0.16†	0 | 18 16 | 1665 (0.9)	---	
25 | 962 (2.6) 38 | 788 (4.8)	0.5 (0.32-0.88) reference	0.01	9 | 946 (0.9) 7 | 757 (0.9)	1.0 (0.38-2.74) reference	0.95
13 | 718 (1.8) 50 | 1033 (4.8)	0.4 (0.2-0.68) reference	0.001	5 | 710 (0.7) 11 | 994 (1.1)	0.6 (0.22-1.82) reference	0.39
10 | 595 (1.7) 53 | 1149 (4.6)	0.4 (0.18-0.71) reference	0.002	3 | 588 (0.5) 13 | 1109 (1.2)	0.4 (0.2-1.52) reference	0.29†
35|744 (4.7) 27|977 (2.7)	1.7 (1.06-2.84) reference	0.02	6|715 (0.84) 10|980 (1.02)	0.8 (0.30-2.25)	0.70
0| 45 56 | 930 (6.0)	--	0.09	1 | 46 (2.2) 6|874 (0.7)	3.1 (0.39-25.93) reference	0.31†
2 | 58 (3.5) 54 | 917 (5.9)	0.6 (0.14-2.34) reference	0.76†	1 | 57 (1.8) 6 | 869 (0.7)	2.5 (0.31-20.74) reference	0.40†

TABLE 4: 7:SUB-ANALYSIS OF 314 SAMPLES OF WOMEN ATTENDING THE ANC: DEMOGRAPHIC PARAMETERS WITH PATENT & SUB-PATENT *P.FALCIPARUM* MONO-INFECTIONS AND WOMEN WITHOUT MALARIA (NEGATIVE BY SMEAR, RDT & PCR)

	Negative* (n=218)	**Patent** (n=34)	**Sub-patent** (n=38)	**Patent *vs* Negative**	**Sub-patent *vs* Negative**	**Patent *vs.* Sub-patent**
DEMOGRAPHICS				*P-value*	*P-value*	*P-value*
Age group						
<20 yr	21|215 (9.8)	2|34 (5.8)	8|38 (21.1)			
20-29 yr	171|215 (79.5)	29|34 (85.3)	25|38 (65.8)	0.7	0.1	0.1
>30 yr	23|215 (10.7)	3|34 (8.8)	5|38 (13.2)			
Caste Category**						
OBC	104|218 (47.7)	17|34 (50.0)	17|38 (44.7)			
SC	39|218 (17.9)	7|34 (20.5)	2|38 (5.3)	0.9	0.1	0.1
ST†	36|218 (16.5)	4|34 (11.7)	7|38 (18.4)			
GEN	39|218 (17.9)	6|34 (17.6)	12|38 (31.6)			
Education						
No Schooling	57|218 (26.2)	12|34 (35.3)	10|38 (26.3)			
Primary	57|218 (26.2)	14|34 (41.2)	17|38 (44.7)	0.02	0.04	0.7
≥Secondary	104|218 (47.7)	8|34 (23.5)	11|38 (28.9)			
Residence						
rural	80|218 (36.7)	31|34 (91.2)	21|38 (55.3)	<0.0001	0.03	0.001
urban	138|218 (63.3)	3|34 (8.8)	17|38 (44.7)			
SES						
Poorest	42|218 (19.3)	6|34 (17.6)	11|38 (28.9)			
Second	39|218 (17.9)	16|34 (47.1)	7|38 (18.4)			
Third	44|218 (20.2)	9|34 (26.5)	9|38 (23.7)	0.0002	0.5	0.02
Fourth	43|218 (19.7)	3|34 (8.8)	5|38 (13.2)			
Richest	50|218 (22.9)	0|34	6|38 (15.8)			
Gravidity						
Primigravidae	77|218 (35.3)	10|34 (29.4)	14|38 (36.8)			
Secundigravidae	74|218 (33.9)	14|34 (41.2)	12|38 (31.6)	0.6	0.9	0.6
Gravidae≥3†	67|218 (30.7)	10|34 (29.4)	12|38 (31.6)			
Survey Site						
Maihar	40|218 (18.3)	27|34 (79.4)	16|38 (42.1)			
Katni	88|218 (40.4)	7|34 (20.6)	12|38 (34.2)	<0.0001	0.004	0.001
Jabalpur	90|218 (41.3)	0|34	9|38 (9.1)			

*Negative for *P.vivax*;**OBC=other backward caste, SC=scheduled caste, ST=scheduled tribe, GEN=general caste;†=pregnancy number;

TABLE 4: 8:SUB-ANALYSIS OF 314 SAMPLES OF WOMEN ATTENDING THE ANC: MORBIDITY FACTORS WITH PATENT AND SUB-PATENT *P.FALCIPARUM* MONO-INFECTION AND WOMEN WITHOUT MALARIA (NEGATIVE BY SMEAR, RDT & PCR)

Morbidity parameters	**Negative*** (n=218)	**Patent** (n=34)	**Sub-patent** (n=38)	**Patent *vs.* negative**	*P-value*	**Sub-patent *vs.* negative**	*P-value*	**Patent *vs.* Sub-patent**	*P-value*
				Difference in means (95% CI) & RR (95% CI)					
Maternal Hb in g/dL, mean (SD)	10.1 (1.9)	8.7 (1.8)	9.8 (2.10	-1.4 (-2.1,-0.6)	0.0002	-0.3 (-0.9, 0.5)	0.5	-1.1 (-2.1, 0.2)	0.01
Mild anaemia (Hb <11.0g/dL), n (%)	142|218 (65.1)	29|34 (85.3)	22|38 (57.9)	1.3 (1.1, 1.5)	<0.0001	0.9 (0.6, 1.2)	0.4	1.5 (1.1, 2.0)	0.01
Moderate-severe anaemia (Hb <9.0g/dL), n (%)	53|218 (24.3)	20|34 (58.8)	14|38 (36.8)	2.4 (1.7, 3.4)	0.01	1.5 (0.9, 2.4)	0.1	1.5 (1.0, 2.6)	0.1
Moderate-severe anaemia (Hb <8.0g/dL), n (%)	25|218 (11.4)	14|34 (41.2)	5|38 (13.2)	3.6 (2.1, 6.2)	<0.0001	1.1 (0.5, 2.8)	0.7	3.1 (1.3, 7.7)	0.01
Fever at visit†	4|216 (1.9)	10|34 (29.4)	4|38 (10.5)	15.5 (5.2, 47.8)	<0.0001	5.5 (1.4, 21.7)	0.01	2.7 (0.9, 8.0)	0.04
History of fever past week	47|214 (21.9)	20|34 (58.8)	9|38 (23.7)	2.6 (1.8, 3.9)	<0.0001	1.1 (0.5, 2.0)	0.8	2.4 (1.3, 4.6)	0.002
History of fever in pregnancy	103|218 (47.2)	27|34 (79.4)	11|38 (28.9)	1.6 (1.3, 2.0)	0.001	0.6 (0.3, 1.0)	0.03	2.7 (1.6, 4.6)	<0.0001
Antimalarial use past week	2|218 (0.9)	1|34 (2.9)	0|38	3.2 (0.3, 34.3)	0.3	---	---	---	---
Any drug in pregnancy	68|213 (31.9)	12|34 (35.3)	6|38 (15.8)	1.1 (0.7, 1.8)	0.6	0.5 (0.2, 1.1)	0.04	2.2 (0.9, 5.3)	0.05
Used any drug past week	21|213 (9.8)	9|34 (26.5)	4|37 (10.8)	2.6 (1.3, 5.3)	0.01	1 (0.4, 3.0)	0.8	2.4 (0.8, 7.2)	0.08
Antimalarial in pregnancy	6|216 (2.7)	1|34 (2.9)	1|38 (2.6)	1 (0.1, 8.3)	0.9	1 (0.1, 2.7)	0.9	1.1 (0.1, 17.2)	0.9

*negative for *P.vivax;* †=axillary temperature ≥37.5^{0}C; RR= Relative Risk; CI= Confidence Interval

4.3A.4.2 Multivariate predictor model of *P.falciparum* malaria

The variables included in the multivariate predictor model of *P.falciparum* infection in antenatal clinic women were: education, residence, socio-economic status, season, gravidity, moderate-severe anaemia, fever at visit, history of fever in past week and in pregnancy, use of antimalarial, MUAC <23cm, iron-supplement and bednet previous night (Table 4: 9). The following variables were predictive of malaria in the final model: residence, season, anaemia, documented fever, and history of fever (Table 4: 10). Residence in rural area was associated with a five-fold increase in the risk of *P.falciparum* malaria (PR 5, 95% CI 2.34-10.7) compared to urban residence. *P.falciparum* infection was 4.5 fold higher in the post-monsoon season than in dry season or peak of the monsoon period. Women with fever at visit, history of fever in the past week or fever anytime during pregnancy were twice as likely to have *P.falciparum* compared to women without fevers. Gravidity was not associated with *P.falciparum* malaria with primigravidae being equally at risk compared to gravidae ≥3 (Table 4: 10).

P.vivax: A model for identifying risk factors for *P.vivax* was not possible because of the low number of positive cases and its associated risk factors.

4.3A.5 Malaria Risk Index for women at antenatal clinic

There were six determinants of *P.falciparum* identified in the multivariate model. These were used to develop a simple composite malaria risk index to identify whether the risk of malaria increased with a combination of risk factors. The frequency of malaria among women with different risk factor combination groups were as follows: in women with one risk factor, malaria prevalence was 0.35% and if five risk factors were present, malaria prevalence was 45% (Figure 4: 6). The risk index in women between sites was also different: in women in Maihar if four risk factors were present the prevalence of malaria was 22.4% and if five factors were present it was 51.3% (20/39). In Katni the presence of four risk factors showed a prevalence of 6.4% and it was 30.0% (3/10) if five risk factors were present (not shown as a figure). Since there was only one case in Jabalpur it was not possible to perform risk index for this site.

TABLE 4: 9: MULTIVARIATE MODEL OF MALARIA RISK FACTORS IN WOMEN AT ANTENATAL CLINICS (PARAMETERS IN INITIAL MODEL)

Dependent variable: ***P.falciparum***

Variable	Level	PR††	95% CI	*P-value*
Education	No schooling	1.6	0.79-3.25	
	Primary	1.6	0.81-3.19	0.37
	≥Secondary	reference		
Residence	Rural	4.7	2.14-10.20	0.0001
	urban	reference		
Socio-economic status	Poorest	0.7	0.37-1.31	
	Second	1.2	0.66-2.11	0.14
	Third and higher	reference		
Season	Cool-months	4.5	1.95-10.47	0.0004
	Summer&monsoon	reference		
Gravidity	Primigravidae	1.0	0.55-1.82	
	Secundigravidae	1.2	0.74-1.98	0.70
	Multigravidae (≥3)†	reference		
Moderate-severe anaemia**	Yes	1.7	0.97-2.92	0.06
	No	reference		
Fever at visit††	Yes	2.2	1.29-3.73	0.003
	No	reference		
History of fever past week	Yes	2.5	1.45-4.16	0.001
	No	reference		
History of fever in pregnancy	Yes	2.4	1.34-4.23	0.003
	No	reference		
Use of antimalarial	Yes	0.7	0.18-2.44	0.53
	No	reference		
MUAC (<23cm)	Yes	1.29	0.80-2.06	0.28
	No	reference	reference	
Use of iron-supplement	No	1.2	0.77-1.93	0.39
	Yes	reference		
Use of bed net previous night	No	1.2	0.59-2.29	0.65
	Yes	reference		

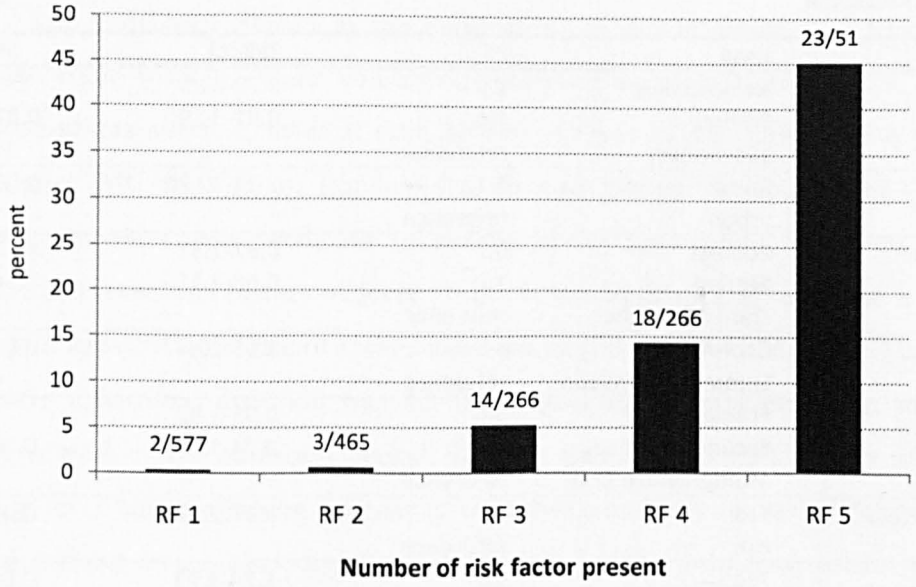
††PR=prevalence Ratio; CI= Confidence Interval; †=pregnancy number; **=Hb <9g/dL; ††=axillary temperature ≥37.5^{0}C;

TABLE 4: 10: MALARIA RISK FACTORS IN WOMEN AT ANTENATAL CLINICS **PARAMETERS IN FINAL MULTIVARIATE MODEL**

Variable	Level	PR††	95% CI	*P-value*
Residence	Rural	5.0	2.34-10.7	<0.0001
	Urban	reference		
Season	Cool months	4.5	1.95-10.5	<0.0001
	Summer & monsoon	reference		
Gravidity	Primigravidae	0.9	0.53-1.71	
	Secundigravidae	1.0	0.65-1.62	0.96
	Multigravidae (≥3) †	reference		
Moderate-severe anaemia**	Yes	1.8	1.05-3.02	0.03
	No	reference		
Fever at visit††	Yes	2.2	1.32-3.75	0.001
	No	reference		
History of fever past week	Yes	2.4	1.43-4.12	0.001
	No	reference		
History of fever in pregnancy	Yes	2.3	1.31-4.16	0.004
	No	reference		

††PR=prevalence Ratio; CI= Confidence Interval; †=pregnancy number; **=Hb <9g/dL; ††= axillary temperature ≥37.5^{0}C;

FIGURE 4: 6: MALARIA RISK INDEX FOR WOMEN AT ANTENATAL CLINIC



***Note**: figures above the bars represent the number of malaria cases and the number of women in each composite risk factor group; RF= risk factor; RF= residence, season, moderate-severe anaemia, fever at visit; h/o fever past week & h/o fever in pregnancy*

4.3B: Results in Delivery Unit Surveys

4.3B.1 Characteristics of enrolled women

2696 women were enrolled in the delivery module (Figure 4:7). This included 414 women during the first 6-week dry season survey in April-May 2006 (survey-1); 611 during the 6-week post-monsoon season survey in October and November 2006 (survey-2); and 1671 women during the extended survey from the end of November 2006 to the end of September 2007(survey-3). Thus, 1025 women were enrolled during the two 6-week rapid assessment surveys 1 and 2, from the three study sites. Because of negligible malaria in Jabalpur during surveys 1 and 2, only Maihar and Katni were included in the extended survey. The characteristics of the study participants and analyses of malaria prevalence include data from all the three surveys and sites (n=2696) (Tables 4: 11 & 4: 12). The subsequent analyses (which provided continuous year-round information from October 2006-September 2007) include data of the surveys 2 and 3 from Katni and Maihar only (N=2080).

The characteristics of the 2696 women enrolled in the delivery module were similar in age, marital status, educational status, and gravidity compared to women enrolled in the antenatal module (Tables 4: 11 & 4: 12). One notable exception was that in the delivery units in Maihar and Katni the proportion of rural women increased considerably during the extended survey, following the introduction of the ASHA program in this region, which promoted delivery at health facilities (see chapter 3). Women tended to be short in stature, with a mean height (SD) of 151cm (4.6) and lean, with a mean MUAC (SD) of 23.0cm (2.1). Blood pressure recordings showed that 20% of delivering women had high blood pressures (systolic $\geq$ 130mm Hg, diastolic $\geq$90mm Hg) (Table 4: 12). The same staff performed the blood pressure recordings using the same machines, but the observations in Katni and Maihar during survey-3 showed an increase in the proportion of women with high blood pressure over time. This may also have reflected the introduction of the ASHA program, resulting in an increase in referrals of high-risk pregnancies and encouragement by the Indian Government to deliver at health facilities.

Self reported frequency of Antenatal clinic visits

Nearly 90% of women reported at least one antenatal visit/contact by the time they came for delivery (Table 4: 12). This number was equally high in all three sites. The median number of antenatal visits was 2 (interquartile range 2-3). 36.6% women reported attending 2 antenatal visits prior to delivery and 32% reported 3 antenatal visits (Figure 4: 8).

FIGURE 4: 7: DIAGRAM SHOWING WOMEN ENROLLED IN THE DELIVERY MODULE DURING THE 3 SURVEYS

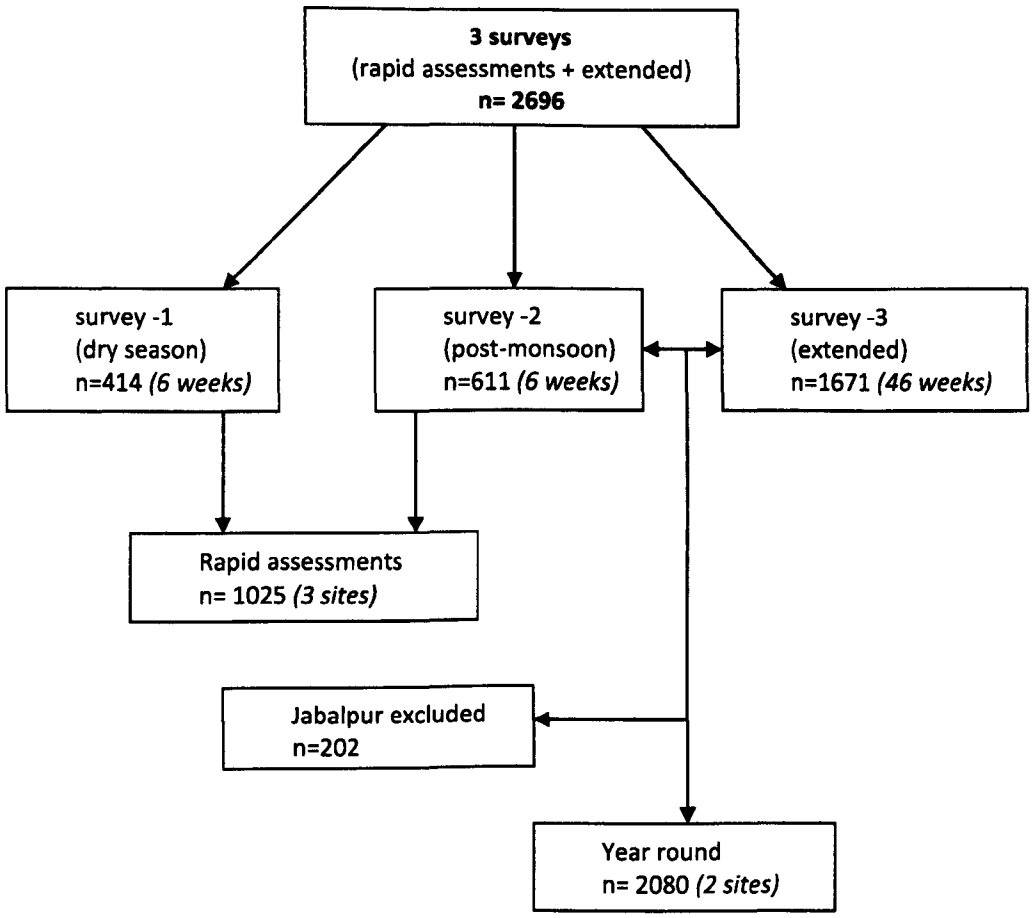
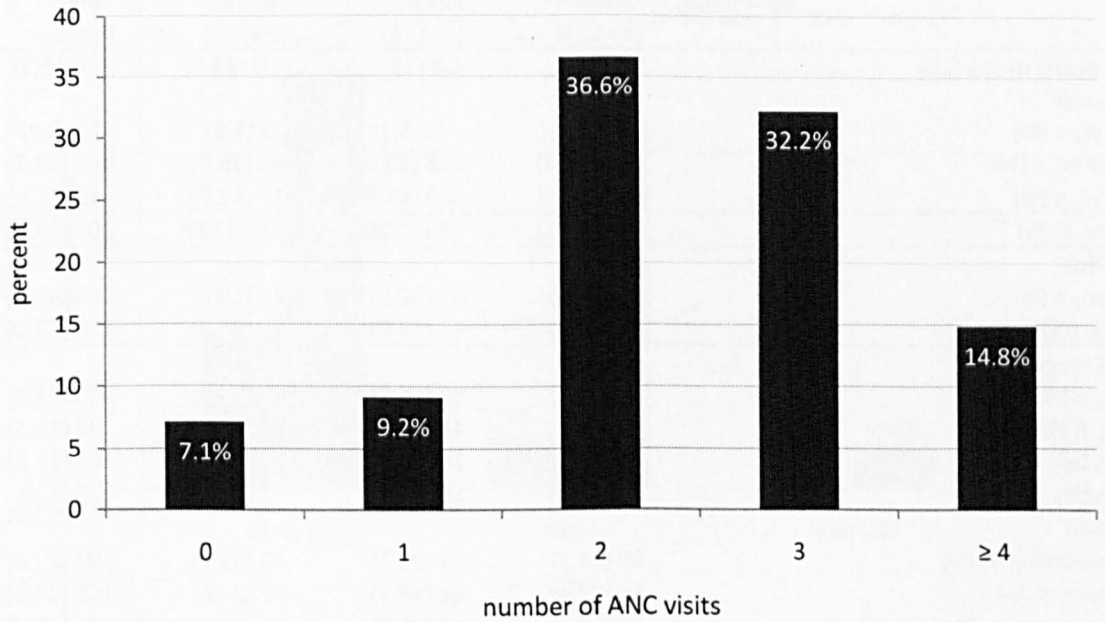


FIGURE 4: 8: ANTENATAL ATTENDANCE PRIOR TO DELIVERY



Self-reported history of malaria and anaemia control measures

In women enrolled from the delivery units, 27.5% used a bed net anytime and 22% reported using it the previous night (Table 4: 12). This was lower than that found among women at the antenatal clinics. The use of ITNs or drugs to prevent malaria in pregnancy was small (0.24%). However, 86.8% women acknowledged using iron and folic acid supplementation[6] compared to 55.3% in the antenatal clinic module.

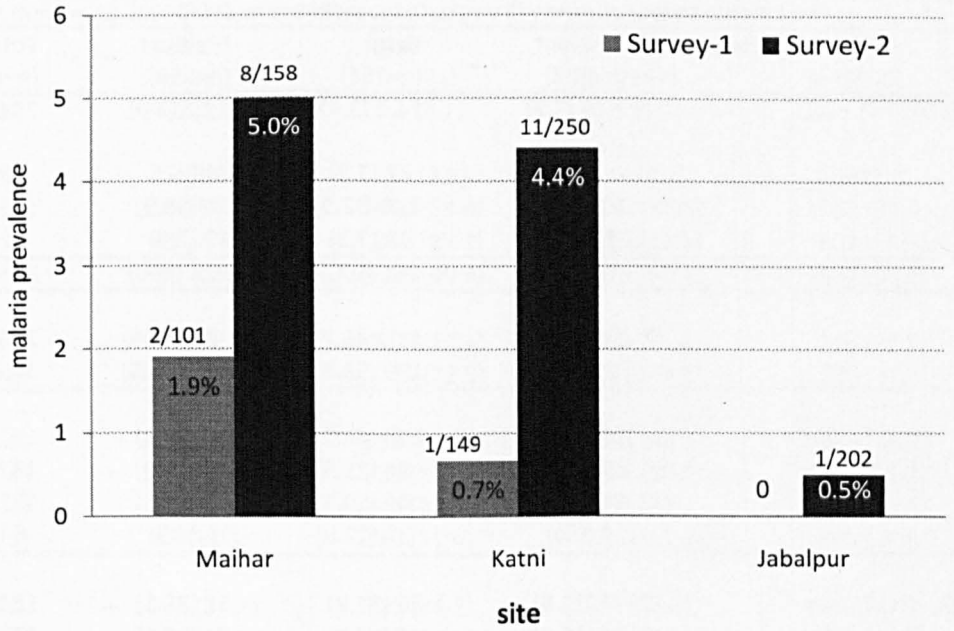
[6] An iron and folic acid combined preparation are provided for pregnant women in Madhya Pradesh.

TABLE 4: 11: CHARACTERISTICS OF 414 WOMEN ENROLLED FROM 3 DELIVERY UNITS (SURVEY 1)

	Dry season survey (6 weeks April -May 2006)			
	Survey site: **Jabalpur** (n= 125)	**Katni** (n=178)	**Maihar** (n=111)	**Total** (n=414)
Age in years, mean (SD)	3.8 (24)	4.0 (24)	4.0 (23.8)	4.0 (23.9)
Age Group				
<20 yr, n (%)	4 (3.2)	9 (5.3)	2 (1.8)	15 (3.7)
20-29 yr, n (%)	107 (86.3)	142 (83.0)	95 (85.6)	344 (84.7)
≥30 yr, n (%)	13 (10.5)	20 (11.7)	14 (12.6)	47 (11.6)
Married, n (%)	123 (99.1)	170 (100)	111 (100)	404 (99.8)
Residence				
Urban, n (%)	59 (47.6)	85 (50.0)	22 (19.8)	166 (40.9)
Rural, n (%)	65 (52.4)	85 (50.0)	89 (80.2)	239 (59.0)
Caste Category[♣]				
OBC, n (%)	55 (44.4)	88 (51.2)	67 (60.4)	210 (51.6)
GEN, n (%)	32 (25.8)	44 (25.5)	23 (20.7)	99 (24.3)
SC, n (%)	19 (15.3)	24 (14.0)	19 (17.1)	62 (15.2)
ST, n (%)	18 (14.5)	16 (9.3)	2 (1.8)	36 (8.9)
Education				
No schooling, n (%)	30 (24.2)	39 (23.1)	39 (35.1)	108 (27.0)
Primary, n (%)	34 (27.4)	84 (49.7)	27 (24.4)	145 (35.6)
≥Secondary[†], n (%)	60 (48.4)	46 (27.2)	45 (40.5)	151 (37.4)
SES rank quintiles, n (%)				
0=Poorest	17 (13.6)	35 (19.6)	32 (28.8)	84 (20.3)
1=Second	11 (8.8)	46 (25.8)	18 (16.2)	75 (18.1)
2=Third	28 (22.4)	26 (14.6)	32 (28.8)	86 (20.8)
3=Fourth	33 (26.4)	35 (19.7)	18 (16.2)	86 (20.8)
4=Richest	36 (28.8)	36 (20.2)	11 (9.9)	83 (20.1)
Gravidity				
Primigravidae, n (%)	60 (48.0)	85 (47.8)	34 (30.6)	179 (43.2)
Secundigravidae, n (%)	36 (28.8)	52 (29.2)	24 (21.6)	112 (27.1)
Gravidae-3, n (%)	19 (15.2)	22 (12.4)	28 (25.3)	69 (16.7)
Gravidae-4, n (%)	6 (4.8)	9 (5.0)	10 (9.0)	25 (6.0)
Multigravidae (≥5)[1] n (%)	4 (3.2)	10 (5.6)	15 (13.5)	29 (7.0)
Self reported ANC ≥1visit, n (%)	112 (90.3)	122 (72.2)	43 (38.7)	277 (68.6)
Maternal height in cm, mean (SD)	153.9 (5.9)	152.0 (6.1)	151.7 (6.3)	152.5 (6.2)
MUAC in cm, mean (SD)	25.0 (3.6)	22.9 (8.2)	23.7 (2.0)	23.8 (5.8)
High BP, n (%) (systolic ≥ 130mm Hg, diastolic ≥ 90mm Hg)	Not measured in this survey			
Bednet use (any), n (%)	69 (55.6)	48 (28.4)	21 (18.9)	138 (34.1)
Used net previous night, n (%)	39 (31.5)	45 (26.6)	8 (7.3)	92 (22.8)
ITN (any), n (%)	0	3 (1.8)	0	3 (0.8)
Used any drug to prevent malaria, n (%)	2 (1.6)	4 (2.4)	1 (0.9)	7 (1.7)
Reported using haematinics, n (%)	110 (89.4)	113 (67.6)	66 (61.1)	289 (72.4)

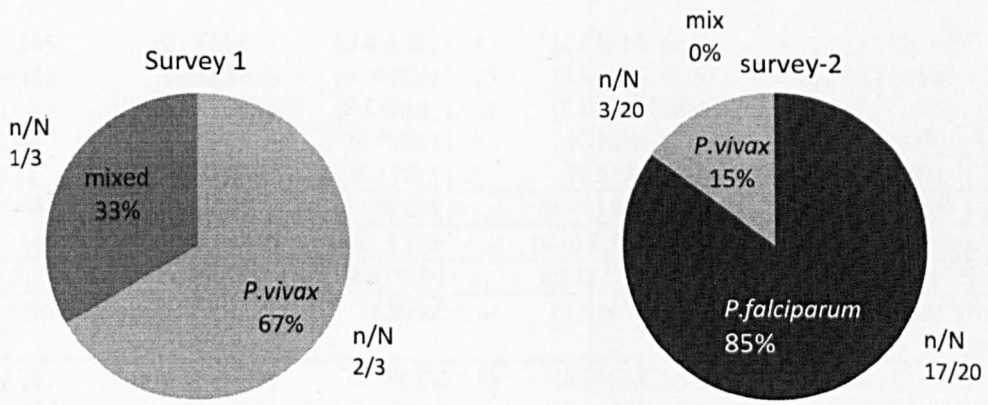
[♣]OBC=Other Backward Caste, GEN=General Caste, SC= Scheduled Caste, ST= Scheduled Tribe;
[†]= secondary and higher combined; SES=socioeconomic status; [1]= pregnancy number;

FIGURE 4: 9: MATERNAL MALARIA PREVALENCE BY SITE & SURVEY AT 3 DELIVERY UNITS



***Note**: numbers above the bars represent the number of malaria positive cases (n/N) by microscopy*

FIGURE 4: 10: PLASMODIUM SPECIES BY SURVEY IN WOMEN AT 3 DELIVERY UNITS



***Note**: estimates are based on microscopy results*

TABLE 4: 12: CHARACTERISTICS OF WOMEN ENROLLED FROM 3 DELIVERY UNITS (SURVEY 2&3)

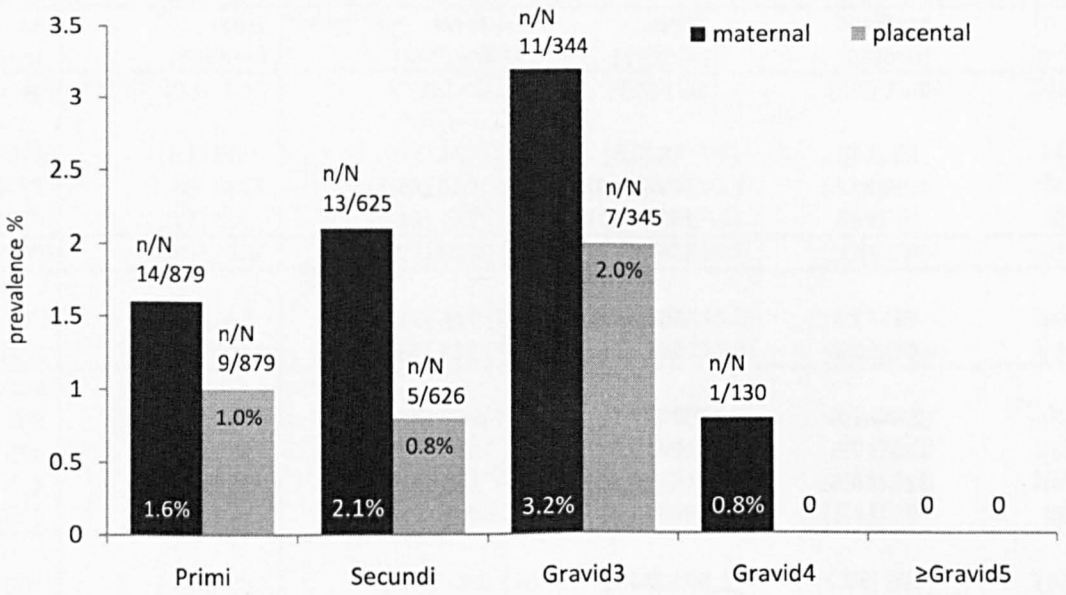
Post monsoon season survey (6 weeks, October-November 2006)				
Survey site:	**Jabalpur** (n=202)	**Katni** (n=251)	**Malhar** (n=158)	**Total** (n=611)
Age in years, mean (SD)	23.9 (3.7)	24.0 (3.4)	24.2 (3.9)	24.0 (3.6)
Age Group				
<20 yr, n (%)	5 (2.6)	7 (2.8)	4 (2.5)	16 (2.7)
20-29 yr, n (%)	169 (86.2)	224 (89.9)	14 (89.9)	535 (88.7)
≥30 yr, n (%)	22 (11.2)	18 (7.3)	12 (7.6)	52 (8.6)
Married, n (%)	199 (99)	251 (100)	158 (100)	608 (99.7)
Residence:				
Urban, n (%)	119 (59.5)	116 (46.2)	29 (18.4)	264 (43.4)
Rural, n (%)	81 (40.5)	135 (53.8)	129 (81.6)	345 (56.6)
Caste Category[♣]				
OBC, n (%)	76 (38.0)	116 (46.2)	59 (37.3)	251 (41.2)
GEN, n (%)	43 (21.5)	54 (21.5)	40 (25.3)	137 (22.5)
SC, n (%)	63 (31.5)	49 (19.5)	45 (28.5)	157 (25.8)
ST, n (%)	18 (9.0)	32 (12.8)	14 (8.9)	64 (10.5)
Education				
No schooling, n (%)	44 (21.8)	80 (31.9)	57 (36.1)	181 (29.6)
Primary, n (%)	34 (16.8)	67 (26.7)	31 (19.6)	132 (21.6)
Secondary, n (%)	68 (33.7)	90 (35.8)	54 (34.2)	212 (34.7)
Higher, n (%)	56 (27.7)	14 (5.6)	16 (10.1)	86 (14.1)
SES rank in quintiles, n (%)				
0=Poorest	31 (15.4)	57 (22.7)	34 (21.5)	122 (19.9)
1=Second	30 (14.8)	58 (23.1)	34 (21.5)	122 (19.9)
2=Third	34 (16.8)	48 (19.1)	41 (26.0)	123 (20.1)
3=Fourth	51 (25.3)	40 (16.0)	31 (19.6)	122 (19.9)
4=Richest	56 (27.7)	48 (19.1)	18 (11.4)	122 (19.9)
Gravidity				
Primigravidae, n (%)	88 (43.5)	97 (38.6)	57 (36.0)	242 (39.6)
Secundigravidae, n (%)	69 (34.2)	102 (40.6)	47 (29.8)	218 (35.7)
Gravidae-3, n (%)	33 (16.3)	32 (12.8)	29 (18.4)	94 (15.3)
Gravidae-4, n (%)	8 (4.0)	12 (4.8)	16 (10.1)	36 (6.0)
Multigravidae (≥5)[1] n (%)	4 (2.0)	8 (3.2)	9 (5.7)	21 (3.4)
Self reported ANC ≥1 visit, n (%)	153 (76.9)	203 (80.8)	133 (84.7)	489 (80.6)
Maternal height in cm, mean (SD)	151.5 (6.9)	151.3 (5.4)	151.1 (5.2)	151.3 (5.9)
MUAC in cm, mean (SD)	23.3 (2.0)	22.7 (2.5)	22.3 (2.3)	22.8 (2.3)
High BP n (%) (systolic ≥130 mmHg, diastolic ≥90 mmHg)	37 (22.3)	18 (8.1)	19 (14.1)	74 (14.2)
Bednet use (any), n (%)	74 (36.4)	79 (31.5)	27 (17.1)	180 (29.5)
Bednet use previous night, n (%)	54 (27.0)	55 (22.1)	12 (7.6)	121 (19.9)
ITN (any), n (%)	1 (0.5)	0.0	0.0	1 (0.2)
Used any drug to prevent malaria, n (%)	0.0	1 (0.4)	3 (1.9)	4 (0.7)
Reported using haematinics, n (%)	149 (75.6)	183 (72.9)	145 (92.4)	477 (78.8)

[♣]OBC = Other Backward Caste, GEN= General Caste, SC= Scheduled Caste, ST=Scheduled Tribe; SES=socioeconomic status;
[1]= pregnancy number;

survey-3 (November 2006-September 2007)			Survey 2 &3*	3-Surveys **	1 Year survey♣
Katni (n=985)	Maihar (n=686)	Total (n=1671)	Total (n=2282)	Total (n=2696)	Total (n=2080)
24.2 (3.8)	24.1 (3.5)	24.2 (3.7)	24.2 (3.7)	24.1 (4.0)	24.2 (3.6)
39 (4.0)	19 (2.8)	58 (3.5)	74 (3.3)	89 (3.3)	69 (3.3)
810 (82.3)	599 (87.3)	1409 (84.4)	1944 (85.5)	2288 (85.4)	1775 (85.5)
135 (13.7	68 (9.9)	203 (12.1)	255 (11.2)	302 (11.3)	233 (11.2)
983 (99.9)	686 (100)	1669 (99.9)	2277 (99.8)	2681 (99.8)	2078 (99.9)
365 (37.4)	85 (12.4)	450 (27.1)	716 (31.4)	716 (32.9)	597 (28.7)
612 (62.6)	601 (87.6)	1213 (72.9)	1561 (68.6)	1800 (67.1)	1480 (71.3)
436 (45.0)	356 (51.9)	792 (47.9)	1043 (46.1)	1253 (46.9)	967 (46.9)
209 (21.6)	120 (17.5)	329 (19.9)	466 (20.6)	565 (21.2)	423 (20.5)
217 (22.4)	127 (18.5)	344 (20.8)	501 (22.1)	563 (21.1)	438 (21.2)
106 (11.0)	83 (12.1)	189 (11.4)	253 (11.2)	289 (10.8)	235 (11.4)
316 (32.1)	255 (37.2)	571 (34.2)	752 (33.0)	860 (32.0)	708 (34.0)
219 (22.3)	174 (25.4)	393 (23.5)	525 (23.0)	670 (25.0)	491 (23.6)
373 (37.9)	235 (34.2)	608 (36.4)	820 (35.9)	971 (36.1)	752 (36.2)
76 (7.7)	22 (3.2)	98 (5.9)	184 (8.1)	184 (6.9)	128 (6.2)
197 (20.0)	136 (19.8)	333 (19.9)	455 (20.0)	539 (20.0)	424 (20.4)
178 (18.1)	158 (23.0)	336 (20.1)	458 (20.1)	533 (20.0)	428 (20.6)
178 (18.1)	180 (26.2)	358 (21.4)	481 (21.0)	567 (21.0)	447 (21.5)
162 (16.4)	142 (20.7)	304 (18.3)	426 (18.7)	512 (19.0)	375 (18.0)
269 (27.4)	70 (10.3)	339 (20.3)	461 (20.2)	544 (20.0)	405 (19.5)
458 (46.5)	274 (40.0)	732 (43.8)	974 (42.7)	1153 (20.0)	886 (42.6)
286 (29.1)	193 (28.1)	479 (28.7)	697 (30.6)	809 (30.0)	628 (30.2)
154 (15.6)	132 (19.2)	286 (17.1)	380 (16.7)	449 (16.7)	347 (16.7)
55 (5.6)	47 (6.9)	102 (6.1)	138 (6.0)	163 (6.0)	130 (6.3)
31 (3.2)	40 (5.8)	71 (4.3)	92 (4.0)	121 (4.5)	88 (4.2)
844 (86.4)	679 (99.1)	1523 (91.6)	2012 (88.7)	2297 (85.9)	1859 (89.8)
151.7 (4.0)	150.5 (4.7)	151.2 (4.3)	151.3 (4.8)	151 (5.1)	151.2 (4.6)
22.9 (2.0)	23.5 (1.9)	23.1 (1.9)	23.1 (2.1)	23.2 (2.2)	23.0 (2.1)
191(24.9)	105 (19.3)	296 (22.6)	370 (20.2)	370 (20.2)	333 (20.0)
344 (35.1)	116 (16.9)	460 (27.6)	646 (28.4)	784 (29.2)	572 (27.5)
315 (32.2)	74 (10.8)	389 (23.4)	510 (22.5)	602 (22.5)	456 (22.0)
5 (0.5)	0.0	5 (0.3)	6 (0.3)	9 (0.3)	5 (0.24)
1 (0.1)	0.0	1 (0.1)	5 (0.2)	12 (0.5)	5 (0.24
814 (88.3)	657 (95.7)	1471 (88.5)	1948 (86.0)	2237 (83.8)	1799 (86.8)

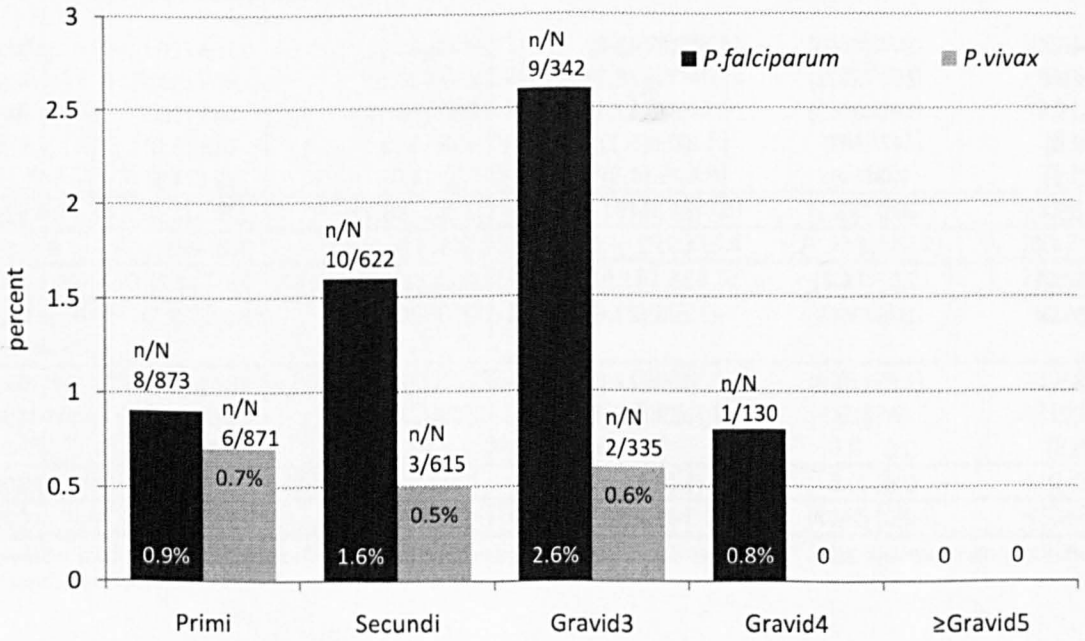
* Includes all sites in surveys 2& 3; ** includes data from all sites in all the 3 surveys; ♣excludes Jabalpur data in survey 2;

FIGURE 4: 11: PREVALENCE OF MATERNAL AND PLACENTAL MALARIA (ANY SPECIES) BY GRAVIDITY



Note: Cochran-Armitage trend test $P = 0.9$ for maternal & placental parasitaemia
Somer's D R|C test for trend, $P = 0.6$ for maternal & $P = 0.7$ for placental parasitaemia

FIGURE 4: 12: MATERNAL PARASITAEMIA BY PLASMODIUM SPECIES



Note: Cochran-Armitage trend test, $P = 0.6$ for *P.falciparum* & $P = 0.3$ for *P.vivax*
Somer's D R|C trend test, $P = 0.2$ for *P.falciparum* & $P = 0.3$ for *P.vivax*

Figures above the bars represent number of malaria positives cases for each species (n/N) by microscopy

4.3B.2 Prevalence of malaria, anaemia at delivery and birth outcome

Maternal Malaria

Rapid assessment surveys 1 and 2 (3 sites): There were only 3 women (0.8%) with evidence of maternal malaria infections (peripheral blood) during the dry season survey-1 (April -May 2006) in the 3 sites. (2 cases of *P.vivax* [66.7%] and 1 case of mixed infection [33.3%]) (Figure 4: 9). By contrast, in the post monsoon survey-2, the prevalence was 3.3% (20 cases) with 85% *P.falciparum* mono-infection (n=17) and 15% *P.vivax* mono-infection (n=3) [*P*=0.0017].

Year-round data (2 sites): Seasonal trends: Among the 2080 women included in the analysis of the full-year data (October 2006-September 2007) the average year-round prevalence of peripheral parasitaemia was 2%; 71.8% were mono-infections of *P.falciparum* and 28.2% were mono-infections of *P.vivax* (*P* = 0.006) (Table 4: 14). There were no mixed infections detected by microscopy.

The monthly observations showed October to December to be the period with the highest prevalence of malaria, while in the rest of the year, the transmission remained low for both species (Figure 4: 12). The peak prevalence of *P.falciparum* occurred in October (5.2%) about 6 weeks after the rainfall peak. There was little seasonal variation for *P.vivax* and the prevalence was low all year round varying between 0 to 1%. Similar to the observations from the antenatal clinics, malaria prevalence was comparable between gravidities (Figure 4: 11& 4:12). The prevalence of maternal *P.vivax* was 0.7%% in primigravidae and 0.6%% in third gravidae (*P*=0.3).

Placental Malaria

The year round prevalence of placental parasitaemia detected microscopically by impression smear was 1% (Table 4: 14). The monthly prevalence of placental parasitaemia showed that the peak for *P.falciparum* occurred in October and November (about 2%) during the post-monsoon months, comparable to peripheral parasitaemia, while the prevalence was less than 1% for the rest of the year (Figure 4: 13). Again a gravidity specific effect was not seen with *P.falciparum* or *P.vivax* (Figure 4:11). Only 2 women (0.1%) had microscopically detected placental malaria with negative peripheral parasitaemia. In contrast, half of the women positive for peripheral parasitaemia (51%) were negative for placental infection indentified by microscopy.

Anaemia

Rapid Assessment Surveys 1&2 (3 sites): The overall anaemia prevalence was 51.1% (Hb <11g/dL). The prevalence of moderate to severe anaemia (Hb <9g/dL) was 13.2% in the dry

season and 23.8% in the post monsoon season (Tables 4: 13 & 4: 14). The anaemia prevalence was higher in Jabalpur than the two rural sites (P=0.001).

Year-round Survey (2 sites): Half the women (50%) had mild anaemia (Hb<11g/dL) and 20% moderate to severe anaemia (Hb < 9g/dL). Moderate to severe anaemia prevalence was higher in Katni (23%) than at Maihar (14%, P=0.0001) (Table 4: 14), which reflects that Katni hospital is equipped with blood transfusion capabilities while Maihar delivery unit can manage only non-risk cases.

Fever and history of antimalarial drug use

Year-round survey (2 sites): Documented fever at delivery was 6.3% and reported history of fever in the previous week was 12.5% (Table 4: 14). The documented fever cases were higher in Katni (9.2%) than at Maihar (2%). The overall use of antimalarials in pregnancy was 2% while self reported use of any drug during pregnancy is 11.5%.

Low Birth Weight

Rapid assessment surveys 1& 2 (3 sites): The overall prevalence of low birth weight (<2500g) was 39.2% and was higher in the post monsoon season (42%) than in the dry season 34% for all gravidity (P=0.023) (Table 4: 13 & 4: 14). The prevalence of low birth weight was higher at Jabalpur, the urban site, while it and lowest at Maihar, the rural site (P=0.013). This reflects the difference between the tertiary and primary site's capability in handling complicated cases. The low birth weight between gravidities were similar: 48% in primigravidae and 42% in grand-multigravidae (≥5) (P=0.23) in survey-2.

Year-round survey (2 sites): The overall prevalence of low birth weight was 33.8% for all gravidities. The mean (SD) birth weight was 2536g (464g). The proportion of low birth weight among primigravidae (38.7%) was higher than in other gravidae (P=0.0001) (Table 4:14).

TABLE 4: 13: MALARIA, ANAEMIA, MATERNAL MORBIDITY & BIRTH OUTCOMES IN WOMEN IN 3 DELIVERY UNITS (SURVEY 1)

Dry season survey (6 weeks April-May 2006)				
Survey sites	**Jabalpur** (n=125)	**Katni** (n=178)	**Maihar** (n=111)	**Total** (n=414)
Overall Maternal Parasitaemia‡, n (%)	0.0	1 (0.67)	2 (1.9)	3 (0.8)
P.falciparum, n (%)[1]	-	0.0	0.0	0.0
P.vivax, n (%)[1]	-	1 (100)	1(50.0)	2 (66.7)
Mixed species, n (%)[1]	-	0.0	1 (50.0)	1 (33.3)
Overall Placental Parasitaemia*, n (%)	0.0	1 (0.63)	1 (0.97)	2 (0.53)
P.falciparum, n (%)[1]	-	0.0	0.0	0.0
P.vivax, n (%)[1]	-	1 (100)	0.0	1 (50.0)
Mixed species, n (%)[1]	-	0.0	1 (100)	1 (50.0)
Cord Parasitaemia, n (%)	*not done in this survey*			
Placental Parasitaemia				
Primigravidae, n (%)	-	1 (1.3)	1 (3.1)	2 (1.2)
Secundigravidae, n (%)	-	-	-	-
Gravidae-3, n (%)	-	-	-	-
Gravidae-4, n (%)	-	-	-	-
Multigravidae (≥5)[2], n (%)	-	-	-	-
Maternal Hb in g/dL, mean (SD)	10.6 (1.9)	11.2 (1.9)	11.4 (1.8)	11.0 (1.9)
Mild anaemia (Hb <11.0g/dL), n (%)	68 (54.4)	64 (38.3)	44 (39.6)	176 (43.6)
Mod-severe anaemia (Hb<9.0g/dL), n (%)	24 (19.2)	20 (11.9)	9 (8.1)	53 (13.2)
Severe anaemia (Hb <7.0g/dL), n (%)	4 (3.2)	3 (1.8)	2 (1.8)	9 (2.2)
Maternal fever[3] at delivery, n (%)	20 (16.0)	33 (19.8)	12 (10.8)	65 (16.1)
Reported fever current pregnancy, n (%)	16 (13.0)	59 (35.1)	11 (10.0)	86 (21.5)
Used antimalarial for fever, n (%)	5 (4.0)	23 (13.6)	1 (0.9)	29 (7.2)
Used any drug for fever, n (%)	9 (7.2)	46 (27.3)	7 (6.4)	62 (15.5)
Reported fever previous week, n (%)	5 (4.0)	20 (11.9)	8 (7.2)	33 (8.2)
Used antimalarial for fever, n (%)	0.0	1 (0.6)	0.0	1 (0.3)
Used any drug for fever, n (%)	1 (0.8)	9 (5.5)	3 (2.7)	13 (3.3)
Birth weight (g), mean (SD)[4]	2566 (457)	2602 (409)	2716 (457)	2626 (440)
Birth weight <2500g, n (%)[4]	35 (34.6)	58 (37.6)	31 (28.7)	124 (34.1)
Low Birth Weight				
Primigravidae, n (%)	15 (33.3)	37 (51.4)	9 (27.3)	61 (40.7)
Secundigravidae, n (%)	10 (31.3)	11 (23.4)	11 (50.0)	32 (31.7)
Gravidae-3, n (%)	5 (31.3)	6 (28.6)	8 (28.6)	19 (29.2)
Gravidae-4, n (%)	2 (40.0)	2 (28.6)	1 (10.0)	5 (22.7)
Multigravidae (≥5)[2], n (%)	3 (100.0)	2 (28.6)	2 (13.3)	7 (28.0)
Baby's gestational age in weeks, mean(SD)	37.5 (1.8)	37.8 (1.3)	37.0 (1.2)	37.5 (1.4)
Preterm babies (<37 weeks)[5], n (%)	21 (20.8)	19 (12.3)	20 (18.5)	60 (16.5)
IUGR, n (%)†	24 (23.7)	47 (30.5)	20 (18.5)	91 (25.0)
SGA, n (%)♣	47 (46.5)	85 (54.8)	30 (27.8)	217 (59.6)
Stillbirths, n (%)	11 (9.3)	5 (3.0)	0.0 (0)	16 (4.0)

‡estimates by microscopy; [1]=Column percentages; *=Placental Impression smear; [2]=pregnancy number; [3]= axillary temperature≥37.5^{0}C; [4]=Singleton births only; [5]=gestational age using Ballard score; † IUGR=gestation ≥37weeks and birth weight <2500g; ♣ SGA calculated using Williams reference score

TABLE 4: 14: MALARIA, ANAEMIA, FEVER & BIRTH OUTCOME IN WOMEN IN 3 DELIVERY UNITS (SURVEYS 2 & 3)

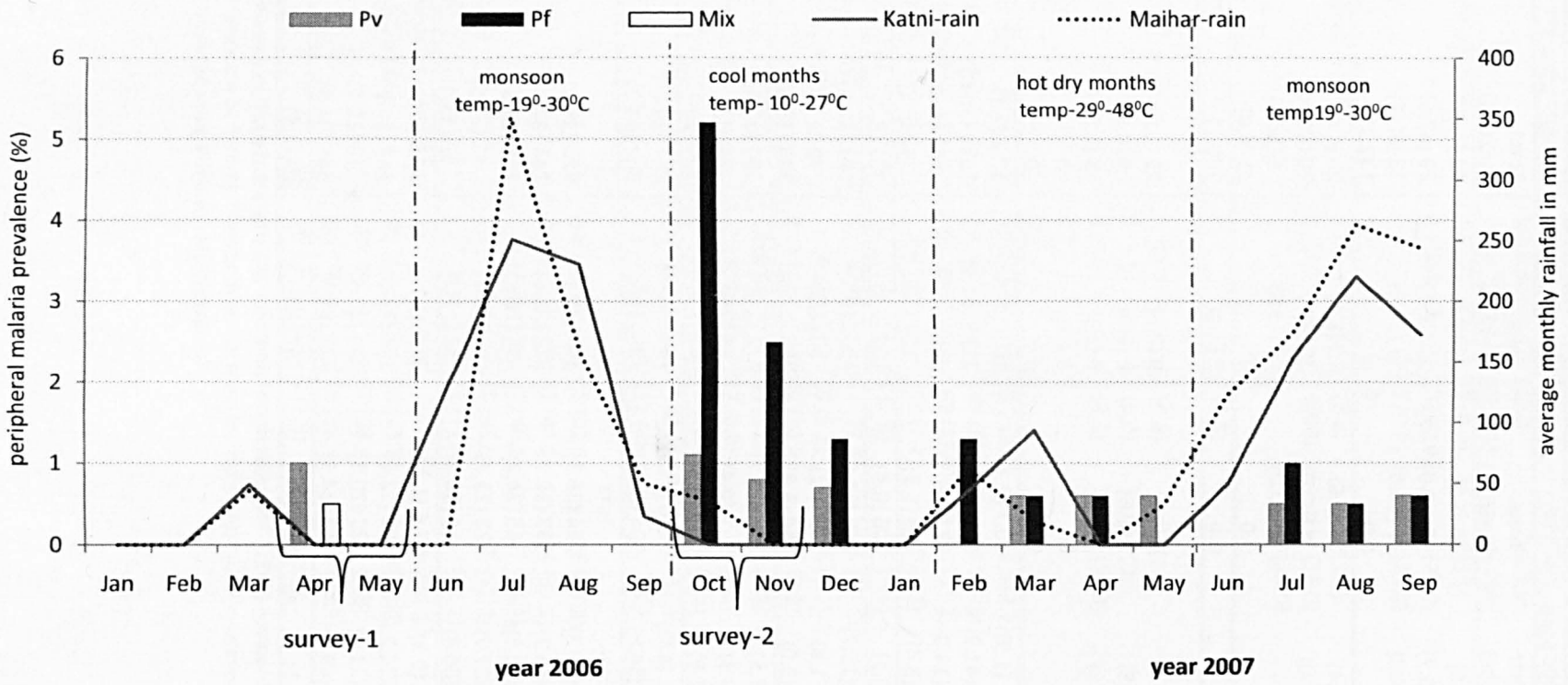
Post monsoon season survey (6 weeks October - November 2006)				
Survey sites:	**Jabalpur** (n=202)	**Katni** (n=251)	**Maihar** (n=158)	**Total** (n=611)
Overall Maternal Parasitaemia‡, n (%)	1 (0.5)	11 (4.4)	8 (5.0)	20 (3.3)
P.falciparum, n (%)[1]	1 (100)	10 (90.9)	6 (75.0)	17 (85.0)
P.vivax, n (%)[1]	0	1 (9.0)	2 (25.0)	3 (15.0)
Mixed species, n (%)[1]	0	0	0	0
Overall Placental Parasitaemia*, n (%)	2 (0.99)	7 (2.8)	4 (2.5)	13 (2.1)
P.falciparum, n (%)[1]	2 (100)	7 (100)	3 (75.0)	12 (91.7)
P.vivax, n (%)[1]	0	0	1 (25.0)	1 (8.3)
Mixed species, n (%)[1]	0	0	0	0
Cord Parasitaemia, n (%)	0	2 (0.8)	0	2 (0.3)
Placental Parasitaemia				
Primigravidae, n (%)	0 (0)	4 (4.2)	1(1.8)	5 (2.1)
Secundigravidae, n (%)	1 (1.5)	2 (2.0)	0 (0)	3 (1.4)
Gravidae-3, n (%)	1 (3.0)	1 (3.1)	3 (10.3)	5 (5.3)
Gravidae-4, n (%)	0 (0)	0	0	0
Multigravidae (≥5)[2], n (%)	0	0	0	0
Maternal Hb in g/dL , mean (SD)	10.2 (2.3)	10.2 (2.2)	10.9 (2.2)	10.3 (2.3)
Mild anaemia (Hb <11.0g/dL), n (%)	121 (59.9)	149 (59.8)	71 (44.9)	341 (55.9)
Mod-severe anaemia (Hb <9.0g/dL), n (%)	52 (25.7)	69 (27.7)	24 (15.2)	145 (23.8)
Severe anaemia (Hb <7.0g/dL), n (%)	16 (7.9)	18 (7.2)	9 (5.7)	43 (7.0)
Maternal fever [3]at delivery, n (%)	27 (13.5)	9 (3.6)	15 (9.6)	51 (8.4)
Reported fever current pregnancy, n (%)	62 (30.8)	94 (37.5)	16 (10.1)	172 (28.2)
Used antimalarials for fever, n (%)	9 (4.5)	13 (5.2)	8 (5.1)	20 (4.9)
Used any drug for fever, n (%)	44 (21.9)	62 (24.9)	13 (8.2)	119 (19.6)
Reported fever previous week, n (%)	12 (6.0)	53 (21.1)	11 (7.1)	76 (12.5)
Used antimalarial for fever, n (%)	1 (0.5)	2 (0.8)	4 (2.5)	7 (1.2)
Used any drug for fever, n (%)	9 (4.5)	18 (7.2)	10 (6.5)	37 (6.1)
Birth weight (g), mean (SD)[4]	2534 (502)	2536 (464)	2623 (367)	2556 (453)
Birth weight <2500g, n (%)[4]	69 (40.1)	111 (48.2)	54 (35.3)	234 (42.1)
Low Birth Weight				
Primigravidae, n (%)	34 (42.5)	48 (52.8)	26 (48.2)	108 (48.0)
Secundigravidae, n (%)	20 (35.1)	41 (44.1)	14 (31.1)	75 (38.5)
Gravidae-3, n (%)	11 (40.7)	12 (40.0)	7 (24.1)	30 (34.9)
Gravidae-4, n (%)	3 (60.0)	5 (55.6)	5 (31.2)	13 (43.3)
Multigravidae (≥5)[3], n (%)	1 (33.3)	5 (71.4)	2 (22.2)	8 (42.1)
Baby's gestational age in weeks, mean SD	37.3 (1.2)	37.2 (1.2)	36.7 (1.1)	37.0 (1.3)
Preterm babies (<37 weeks)[5], n (%)	30 (17.6)	58 (25.2)	44 (28.8)	132 (23.8)
IUGR, n (%)[†]	48 (28.2)	57 (24.8)	12 (7.8)	117 (21.1)
SGA, n (%)[♣]	64 (37.7)	88 (38.4)	26 (16.9)	178 (32.3)
Stillbirths, n (%)	20 (10.3)	6 (2.5)	3 (1.9)	29 (4.9)

‡= estimates by microscopy; [1]=Column percentages; *=Placental Impression smear; [2]=pregnancy number; [3]= axillary temperature≥37.5^{0}C; [4]=Singleton births only; [5]=gestational age using Ballard score; [†] IUGR=gestation ≥37weeks and birth weight ≤2500g; [♣] SGA calculated using Williams reference score

Survey 3- November 2006-September2007			Survey* 2 & 3	Survey** 1,2 & 3	1 Year survey♣
Katni (n=985)	Maihar (n=686)	Total (n=1671)	Total (n=2282)	Total (n=2696)	Total (n=2080)
13 (1.3)	7 (1.0)	20 (1.2)	40 (1.8)	43 (1.6)	39 (2.0)
6 (46.2)	6 (85.7)	12 (60.0)	29 (72.5)	29 (67.5)	28 (71.8)
7 (53.8)	1 (14.3)	8 (40.0)	11 (27.5)	13 (30.2)	11 (28.2)
0.0	0.0	0.0	0	1 (2.3)	0
7 (0.7)	3 (0.4)	10 (0.6)	23 (1.0)	25 (1.0)	21 (1.0)
5 (71.4)	3 (100)	8 (80.0)	20 (87.0)	20 (80.0)	18 (85.7)
2 (28.6)	0.0	2 (20.0)	3 (13.0)	4 (16.0)	3 (14.3)
0.0	0.0	0.0	0	1 (4.0)	0
0.0	0.0	0.0	2 (0.1)	2 (0.1)	2 (0.1)
3 (0.7)	1 (0.4)	4 (0.6)	9 (0.9)	11 (1.0)	9 (1.0)
2 (0.7)	1 (0.5)	3 (0.6)	6 (0.9)	6 (0.8)	5 (0.8)
2 (1.3)	1 (0.8)	3 (1.1)	8 (2.1)	8 (1.8)	7 (2.0)
0	0	0	0	0	0
0	0	0	0	0	0
10.4 (2.1)	11.1 (1.9)	10.7 (2.1)	10.6 (2.1)	10.7 (2.1)	10.7 (2.1)
540 (54.9)	282 (41.2)	822 (49.3)	1163 (51.1)	1339 (50.0)	1042 (50.2)
225 (22.9)	96 (14.0)	321 (19.3)	466 (20.5)	519 (19.4)	414 (19.9)
65 (6.6)	23 (3.4)	88 (5.3)	131 (5.8)	140 (5.2)	115 (5.5)
91 (9.2)	15 (2.1)	106 (6.4)	157 (6.9)	222 (8.3)	130 (6.3)
155 (15.8)	108 (15.7)	263 (15.8)	435 (19.1)	521 (19.5)	373 (18.0)
8 (0.8)	12 (1.8)	20 (1.2)	50 (2.2)	79 (3.0)	41 (2.0)
102 (10.4)	62 (9.0)	164 (9.8)	283 (12.5)	346 (13.0)	239 (11.5)
171 (17.5)	24 (3.5)	195 (11.7)	271 (11.9)	304 (11.4)	259 (12.5)
4 (0.4)	2 (0.3)	6 (0.4)	13 (0.6)	14 (0.5)	12 (0.6)
37 (3.8)	13 (1.9)	50 (3.0)	87 (3.8)	100 (3.8)	78 (3.8)
2680 (404)	2671 (421)	2676 (411)	2646 (425)	2643 (427)	2656 (417)
271 (29.3)	233 (34.8)	504 (31.6)	738 (34.3)	862 (34.3)	669 (33.8)
141 (32.4)	113 (42.3)	254 (36.1)	362 (39.1)	423 (39.3)	328 (38.7)
68 (25.4)	69 (36.7)	137 (30.0)	212 (32.6)	244 (32.4)	192 (32.3)
40 (20.0)	26 (20.3)	66 (24.2)	96 (26.8)	115 (27.1)	85 (25.6)
10 (20.0)	13 (27.7)	23 (23.7)	36 (28.4)	41 (27.5)	33 (27.1)
12 (44.4)	12 (30.8)	24 (36.4)	32 (37.7)	39 (35.4)	31 (37.8)
37.8 (1.2)	37.3 (0.9)	37.6 (1.1)	37.5 (1.2)	37.5 (1.3)	37.2 (1.5)
104 (11.2)	88 (13.2)	192 (12.0)	324 (15.1)	384 (15.3)	294 (14.8)
174 (18.8)	147 (21.9)	321 (20.1)	438 (20.4)	529 (21.1)	390 (19.7)
335 (36.2)	461 (69.0)	543 (34.1)	721 (33.6)	868 (34.6)	289 (14.6)
36 (3.7)	9 (1.3)	45 (2.7)	74 (3.3)	90 (3.4)	54 (2.6)

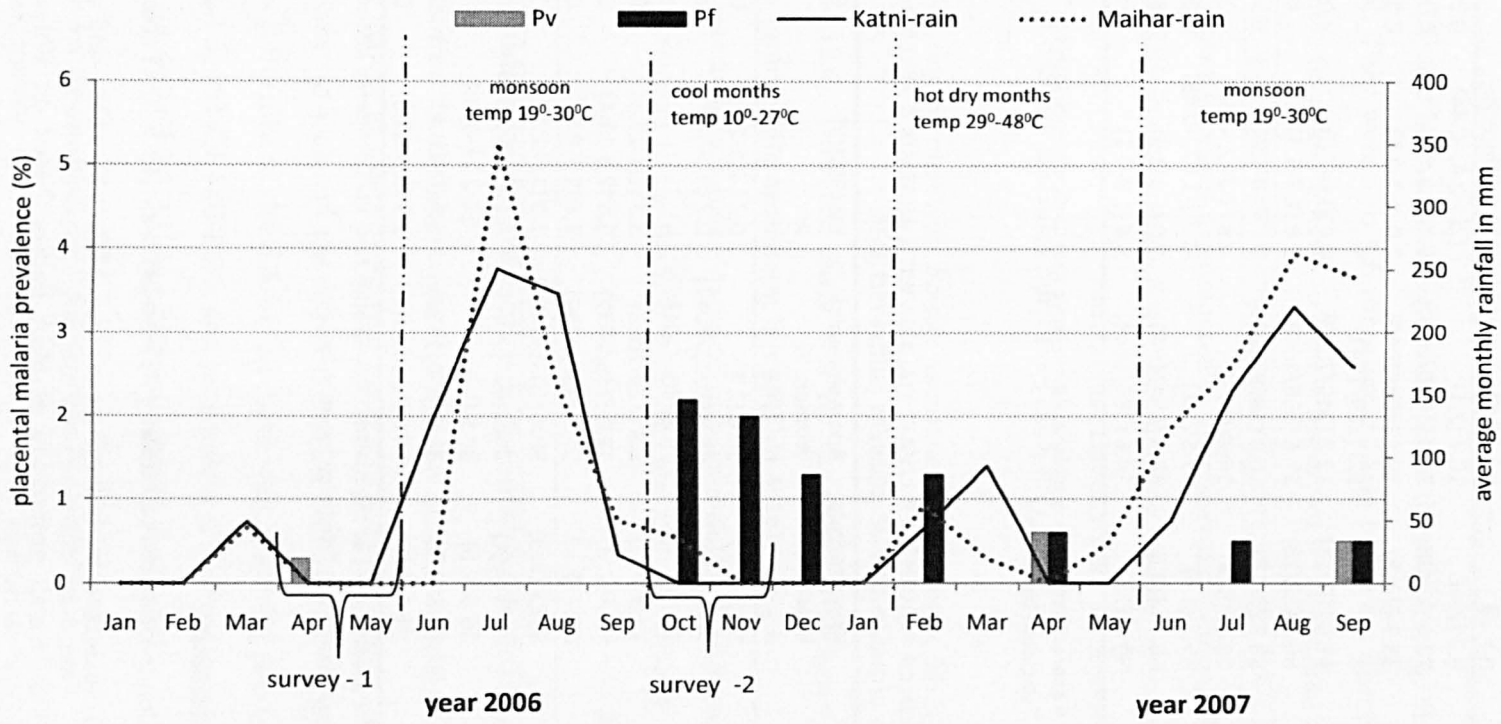
* Includes all sites in survey 2& 3; ** includes data from 3 sites in all the 3 surveys; ♣excludes Jabalpur data in survey 2

FIGURE 4: 13: MATERNAL MALARIA PREVALENCE AT DELIVERY (SURVEYS 2 & 3) WITH RAINFALL



***Note**: malaria detected by microscopy*

FIGURE 4: 14: PLACENTAL MALARIA PREVALENCE AT DELIVERY (SURVEYS 2 & 3) WITH RAINFALL



***Note**: placental malaria= detected by impression smear; temp= temperature*

TABLE 4: 15: ASSOCIATION OF **PLACENTAL *P.FALCIAPRUM*** MALARIA WITH ANAEMIA, FEVER, MUAC & BIRTH OUTCOME AT DELIVERY (SURVEYS 2 & 3)

	Parasitaemic Women, % n = 18	**Aparasitaemic‡ Women, %** n = 2048	**RR (95%CI)** *or* mean difference	***P-value***
Haemoglobin mean (SD)	9.2 (2.4)	10.7 (2.1)	-1.5 (-2.4, -0.44)	0.005
Anaemia (Hb<11g/dL)	72.2 (13/18)	50.0 (1020/2040)	1.4 (1.1-1.9)	0.06
Moderate- severe anaemia*	38.9 (7/18)	19.8 (403/2040)	2.0 (1.1-3.5)	0.04
Fever at visit††	0	6.3 (129/2041)	--	--
History of fever previous week	55.6 (10/18)	12.0 (245/2037)	4.6 (3.0-7.0)	<.0001
History of fever in pregnancy	66.7 (12/18)	17.6 (358/2044)	3.8 (2.7-5.3)	<.0001
MUAC <23cm	33.3 (6/18)	50.6 (1035/2045)	0.7 (0.3-1.2)	0.14
Birth weight mean (SD)**	2173 (524)	2660 (415)	-487 (-722, -250)	<.0001
Gestation mean (SD)**	36.3 (1.4)	37.5 (1.2)	-1.2 (-1.87, -0.48)	0.001
Low birth weight (<2500g)**	66.7 (8/12)	33.5 (654/1949)	2.0 (1.3-2.9)	0.02
Preterm (<37 weeks)**	50 (6/12)	14.6 (284/1948)	3.4 (1.9-6.1)	0.0006

♣=Placental impression smear; ‡ = aparasitaemic of any malaria; RR= Relative risk; CI=Confidence Interval; *Hb <9g/dL; ††=axillary temperature ≥37.5^{0}C; **singleton babies only

TABLE 4: 16: ASSOCIATION OF **PLACENTAL *P.VIVAX*** MALARIA WITH ANAEMIA & FEVER, MUAC & BIRTH OUTCOME AT DELIVERY (SURVEYS 2 & 3)

	Parasitaemic Women, % n = 3	**Aparasitaemic‡ Women, %** n = 2048	**RR (95%CI)** *or* mean difference	***P-value***
Haemoglobin mean (SD)	10.9 (1.7)	10.6 (2.1)	0.3 (-2.1-2.6)	0.8
Anaemia (Hb<11g/dL)	33.3 (1/3)	49.9 (1021/2043)	0.7 (0.1-3.3)	0.56†
Moderate-severe anaemia*	0	19.8 (404/2046)	--	--
Fever at visit††	33.3 (1/3)	6.3 (129/2044)	5.3 (1.1-26.6)	0.05†
History of fever previous week	66.7 (2/3)	12.0 (245/2037)	5.5 (2.5-12.4)	0.004†
History of fever in pregnancy	66.7 (2/3)	17.5 (358/2044)	3.8 (1.7-8.5)	0.25†
MUAC <23cm	100.0(3/3)	50.6 (1035/2045)	1.9 (1.8-2.1)	0.08
Birth weight mean (SD)**	2533(144)	2659(414)	-126(-596, -343)	0.3
Gestation mean (SD)**	36.5 (0.6)	37.5 (1.2)	-1 (-2.23-0.54)	0.13
Low birth weight (<2500g)**	66.6 (2/3)	33.6 (656/1952)	1.9 (0.9-4.4)	0.22
Preterm (<37 weeks)**	33.3 (1/3)	14.6 (285/1951)	2.3 (0.5-11.3)	0.37

♣=Placental impression smear; ‡ = aparasitaemic of any malaria; RR= Relative Risk; CI= Confidence Interval; †Fisher's Exact test *Hb<9g/dL; ††=axillary temperature ≥37.5^{0}C; **singleton babies only;

Preterm & other Birth Outcomes

The overall prevalence of preterm babies (<37weeks) was (14.5%) (Table 4: 14). The mean gestational age (SD) (assessed by Ballard score) was 37.2 (1.5) weeks and did not differ between sites (P=0.21). The overall proportion of SGA babies (based on Williams reference score) was 14.6%. 19.7% babies were IUGR, defined as term babies with weight <2500g and stillbirth in 2.6%.

4.3B.3 Associations between placental malaria, morbidity symptoms and birth outcome

P.falciparum: Women with placental *P.falciparum* parasitaemia were twice as likely to be moderate to severely anaemic (<9g/dL) compared to women without any parasitaemia (RR 2.0, 95% CI 1.1-3.5) and had mean haemoglobin levels that were 1.5 g/dL lower (95%CI -2.4, -0.44) (Table 4: 15). They were also 4.6 times more likely to have a history of fever in the previous week, and three times more likely to have a history of fever anytime during pregnancy. The risk of low birth weight was double and the risk of preterm birth 3.4 fold higher for women with placental *P.falciparum* malaria as compared to aparasitaemic women. Mean birth weight was 487g (95%CI -722,-250) lower in women with parasitaemia, and the mean gestation was 1.2 weeks less (95%CI -1.87,-0.48) (Table 4:15). Similar associations were observed in women with peripheral parasitaemia at delivery and birth outcome (Table 4:17).

P.vivax: Delivering women with *P.vivax* parasitaemia were more likely to have documented fever at visit (RR 5.3, 95% CI 1.1-26.6) as well as a history of fever during the previous week (RR 5.5, 95% CI 2.5- 12.4). However, there was no significant association with haemoglobin levels, anaemia, low birth weight or preterm birth (Table 4: 16).

The associations between maternal parasitaemia (for *P.falciparum & P.vivax*) and morbidity symptoms were similar to the findings with placental malaria (Table 4: 18).

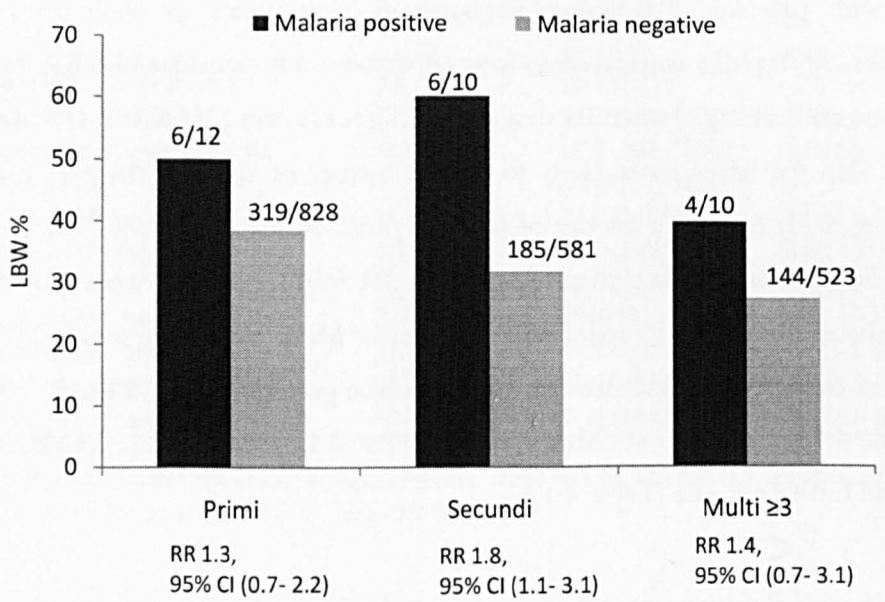
4.3B.4 Determinants of Maternal and Placental *P.falciparum* malaria at delivery

Maternal malaria: In the univariate analysis the factors associated with maternal *P.falciparum* malaria were similar to that of placental malaria (Tables 4:19 & 4: 20). There was no difference between gravidity groups and the effect of malaria on low birth weight (Figure 4: 15). Likewise, there was no difference in the risk of low birth weight among paucigravidae (with and without malaria) (RR 1.5, 95%CI 1.03-2.24) and multigravid (≥3 pregnancies) women with and without malaria (RR 1.4, 95%CI 0.7-3.1) (results not shown in the figure).

4.3B.4.1 Univariate analysis for P.falciparum Placental malaria

Societal, demographic and seasonal factors: The univariate analysis showed a significant association between placental parasitaemia and the following factors: caste, rural residence, socioeconomic status, antenatal attendance and season (Table 4: 21). Women of the Scheduled tribe had higher risk of placental malaria compared to women of the General caste. Antenatal attendance was strongly associated with placental malaria; women who had not attended

FIGURE 4: 15: ASSOCIATION OF LOW BIRTH WEIGHT WITH MATERNAL MALARIA (ANY SPECIES)



***Note**: singleton deliveries only*

antenatal care was more at risk of malaria at delivery (PR 5.6, 95% CI 2.1-14.2). Age, gravidity and educational level were not significantly associated with placental malaria infection.

Malaria Morbidity and Control factors: Women with moderate-severe anaemia were more likely to have placental *P.falciparum* malaria. Similarly, a history of fever during pregnancy (PR 9.1, 95% CI 3.4-24.2) or fever in the past week (PR 8.8, 95% CI 3.5-22.1) was also strongly predictive of the presence of placental malaria (Table 4: 22). Likewise, the risk of placental *P.falciparum* malaria increased in women with a history of antimalarial use for fever in pregnancy. Unlike in the ANC based analysis, fever at the time of delivery was not associated with placental malaria. Self reported use of bed net and use of haematinics was not associated with placental malaria.

P.vivax: Caste was the only socio-demographic factor associated with maternal parasitaemia. A history of fever and drug use in fever in the previous week was the morbidity factors strongly associated with *P.vivax* infection (Tables 4: 19 & 4: 20). None of the other parameters had a significant association with *P.vivax* infection.

TABLE 4: 17: ASSOCIATION OF **MATERNAL *P.FALCIPARUM*** MALARIA WITH ANAEMIA, FEVER, MUAC & BIRTH OUTCOME AT DELIVERY (SURVEY 2&3)

	Parasitaemic Women, % n = 28	**Aparasitaemic†† Women, %** n = 2028	**RR (95% CI)** *or* mean difference	***P-value***
Haemoglobin mean (SD)	9.5 (2.5)	10.7 (2.1)	-1.2 (-1.9,-0.4)	0.003
Anaemia (Hb<11g/dL)	64.3 (18/28)	49.9 (1009/2020)	1.2 (0.9-1.7)	0.1
Moderate-severe anaemia♣	35.7 (10/28)	19.6 (398/2020)	1.8 (1.1-3.0)	0.03
Fever at visit*	3.7 (1/27)	6.3 (127/2021)	0.6 (0.1-4.1)	0.5†
History of fever previous week	50.0 (14/28)	11.7 (236/2017)	4.2 (2.8-6.1)	<.0001
History of fever in pregnancy	53.8(15/28)	17.5 (353/2024)	3.1 (2.1-4.4)	<.0001
MUAC <23cm	78.7(22/28)	48.9 (992/2025)	1.6 (1.3-1.9)	0.002
Low birth weight (<2500g)**	54.5 (12/22)	33.5 (647/1930)	1.6 (1.1-2.4)	0.04
Preterm (<37 weeks)**	45.5 (10/22)	14.4 (278/1929)	3.1 (1.9-5.0)	<0.0001

††= aparasitaemic of any malaria; RR=Relative Risk; CI= Confidence Interval; †=Fisher's Exact test ♣Hb <9g/dL; *=axillary temperature ≥37.5^{0}C; **singleton babies only;

TABLE 4: 18: ASSOCIATION OF **MATERNAL *P.VIVAX*** MALARIA WITH ANAEMIA, FEVER, MUAC & BIRTH OUTCOME AT DELIVERY (SURVEYS 2 & 3)

	Parasitaemic Women, % n = 11	**Aparasitaemic†† Women, %** n = 2028	**RR (95% CI)** *or* mean difference	***P-value***
Haemoglobin mean (SD)	10.9 (1.7)	10.6 (2.1)	0.3 (-1.0-1.5)	0.7
Anaemia (Hb<11g/dL)	45.5 (5/11)	49.9 (1009/2020)	0.9 (0.5-1.7)	0.8
Moderate-severe anaemia♣	0	19.7 (398/2020)	-	-
Fever at visit*	18.2 (2/11)	6.3 (127/2021)	2.9 (0.8-10.3)	0.1†
History of fever previous week	54.6 (6/11)	11.7 (236/2017)	4.7 (2.7-8.1)	<.0001
History of fever in pregnancy	36.4 (4/11)	17.5 (353/2024)	2.0 (0.9-4.4)	0.1†
MUAC <23cm	63.6 (7/11)	48.9 (992/2025)	1.2 (0.8-2.0)	0.3
Low birth weight (<2500g)**	40.0 (4/10)	33.6 (649/1933)	1.1 (0.6-2.5)	0.7†
Preterm (<37weeks)**	20.0 (2/10)	14.4 (279/1932)	1.3 (0.4-4.8)	0.6†

††= aprasitaemic for any malaria; RR=Relative Risk; CI=Confidence Interval; †= Fisher's Exact test ♣Hb <9g/dL; *=axillary temperature ≥37.5^{0}C; **singleton babies only;

4.3B.5 Multivariate Predictor model of Placental *P.falciparum* Malaria

The results of the multivariate model on the determinants of maternal *P.falciparum* malaria were comparable to the outcome of the placental malaria model. Therefore, only the results of the placental *P.falciparum* model are presented. Eleven factors fitted the initial model (Table 4: 23). In the final multivariate model, caste, antenatal attendance, season, history of fever in the past week and fever in pregnancy were predictive of placental malaria, as it remained strongly associated with placental *P.falciparum* infection (Table 4: 24).

TABLE 4: 19: UNIVARIATE ANALYSIS OF DEMOGRAPHIC FACTORS ASSOCIATED WITH **MATERNAL MALARIA** AT DELIVERY (1 YEAR SURVEY)

	any malaria infection		
	Parasitaemia | n (%) n=39 (positive)	**PR (95% CI)**	***P-value***
Age group			
<20 yr	1 | 68 (1.4)	3.4 (0.22-54.06)	0.21
20-29 yr	37 | 1763 (2.1)	4.9 (0.67-35.47)	
≥30 yr	1 | 233 (0.4)	reference	
Caste Category*			
OBC	18 | 962 (1.9)	2.0 (0.67-5.81)	
SC	6 | 433 (1.4)	1.5 (0.41-5.15)	0.005
ST	11 | 233 (4.7)	5.0 (1.60-5.51)	
GEN	4 | 423 (0.9)	reference	
Education			
No Schooling	13 | 702 (1.8)		
Primary	13 | 487 (2.7)	-----	0.24
Secondary	13 | 749 (1.7)		
Higher	0	reference	
Residence			
rural	35 | 1472 (2.4)	3.5 (1.25-9.85)	0.01
urban	4 | 592 (0.7)	reference	
SES			
Poorest	14 | 424 (3.3)	13.3 (1.76-100.9)	
Second	8 | 423 (1.9)	7.6 (0.95-60.81)	
Third	8 | 443 (1.8)	7.2 (0.91-58.07)	0.03
Fourth	8 | 372 (2.2)	8.6 (1.1 -69.13)	
Richest	1 | 404 (0.3)	reference	
Gravidity			
Primigravidae	14 | 879 (1.6)		
Secundigravidae	13 | 625 (2.1)		0.17
Gravidae-3	11 | 344 (3.2)		
Gravidae-4	1 | 130 (0.8)		
Multigravidae (≥5)**	0 | 88 (-)	reference	
Attended ANC (any)			
No	11 | 209 (5.3)	3.4 (1.75-6.91)	0.0002
Yes	28 | 1850 (1.5)	reference	
Possessed ANC card at visit			
No	33 | 1604 (2.1)	1.5 (0.64-3.60)	0.33
Yes	6 | 446 (1.4)	reference	
Survey Site			
Katni	24 | 1226 (1.9)	1.0 (0.57-2.07)	0.7
Maihar	15 | 841 (1.8)	reference	
Season			
Cool humid months	25 | 720 (3.5)	3.2 (1.40-7.42)	0.001
Monsoon	7 | 694 (1.0)	0.9 (0.33-2.71)	
Hot dry months	7 | 652 (1.1)	reference	

PR= Prevalence Ratio; CI= Confidence Interval; *OBC = Other backward caste, SC = Schedule caste, ST= Schedule Tribe, GEN=General caste; SES=socioeconomic status; **pregnancy number;

P.falciparum infection			*P.vivax infection*		
Parasitaemia |n (%) n =28 (positive)	**PR (95% CI)**	***P -value***	**Parasitaemia |n (%)** n = 11 (positive)	**PR (95%CI)**	***P-value***
0 | 67 (-)			1 | 68 (1.5)	-----	0.3
27 | 1753 (1.5)	3.5 (0.48-26.2)	0.24	10 | 1736 (0.6)		
1 | 233 (0.4)	reference		0	reference	
8 | 952 (0.8)	0.9 (0.26-2.93)		10 | 954 (1.1)		
6 | 433 (1.4)	1.5 (0.41-5.15)	0.006	0 | 427 (-)	-----	0.02
10 | 232 (4.3)	4.5 (1.40-14.37)		1 | 233 (0.5)		
4 | 423 (0.9)	reference		0 | 419 (-)	reference	
13 | 702 (1.9)			0 | 689 (-)		
8 | 482 (1.7)		0.22	5 | 479 (1.0)	-----	0.05
7 | 743 (0.9)			6 | 742 (0.8)		
0 | 128 (-)	reference		0 | 128 (-)	reference	
26 | 1463 (1.8)	5.2 (1.2-22.01)	0.01	9 | 1446 (0.6)	1.8 (0.39-8.47)	0.42
2 | 590 (0.3)	reference		2 | 590 (0.3)	reference	
13 | 423 (1.6)	12.4 (1.63-94.5)		1 | 411 (0.2)		
5 | 420 (1.2)	4.8 (0.56-40.98)		3 | 418 (0.7)		
6 | 441 (1.4)	5.4 (0.66-45.45)	0.008	2 | 437 (0.5)	-----	0.10
3 | 367 (0.8)	3.3 (0.34-31.60)		5 | 369 (1.4)		
1 | 404 (0.3)	reference		0 | 403 (-)	reference	
8 | 873 (0.9)			6 | 871 (0.7)		
10 | 622 (1.6)			3 | 615 (0.5)		
9 | 342 (2.6)	-----	0.12	2 | 335 (0.6)	-----	0.81
1 | 130 (0.8)			0 | 129 (-)		
0 | 88 (-)	reference		0 | 88 (-)	reference	
10 | 208 (4.8)	4.9 (2.20-10.51)	<0.0001	1 | 199 (0.5)	0.9 (0.11-7.15)	0.9
18 | 1840 (0.9)	reference		10 |1832 (0.6)	reference	
25 | 1596 (1.6)	2.3 (0.70-7.60)	0.15	8 | 1579 (0.5)	0.74 (0.19-2.81)	0.6
3 | 443 (0.7)	reference		3 | 443 (0.7)	reference	
16 | 1218 (1.3)	0.9 (0.40-1.91)	0.8	8 | 1210 (0.7)	1.8 (0.48-6.8)	0.36
12 | 838 (1.4)	reference		3 | 829 (0.4)	reference	
20 | 715 (2.8)	4.5 (1.55-13.2)		5 | 700 (0.7)	1.5 (0.37-6.4)	
4 | 691 (0.6)	0.9 (0.23-3.71)	0.0002	3 | 690 (0.4)	0.9 (0.10-4.6)	0.73
4 | 649 (0.6)	reference		3 | 648 (0.5)	reference	

TABLE 4: 20: UNIVARIATE ANALYSIS OF ANAEMIA, FEVER & MALARIA CONTROL MEASURES ASSOCIATED WITH **MATERNAL MALARIA** AT DELIVERY (1 YEAR SURVEY)

	any malaria species		
	Aparasitaemia | n (%) n= 39 (positive)	**PR (95% CI)**	***P-value***
Anaemia (Hb <11g/dL)			
Yes	23 | 1033 (2.2)	1.4 (0.76-2.69)	0.26
No	16 | 1029 (1.6)	reference	
Mod-severe anaemia (Hb <9g/dL)			
Yes	10 | 409 (2.4)	1.3 (0.68-2.83)	0.35
No	29 | 1653 (1.7)	reference	
Fever at visit[†]			
Yes	3 | 130 (2.3)	1.3 (0.39-4.08)	0.68
No	35 | 1932 (1.8)	reference	
History of fever past week			
Yes	20 | 256 (7.8)	7.4 (4.00-13.67)	<0.0001
No	19 | 1800 (1.1)	reference	
Used antimalarial past week			
Yes	3 | 11 (27.3)	15.6(5.62-43.08)	<0.0001
No	36 | 2055 (1.8)	reference	
Used any drug past week			
Yes	14 | 77 (18.2)	14.4 (7.80-26.6)	<0.0001
No	25 | 1981 (1.3)	reference	
History of fever in pregnancy			
Yes	19 | 372 (5.1)	4.3 (2.32-8.00)	<0.0001
No	20 | 1691 (1.2)	reference	
Used antimalarial in pregnancy			
Yes	4 | 41 (9.76)	5.6 (2.10-15.14)	0.0002
No	35 | 2025 (1.70)	reference	
Used any drug in pregnancy			
Yes	11 | 239 (4.6)	2.9 (1.51-5.90)	0.001
No	28 | 1819 (1.5)	reference	
Used any drug to prevent malaria			
Yes	1 | 5 (20.0)	10.8 (1.80-64.20)	0.003
No	38 | 2057 (1.9)	reference	
Used haematinics			
Yes	32 | 1794 (1.8)	0.68 (0.30-1.52)	0.35
No	7 | 267 (2.6)	reference	
Used bednet in pregnancy			
Yes	8 | 508 (1.6)	0.78 (0.36-1.70)	0.54
No	31 | 1555(1.9)	reference	
Used bednet previous night			
Yes	6 | 418 (1.4)	0.71 (0.31-1.69)	0.44
No	33 | 1641 (2.0)	reference	
MUAC (<23cm)			
Yes	29 | 1021 (2.8)	2.96 (1.45-6.04)	0.002
No	10 | 1043 (0.9)	reference	
BP Systolic (≥130mm Hg)			
Yes	6 | 621 (0.9)	0.42 (0.17-1.00)	0.04
No	33 | 1445 (2.3)	reference	
BP Diastolic (≥90mm Hg)			
Yes	4 | 453 (0.9)	0.4 (0.14-1.13)	0.07
No	35 | 1613 (2.2)	reference	

PR= Prevalence Ratio; CI = Confidence Interval; [†]axillary temperature≥37.5^{0}C;

P.falciparum infection			*P.vivax infection*		
Parasitaemia | n (%) n = 28 (positive)	**PR (95% CI)**	***P-value***	**Parasitaemia |n (%)** n= 11 (positive)	**PR (95%CI)**	***P-value***
18 | 1028 (1.6) 10 | 1023 (0.9)	1.8 (0.83-3.86) reference	0.13	5 | 1015 (4.9) 6 | 1019 (5.9)	0.83 (0.25-2.71) reference	0.76
10 | 409 (2.4) 18 | 1642 (1.1)	2.2 (1.03 - 4.79) reference	0.03	0 | 399 11 | 1635 (6.7)	----	---
1 | 128 (0.8) 26 | 1923 (1.4)	0.6 (0.07 - 4.20) reference	1.0	2 | 129 (1.6) 9 | 1906 (0.5)	3.3 (0.71-15.03) reference	0.15
14 | 250 (5.6) 14 | 1795 (0.8)	7.2 (3.46 -14.91) reference	<0.0001	6 | 242 (2.5) 5 | 1786 (0.3)	8.8 (2.72-28.79) reference	<0.0001
3 | 11 (27.3) 25 | 2044 (1.2)	22.3 (7.80-63.1) reference	<0.0001	0 | 8 11 | 2030 (0.5)	----	---
11 | 74 (14.9) 17 | 1973 (0.9)	17.2 (8.40-35.5o) reference	<0.0001	3 | 66 (4.6) 8 | 1964 (0.4)	11.1 (3.02-41.1) reference	0.004
15 | 368 (4.1) 13 | 1684 (0.8)	5.2 (2.53-11.00) reference	<0.0001	4 | 357 (1.1) 7 | 1678 (0.4)	2.7 (0.79-9.1) reference	0.11
4 | 41 (9.8) 24 | 2014 (1.2)	8.1 (2.97-22.50) reference	<0.002	0 | 37 11 | 2001 (0.5)	----	---
10 | 238 (4.2) 18 | 1809 (1.0)	4.2 (1.97-9.00) reference	<0.0001	1 | 229 (0.44) 10 | 1801 (0.56)	0.78 (0.11-6.10) reference	1.0
1 | 5 (20.0) 27 | 2046 (1.3)	15.1 (2.52-91.01) reference	0.0003	0 | 4 (-) 11 | 2030 (0.5)	----	---
5 | 265 (1.9) 23 | 1785 (1.3)	1.46 (0.56-3.80) reference	0.43	9 | 1771 (0.51) 2 | 262 (0.76)	0.66 (0.14-3.06) reference	0.64
6 | 506 (1.19) 22 | 1546 (1.42)	0.83 (0.33-2.04) reference	0.68	2 | 502 (0.4) 9 | 1533 (0.6)	0.67 (0.14-3.11) reference	1.0
4 | 416 (0.9) 24 | 1632 (1.5)	0.65 (0.22-1.87) reference	0.63	2 | 414 (0.5) 9 | 1617 (0.6)	0.86 (0.18-4.00) reference	1.0
22 | 1041 (2.2) 6 | 1039 (0.5)	3.75 (1.52-9.22) reference	0.002	7 | 999 (0.7) 4 | 1037 (0.4)	1.81 (0.53-6.18) reference	0.3
3 | 618 (0.5) 25 | 1437 (1.7)	0.27 (0.08-0.92) reference	0.24	3 | 618 (0.5) 8 | 1420 (0.6)	0.86 (0.20-3.20) reference	1.0
3 | 452 (0.7) 25 | 1603 (1.6)	0.42 (0.12-1.40) reference	0.17	1 | 450 (0.2) 10 | 1588 (0.6)	0.35 (0.04-2.71) reference	0.47

TABLE 4: 21: UNIVARIATE ANALYSIS OF DEMOGRAPHIC FACTORS ASSOCIATED WITH **PLACENTAL MALARIA** AT DELIVERY (1 YEAR SURVEY)

	Any malaria infection		
	Parasitaemia | n (%) n=21 (malaria positive)	**PR (95% CI)**	***P-value***
Age group			
<20 yr	0 | 68 (-)		0.41
20-29 yr	20 | 1765 (1.1)	2.6 (0.35-19.58)	
>30 yr	1 | 233 (0.4)	reference	
Caste Category*			
OBC	7 | 962 (0.7)	1.5 (0.35-7.30)	
SC	5 | 434 (1.2)	2.4 (0.47-12.49)	0.01
ST	7 | 234 (2.9)	6.3 (1.32-30.2)	
GEN	2 | 423 (0.5)	reference	
Education			
No Schooling	8 | 703 (1.1)	1.4 (0.18-11.5)	
Primary	6 | 488 (1.2)	1.5 (0.19-12.9)	0.86
Secondary	6 | 749 (0.8)	1.0 (0.12-8.4)	
Higher	1 | 128 (0.8)	reference	
Residence			
rural	19 | 1473 (1.3)	3.8 (0.89-16.36)	0.05
urban	2 | 593 (0.3)	reference	
SES			
Poorest	10| 424 (2.4)		
Second	5 | 424 (1.2)	-----	0.01
Third	4 | 444 (0.9)		
Fourth	2 | 372 (0.5)		
Richest	0 | 404	reference	
Gravidity			
Primigravidae	9 | 879 (1.0)		
Secundigravidae	5 | 626 (0.8)	-----	0.19
Gravidae-3	7 | 345 (2.0)		
Gravidae-4 Multigravidae (≥5)**	0 | 88	reference	
Attended ANC (any)			
No	7 | 210 (3.3)	4.4 (1.79-10.79)	0.0004
Yes	14 | 1851 (0.8)	reference	
Possessed ANC card at visit			
No	18 | 1605 (1.1)	1.7 (0.49-5.61)	0.40
Yes	3 | 447 (0.7)	reference	
Survey Site			
Katni	14 | 1227 (1.1)	1.4 (0.55-3.38)	0.49
Maihar	7 | 842 (0.8)	reference	
Season			
Cool humid months	14 | 720 (1.9)	3.2 (1.04-9.58)	0.008
Monsoon	3 | 696 (0.4)	0.7 (0.15 - 3.12)	
Hot dry months	4 | 652 (0.6)	reference	

PR=Prevalence Ratio; CI= Confidence Interval; *OBC = Other backward caste, SC = Schedule caste, ST= Schedule Tribe, GEN=General caste; SES=socioeconomic status;**pregnancy number;

P.falciparum infection		
Parasitaemia | n (%) n=18 (positive)	**PR (95% CI)**	***P-value***
0 | 68 (-)		
17 | 1762 (0.9)	2.2 (0.3-16.8)	0.52
1 | 233 (0.4)	reference	
4 | 959 (0.4)	0.9 (0.16-4.79)	
5 | 434 (1.2)	2.4 (0.47-12.4)	
7 | 234 (2.9)	6.3 (1.32-30.2)	0.001
2 | 423 (0.5)	reference	
8 | 703 (1.1)	1.4 (0.18-11.54)	
5 | 487 (1.0)	1.3 (0.15-11.14)	
4 | 747 (0.5)	0.7 (0.07-6.08)	0.63
1 | 128 (0.8)	reference	
17 | 1471 (1.2)	6.8 (0.91-51.2)	0.02
1 | 592 (0.2)	reference	
10 | 424 (2.4)		
4 | 423 (0.9)		0.001
4 | 444 (0.9)	------	
0 | 370 (-)		
0 | 404 (-)	reference	
7 | 877 (0.8)		
5 | 626 (0.8)		0.20
6 | 344 (1.7)	----	
0 | 130		
0 | 88	reference	
7 | 210 (3.3)	5.6 (2.19-14.29)	<0.0001
11 | 1848 (0.6)	reference	
16 | 1603 (1.0)	2.2 (0.51-9.61)	0.27
2 | 446 (0.4)	reference	
12 | 1225 (0.9)	1.4 (0.51-3.60)	0.50
6 | 841 (0.7)	reference	
13 | 719 (1.8)	3.9 (1.12-12.70)	0.003
2 | 695 (0.3)	0.6 (0.1-3.71)	
3 | 651 (0.5)	reference	

TABLE 4: 22: UNIVARIATE ANALYSIS OF ANAEMIA, FEVER & CONTROL MEASURES ASSOCIATED WITH **PLACENTAL MALARIA** AT DELIVERY (1 YEAR SURVEY)

	any malaria infection		
	Parasitaemia | n (%) n=21 (positive)	**PR (95%CI)**	***P-value***
Anaemia (Hb <11g/dL)			
Yes	14 | 1035 (1.4)	1.9 (0.80-4.90)	0.12
No	7 | 1029 (0.7)	reference	
Mod-severe anaemia (Hb <9g/dL)			
Yes	7 | 411 (1.7)	2.0 (0.81-4.92)	0.12
No	14 | 1653 (0.9)	reference	
Fever at visit (temp≥37.5^{0}C)			
Yes	1 | 130 (0.8)	0.8 (0.10-5.80)	0.81
No	19 | 1934 (0.9)	reference	
History of fever past week			
Yes	12 | 257 (4.7)	9.3 (3.97-21.91)	<0.0001
No	9 | 1801 (0.5)	reference	
Used antimalarial past week			
Yes	0 | 11 (-)	---	----
No	21 | 2057 (1.0)		
Used any drug past week			
Yes	8 | 77 (10.4)	15.8 (6.71-37.10)	<0.0001
No	13 | 1983 (0.7)	reference	
History of fever in pregnancy			
Yes	14 | 372 (3.8)	9.1 (3.69-22.39)	<0.0001
No	7 | 1693 (0.4)	reference	
Used antimalarial in pregnancy			
Yes	3 | 41 (7.3)	8.2 (2.52-26.8)	<0.0001
No	18 | 2027 (0.9)	reference	
Used any drug in pregnancy			
Yes	9 | 239 (3.8)	5.7 (2.4-13.4)	<0.0001
No	12 | 1821 (0.7)	reference	
Used any drug to prevent malaria			
Yes	0 | 5 (-)	----	----
No	21 | 2059 (1.0)		
Used haematinics			
Yes	17 | 1795 (0.9)	0.6 (0.2-1.04)	0.40
No	4 | 268 (1.5)	reference	
Used bednet in pregnancy			
Yes	6 | 508 (1.2)	1.2 (0.45-3.30)	0.67
No	15 | 1557 (0.9)	reference	
Used bednet previous night			
Yes	5 | 418 (1.2)	1.2 (0.45-3.30)	0.68
No	16 | 1643 (0.9)	reference	
MUAC (<23cm)			
Yes	12 | 1022 (1.2)	1.4 (0.57-3.20)	0.47
No	9 | 1044 (0.9)	reference	
BP Systolic (≥130mm Hg)			
Yes	2 | 622 (0.3)	0.2 (0.05-1.04)	0.04
No	19 | 1446 (1.3)	reference	
BP Diastolic (≥90mm Hg)			
Yes	4 | 454 (0.9)	0.8 (0.28-2.47)	0.74
No	17 | 1614 (1.1)	reference	

P.falciparum infection		
Parasitaemia | n (%) n= 18 (positive)	**PR (95%CI)**	***P-value***
13 | 1034 (1.3)	2.5 (0.92-7.2)	0.06
5 | 1027 (0.5)	reference	
7 | 411 (1.7)	2.5 (0.99-6.5)	0.04
11 | 1650 (0.7)	reference	
0 | 129 (-)	----	----
17 | 1932 (0.9)		
10 | 255 (3.9)	8.8 (3.5-22.1)	<0.0001
8 | 1800 (0.4)	reference	
0 | 11 (-)	----	----
18 | 2052 (-)		
7 | 76 (9.2)	16.5 (6.61-41.6)	<0.0001
11 | 1981 (0.6)	reference	
12 | 370 (3.2)	9.1 (3.45-24.2)	<0.0001
6 | 1692 (0.4)	reference	
3 | 41 (7.3)	9.8 (2.97-32.79)	<0.0001
15 | 2024 (0.7)	reference	
8 | 238 (3.4)	6.1 (2.43-15.3)	<0.0001
10 | 1819 (0.6)	reference	
0 | 5 (-)	----	----
18 | 2056 (-)		
15 | 1793 (0.8)	0.8 (0.21-2.56)	0.63
3 | 267 (1.1)	reference	
5 | 507 (0.9)	1.1 (0.42-3.29)	0.75
13 | 1555 (0.8)	reference	
4 | 417 (0.9)	1.1 (0.37-3.3)	0.83
14 | 1641 (0.9)	reference	
12 | 1022 (1.2)	2.0 (0.76-5.40)	0.14
6 | 1041 (0.6)	reference	
2 | 622 (0.3)	0.3 (0.06-1.25)	0.07
16 | 1443 (1.1)	reference	
4 | 454 (0.9)	1.0 (0.33-3.06)	0.98
14 | 1611 (0.9)	reference	

PR=Prevalence Ratio; CI= Confidence Interval

TABLE 4: 23: MULTIVARIATE ANALYSIS FOR RISK FACTORS ASSOCIATED WITH PLACENTAL MALARIA PARAMETERS IN THE INITIAL MODEL

Dependent variable: ***P.falciparum***

Variable	Level	Prevalence ratio	95%CI	*P-value*
Caste category[♣]	GEN	1.7	0.32-9.27	
	SC	2.9	0.76-10.76	0.06
	ST	6.3	1.64-23.81	
	OBC	reference		
Residence	Rural	4.4	0.54-34.79	0.16
	urban	reference		
Socio-economic status	Poorest	2.8	0.74-10.45	
	Second	1.5	0.36-5.97	0.29
	Third & higher	reference		
Education	No schooling	0.5	0.13-1.95	
	Primary	0.7	0.18-2.84	0.61
	≥Secondary	reference		
Attended ANC	No	2.8	1.00-8.01	0.05
	Yes	reference		
Season	Cool months	2.5	0.84-7.63	0.09
	Summer-monsoon	reference		
Gravidity	Primigravidae	1.3	0.39-4.51	
	Secundigravidae	0.9	0.27-3.26	0.83
	Multigravidae (≥3)	reference		
History of fever past week	Yes	2.8	0.91-8.64	0.07
	No	reference		
History of fever in pregnancy	Yes	3.7	1.11-12.65	0.03
	No	reference		
Use of antimalarial	Yes	3.1	0.70-13.59	0.14
	No	reference		
Moderate-severe anaemia[†]	Yes	1.5	0.50 - 4.60	0.45
	No	reference		

[♣]GEN = General caste, SC= Scheduled caste, ST =Schedule Tribe, OBC = Other backward caste; [†]Hb <9g/dL;
CI= Confidence Interval

TABLE 4: 24: RISK FACTORS ASSOCIATED WITH PLACENTAL MALARIA: PARAMETERS IN THE FINAL MODEL

Variable	Level	Prevalence ratio	95%CI	*P-value*
Caste category[♣]	GEN	1.4	0.27-7.51	0.01
	SC	3.3	0.92-12.05	
	ST	6.6	1.98-22.06	
	OBC	reference		
Attended ANC	No	3.1	1.20-8.16	0.02
	Yes	reference		
Season	Cool- months	2.9	1.00-8.56	0.05
	Summer-monsoon	reference		
Gravidity	Primigravidae	1.3	0.43-3.72	0.78
	Secundigravidae	0.8	0.27-2.60	
	Multigravidae	reference		
History of fever past week	Yes	4.2	1.50-11.95	0.006
	No	reference		
History of fever in pregnancy	Yes	4.7	1.52-14.4	0.007
	no	reference		

[♣]GEN = General caste, SC= Scheduled caste, ST =Schedule Tribe, OBC = Other backward caste; CI=Confidence Interval

4.3C Public health impact of malaria at antenatal & delivery unit women

Attributable fractions (AF) which represent the proportion of moderate to severe anaemia and birth outcomes attributable to women with *P.falciparum* exposure were calculated. In women at antenatal clinics the AF for moderate to severe anaemia was 61.5% (Table 4: 25). It suggests that *P.falciparum* accounted for 61.5% of all cases of moderate to severe anaemia among those with *P.falciparum* malaria in the ANC. In the delivery unit the AF for moderate to severe anaemia was 50%. 50% of all low birth weight and 70% of all cases of preterm babies among women with placental malaria at delivery could be attributable to *P.falciparum* malaria.

PAF calculations assessed the overall impact of malaria exposure at population level on moderate to severe anaemia and birth outcome. PAF showed that *P.falciparum* exposure explained 8.4% of moderate to severe anaemia at population level, which indicate that malaria control can prevent 8.4% of moderate to severe anaemia among the antenatal women. Likewise, placental malaria exposure explains only 4.6% low birth weight and 6.6% preterm babies at population level.

TABLE 4: 25: MALARIA ATTRIBUTABLE FRACTIONS

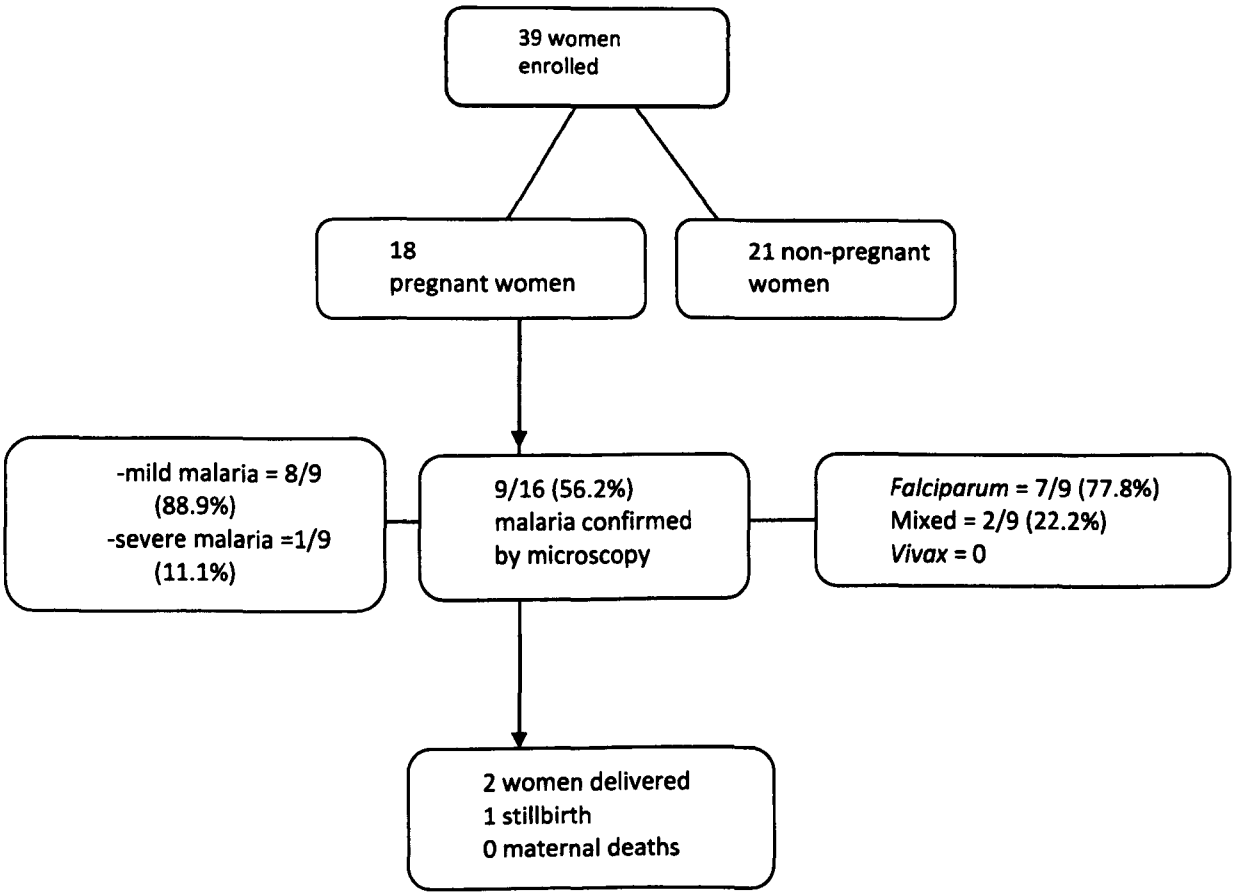
	Outcome	Exposure	Exposure Prevalence	RR (95% CI)	Attributable fractions	
					AF	PAF
ANC	moderate-severe anaemia†	*P.falciparum*	3.5%	2.6 (2.1-3.4)	61.5%	8.4%
DU	moderate-severe anaemia†	*P.falciparum* (placenta)	0.86%	2.0 (1.1-3.5)	50%	4.6%
	LBW (<2500g)			2.0 (1.3-2.9)	50%	4.6%
	Preterm (<37week)			3.4 (1.9-6.1)	70%	6.6%

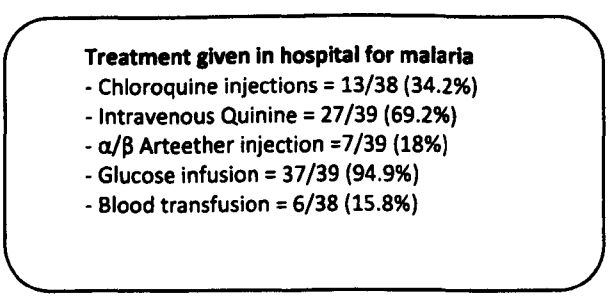
†=Hb <9g/dL

4.3D Malaria surveillance among Inpatient Women

Women of reproductive age, admitted with a suspected or confirmed malaria illness during the two rapid assessment surveys were enrolled from the 3 facilities. 39 women fulfilled the enrolment criteria out of which 18 were pregnant (46%) and 16 of them had the required data recorded in their hospital history sheet. Descriptive information relating to their malaria illness are provided (Figure 4: 14). 9/16 (56%) pregnant women had a documented microscopically confirmed malaria diagnosis: 77.8% mono-infection with *P.falciparum* and 22.2% mixed species. Two of the nine women with confirmed malaria delivered while admitted in hospital; one was a

FIGURE 4: 16: MALARIA SURVEILLANCE IN WOMEN ADMITTED IN THE HOSPITAL (SURVEY 1 & 2)





stillbirth. There was 1 case with diagnosis of severe malaria. There were no document cases of cerebral malaria or death among the admitted pregnant women during the survey period. The use of antimalarial drugs in the hospital was as follows: chloroquine injections 34%, intravenous quinine 69% and 18% received αβ artether (Emal) injections. It is should be noted that many of the pregnant women were cases diagnosed with malaria in the antenatal clinic by the study team, and admitted for treatment, particularly in the rural sites. The detection of malaria by the study team leading to initiation of treatment partly might have contributed to the reduced number of severe malaria cases in these facilities.

4.4 Discussion

The burden of malaria in pregnancy in Madhya Pradesh was determined by conducting two rapid assessment surveys followed by a year round survey in an urban, semi-urban, and rural site. The overall prevalence of malaria, as assessed by microscopy among the women attending the antenatal clinics during the two 6-week surveys was low (4.5%); 6.5% during the post-monsoon peak transmission season and 1.9% during the dry season. The year-round survey conducted only in the delivery units of the semi-urban and the rural sites confirmed the overall low prevalence with findings of 2% and 1% respectively for peripheral and placental parasitaemia. This is the first time active screening of both symptomatic and asymptomatic pregnant women was used in this region. The recent study from the adjacent state of Jharkhand, which used similar screening methodology, also reported a comparable malaria prevalence of 1.8% and 1.7% respectively among the antenatal and delivering women (Hamer, *et al* 2009).

The majority of infections were due to *P.falciparum* (80%). There was a marked variation between the three study sites with the highest prevalence of infection seen in Maihar, the rural site. In contrast, in Jabalpur, a city with over one million inhabitants (Census-India 2001), the prevalence was 0.3% in the post monsoon transmission peak, with no infection in the dry season. As expected a distinct seasonal trend was observed for *P.falciparum*. 69% of the infections occurred during the 3 months between October and December, starting approximately 6 weeks after the peak of the monsoon rains, while 83% occurred in the 6 month period, fulfilling the definition of 'marked seasonality'(Roca-Feltrer, *et al* 2009). The *P.vivax* infections were low throughout the year (<1% for peripheral and placental smears) with little seasonal variation. The *P.vivax* and *P.falciparum* ratio was lowest in the post-monsoon season (1:8) and highest in the dry season with 60% of infections being *P.vivax*. The lack of seasonal variation in *P.vivax* infection is

likely due to relapses from the dormant liver stages persisting throughout the year, rather than ongoing transmission of *P.vivax* in the dry season (Mayxay, *et al* 2004, Snounou and White 2004).

Almost two-thirds (63%) of the women attending the antenatal clinics with *P.falciparum* infection were symptomatic (documented fever or history of fever in the previous week). This contrasts with findings from malaria endemic regions in most of sub-Saharan Africa. The findings reflect the low level of malaria transmission and corresponding low levels of acquired malaria immunity in this population, which is in keeping with previous studies from India and, other low or unstable transmission regions (Das 2000, Hamer, *et al* 2009, Kochar DK, *et al* 1998, Nosten, *et al* 2004, Singh, *et al* 1999a)

Previous studies from India have reported higher prevalence of parasitaemia in paucigravidae and in younger women (<20years) (Hamer, *et al* 2009, Singh, *et al* 1999a). In the present study, age was not a determinant of malaria risk. Similarly, a gravidity specific effect, which is common in areas with high transmission was not seen in this study, reflecting the low risk of exposure and lack of acquisition of pregnancy specific immunity in first pregnancies. The risk of low birth weight and maternal anaemia associated with malaria was also similar among the different gravidity and age groups. This is consistent with studies in the highlands of Madagascar with comparably low prevalence (2%) and in areas with low or unstable transmission such as in Ethiopia, Brazil and Sudan (Cot, *et al* 2002, Newman, *et al* 2003, Martinez-Espinosa, *et al* 2004, Adam, *et al* 2009).

Although the infection risk appeared to be low in this study population, the impact of patent infection was considerable. Infected women were much more likely to be anaemic, or give birth to a preterm or low birth weight babies. Anaemia was common in this setting throughout the year, including among women without malaria (62%) and equally prevalent in the three study sites, implying that aetiologies other than malaria are dominant. Because haemoglobin levels are generally low in this population, malaria has the potential to exacerbate and was associated with a 1.3g/dL and 1.6g/dL lower haemoglobin (placental and maternal) and doubling the risk of moderate to severe anaemia compared to uninfected women. Overall, *P.falciparum* accounted for 61% of moderate to severe cases of anaemia in *P.falciparum* infected women. Because severe anaemia is associated with increased maternal morbidity, mortality and low birth weight (Brabin, *et al* 2001a, Shulman 1999, Steketee, *et al* 2001), the level of malaria induced anaemia observed in this population is likely to have a significant adverse impact on the health of individual pregnant women and their newborns.

The adjusted mean birth weight of babies born to women with placental *P.falciparum* malaria was 487g (95% CI 250-722g) lower and the risk of low birth weight was twice as high compared to women without placental malaria; this was evident in all gravidity groups. This

impact on birth weight was greater than previously reported from India and other Asian countries (India [350g], Indonesia [192g], Thailand [151g]) and of stable transmission areas of Africa [169g] (Guyatt and Snow 2004, Nosten, *et al* 1991, Poespoprodjo, *et al* 2008, Singh, *et al* 1999a). This study also found 3.4 fold increased risk of preterm delivery and a 1.2 weeks reduction in the average duration of pregnancies, which might explain the relatively large reductions in birth weight associated with *P.falciparum* infections. *P.falciparum* was responsible for 70% of the preterm deliveries in parasitaemic women. A history of fever was associated with preterm delivery (RR 1.7 CI 1.4-2.2) similar to earlier studies in other Asian countries (Luxemburger, *et al* 2001, Menon, 1972, Wickramasuriya 1937). Fever induces inflammatory cytokines which trigger uterine contractions that can result in premature deliveries, and is the mechanism thought to occur in cases of placental malaria (Menendez, *et al* 2000).

At delivery, caste, season, lack of antenatal clinic attendance, and history of fever anytime in pregnancy were factors predictive of placental malaria. The women of the Scheduled caste had a higher risk of malaria. Women of this caste were more likely to live in remote rural areas and get more exposure to mosquitoes. Likewise, women who had not attended antenatal care were those living in the more remote areas with less accessibility. Similar factors together with residence were predictive of malaria in antenatal women suggesting that the determinants of malaria in this population were similar despite the wide difference in prevalence seen between the rural and urban antenatal women.

Even though the number of *P.vivax* cases was small, the observations suggest *P.vivax* infections are also associated with reductions in mean birth weight (126 g, 95%CI -596, -343) and mean gestational age (7 days), although to a lesser degree than that observed with *P.falciparum.* While these differences were not statistically significant, the findings are consistent with previous reports from Thailand and Indonesia (Nosten, *et al* 1991, Poespoprodjo, *et al* 2008). There was also a strong association between *P.vivax* infection and history of fever in the past week and this association was stronger than that observed with *P.falciparum* infections in delivering women. *P.vivax* is not known to sequester in the placenta and none of the placental histopathology examinations found evidence of sequestration or pooling of infection in the placenta (see Chapter 5). Although the cross sectional nature of the study does not permit to make inferences about causality and time line of events, the effect on birth weight with *P.vivax* maybe a result of systematic infection leading to fever inducing early labour. In contrast to previous studies from the region, there was no evidence that *P.vivax* infections were associated with anaemia (Singh, *et al* 1999a).

Placental *P.falciparum* infections in the absence of peripheral malaria were rare: only 2 women (0.1%) with negative peripheral parasitaemia had placental infections, detected by

impression smears. This is consistent with observations reported from Thailand, but contrasts with reports from areas of high transmission such as a study of Tanzanian women, where 46% of women with parasitized placentas had no evidence of peripheral parasitaemia (Ismail, *et al* 2000, McGready, *et al* 2004). However, placental histopathology which is known to be more sensitive than standard microscopy found 4.6% of the placental impression smear negative women had histological evidence of active infections, and this figure was 2.8% among women who had negative peripheral smears at delivery (described in detail in the next chapter).

Interestingly, the diagnostic PCR studies suggested a very high prevalence of sub-microscopic infections among women attending antenatal care. During the peak transmission period, 16.0% and 6.4% of the 262 smear negative cases were PCR positive for mono-*P.falciparum* and mono-*P.vivax* respectively. The PCR study excluded dry season ANC samples. Women in the rural areas had the highest frequency of sub-microscopic infections, but interestingly, in Jabalpur, the urban site, 9% of the smear negative women were PCR positive for *P.falciparum* in the post monsoon transmission season. This difference could not be explained by antimalarial drug use which is low and as the variation of antimalarial use between patent and sub-patent infection did not differ by sites. As opposed to patent infections, a higher rate of mixed species infections was detected by PCR implying that cross-species suppression maybe occurring (Duffy 2001, Snounou and White 2004). Experimental and other studies have shown lower parasite densities for *P.vivax* and inhibition of *P.vivax* from co-infection with *P.falciparum*. Examiner error is also possible, with one species space overlooked because of difficulty of differentiating the ring-stages of the two parasite species. The level of sub-microscopic infections suggests that exposure to malaria might be considerably higher than suggested by prevalence of the patent infections. It is unclear from this cross-sectional study, whether women contain their infections or, if left untreated, whether these eventually become patent and symptomatic. There are very few publications on sub-patent infections and they differ according to setting and immune status of the pregnant population (Mockenhaupt, *et al* 2000, Saute, *et al* 2002). The current study was not designed to assess the association of sub-microscopic infections and morbidity (only a sub-selection of women in the ANC component was assayed for PCR to assess RDTs; ANC component was chosen because ANC is where RDTs would be used to screen pregnant women in MiP control programmes). But, among this sub-group, sub-microscopic *P.falciparum* infections were associated with documented fever (RR5.5, 95%CI 1.4, 21.7) and a marginally significant association with moderate-severe anaemia (RR 1.5, 95%CI 0.9, 2.4, *P=0.1*).

The estimates in our study are likely to be representative of the women attending antenatal care, or for delivery, for the larger region, at the time of conducting the study. The general characteristics of the women in the 3 surveys were comparable to that reported in the

NFHS III (NFHS-3 2005-2006) for Madhya Pradesh. The rainfall average of the study years (2006-2007) was within the average for the previous 8-10 years as defined by the rainfall anomaly index (refer to chapter 3) (van Rooy, 1965 cited in, (Tilahun 2006). The low coverage of ITNs and inconsistent implementation of IRS in the study districts, reflect the current pattern of malaria control in the state.

Because of the low prevalence of *P.falciparum* malaria, the impact on the prevalence of maternal malarial anaemia, low birth weight and thus the overall infant health will be modest. For example, as many as 60% of the moderate to severe anaemia in parasitaemic women could be attributed to malaria. However, the population attributable fraction, which is a function of the prevalence of the risk factor (malaria) suggest that elimination of malaria would only reduce the prevalence of moderate to severe anaemia by 8.4%. Similarly, the etiological fraction of malaria towards low birth weight and preterm deliveries was 50% and 70% respectively, but elimination of malaria would only result in 4.6% and 6.6% reductions in the prevalence of low birth weight and preterm deliveries in this population. Madhya Pradesh is the largest state in India, and the absolute number of women and infants that are likely to benefit is high. In this regard, a recent study based on the prevalence of 6.4% as found in this study, estimated 183,000 cases of malaria in pregnancy could be averted each year (Diamond-Smith, *et al* 2009). It is possible that these are underestimates and the true impact may be greater, particularly if accounted for the impact of sub-microscopic infections. This has important implications for the anticipated impact of successful malaria control on maternal and newborn health.

Although the national recommendation in India, at the time of this study, was to use chemoprophylaxis with chloroquine during pregnancy, the study showed that these guidelines were not implemented. Malaria control in pregnancy consisted mainly of passive surveillance, and diagnosis and treatment in symptomatic women. A new control policy is essential for this region. Considering the low prevalence and the short seasonal predominance of transmission, prevention with IPTp might not be the most feasible approach, unless timed to implement only in the peak malaria transmission season. General use of IPTp, throughout the year could lead to many women receiving antimalarials needlessly. Seasonal implementation of interventions however is a logistical challenge. ITN distribution is a choice that is likely to be acceptable by this population, because the use of non-treated bednet is already common. However ITN alone may not be as effective as observed in Africa, because the predominant vector populations (*A.fluviatilis* and *A.culicifacies*) in this region are mainly dusk biters and are exophilic and zoophilic. An additional strategy to consider is the expansion of the current methods of case detection to screening approaches. One such option is scheduled intermittent screening and treatment (IST) provided as part of focused antenatal care. IST consists of screening women at regular intervals using RDTs

and treating the RDT positive women with a long-acting ACT. It may be a particularly suitable intervention in areas where there is a lot of heterogeneity in exposure risk (e.g. 20% of the population has 80% of the malaria burden) as it would limit drug use to the highest risk groups and the peak transmission seasons. Studies are planned to assess the efficacy and feasibility of IST in India (Chandramohan, personal communication).

Many limitations need to be considered in this study. The rapid assessment surveys have proven to be informative in determining the seasonal peak malaria burden. The surprising variation between study sites that are less than 100 km apart, and the marked seasonal variation in malaria prevalence stress the limitations of rapid assessment cross-sectional surveys. They do not solve the lack of information of the trend and the total length of the transmission seasons for *P. falciparum* and *P. vivax*. Although a year round survey was conducted to capture the transmission duration, it had to be restricted to the delivery units due to funding limitations. The rapid assessment methodology with 6-8 weeks duration per survey was also not practical to capture the severity of disease in pregnant women in low transmission areas as found in Madhya Pradesh. The cross-sectional design of the surveys could provide an estimate of malaria at a given moment in time only. *P.vivax* infections were relatively low, and as a result, the MiP burden associations with *P.vivax* malaria could not be conclusively established.

In conclusion, the prevalence of malaria in these three sites in Madhya Pradesh was low with wide variation between sites and markedly seasonal with predominance of *P.falciparum*. The majority of infections were symptomatic, reflecting the relative low level of background immunity that may predispose women to developing fever and delivering prematurely, doubling the risk of low birth weight. Despite the low prevalence of malaria, malaria in pregnancy was associated with major adverse effects to the mother and newborn and, unlike in areas with stable transmission, primigravidae and multigravidae were equally affected. Even though the exposure risk to the individual is relatively low, the potential public health impact of successful control of malaria in pregnancy is considerable, because of the large population of reproductive age women at risk of malaria in Madhya Pradesh.

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Chapter 5

Placental histopathological changes and the association with birth outcome & maternal anaemia

5.1 Introduction

The pathognomonic feature of placental malaria centres on the cytoadhesion of *P.falciparum* infected erythrocytes to chondroitin sulphate A (CSA) and other placental receptors and the associated inflammatory response involving a homing in of monocytes and macrophages. The presence of parasites, inflammatory cells and pigment (haemozoin) in immune cells and fibrin are the characteristic features of placental malaria and have been used to classify the chronology of placental infection (Bulmer, *et al* 1993a, Ismail, *et al* 2000).

The association between these placental changes and low birth weight and maternal anaemia has been documented for *P.falciparum* infections, however, the exact mechanism is not yet clear (Leopardi, *et al* 1996, Menendez, *et al* 2000, Rogerson, *et al* 2003b, Watkinson, *et al* 1983). Early investigations showed a strong association between the presence of parasites and low birth weight and that the accumulation of parasites *per se* may affect placental function (Brabin, *et al* 2004, Leopardi, *et al* 1996). There is now clear evidence that the placental inflammatory response to the sequestration of parasites and the presence of pigment are associated with fetal growth restriction, although it is not clear whether this is causal with placental function, or whether these are proxies for disease severity or the duration of infection (Abrams, *et al* 2003, Duffy 2001, Muehlenbachs, *et al* 2010). These previous studies of *P.falciparum* were conducted in highly endemic regions in sub-Saharan Africa.

There is far less information on malaria associated placental histological changes with placental *P.falciparum* in low transmission areas and those associated with *P.vivax* malaria. Only two studies have reported on histopathological findings associated with *P.vivax* (McGready, *et al* 2004, Parekh, *et al* 2010). *P.vivax* is not known to cytoadhere in the placenta, yet has been found to have an association with a significant reduction in maternal haemoglobin levels and reductions in birth weight, although the effects appear less pronounced than with *P.falciparum*. The present study describes both *P.falciparum* and *P.vivax* associated morphological changes in the placenta and the associated impact of placental *P.falciparum* infection on birth outcome and maternal anaemia in a low malaria transmission area.

5.2 Material

5.2.1 Sample Collection

Women were enrolled at delivery during a one year period between October 2006 and September 2007. A finger prick blood sample was collected for malaria microscopy and RDT before delivery. The placental samples included incision and impression smears for microscopic

examination, a biopsy for histological examination and placental blood for PCR. The different sampling techniques are described in chapters 3 and 8.

Placental histopathology sections: Two 2x2x1cm biopsies were cut from the maternal side of the placenta at opposite ends half-way between the umbilical cord insertion and the edge of the placenta. The two sections were placed in jars containing 100ml of 10% phosphate buffered formalin and fixed overnight. Neutral buffered formalin was used in order to avoid formation of formalin pigments. The following day the fixed placental tissues were trimmed to make about 3mm thick sections. The trimmed sections were placed in labelled cassettes and in fresh 10% buffered formalin fixative and transported to the central field station in Jabalpur and stored in an air conditioned room until processed to wax blocks. While stored, the fixative was changed monthly to ensure pH stability, and all specimens were processed within a maximum of 3 months. The sections were processed using standard techniques and embedded in paraffin wax. The paraffin blocks were cut into 5μm thick sections for each of the two biopsies, mounted on slides and stained with haematoxylin and eosin (H&E).

5.3 Methods

5.3.1 Sample Selection for histopathology examination

Placental histology sections were processed from 2282 women. The sections corresponding to malaria positive results by any of the following diagnostic tests: RDT, peripheral, placental incision and placental impression smears, were selected for histopathological examination. 50 malaria positive sections were selected for histological examination, including 40 from women with positive peripheral smears and an additional 10 from women with positive placental smears (7) or RDT (3).

In addition to the positive smears, 456 negative sections were selected at random using a random selection procedure in SAS Software. To minimize selection bias and to allow extrapolation of the results to the total sample, the number of negative controls was selected proportional to the number of positive samples, stratified by site and survey. The number of negative sections required from each site was: 18, 91 and 91 for Jabalpur (9.1% of 200), Katni (45.1%) and Maihar (45.1%) in survey 2 and 159 and 97 for Katni (63.6% of 250) and Maihar (38.6%) in survey 3 respectively. A spare list of 10 sections was generated for each site to replace any missing sections.

5.3.2 Histopathological Examination

The histology slides were first examined using standard light microscopy, and thereafter using polarized light to increase the sensitivity of haemozoin detection (Romagosa, *et al* 2004). The birefringence of haemozoin pigment under polarized light makes identification of parasitized cells within the placental structures easier, particularly in cases with scanty parasitaemia. The slides were read by the study investigator (RA) who was aware of blood smear results, but was unaware (at the time of slide examination) of the maternal characteristics, obstetric history and pregnancy outcome corresponding to the sections examined. The histological examination was done according to the approach described previously (Bulmer, *et al* 1993a, Ismail, *et al* 2000).

Parasitized cells and pigment: The intervillous spaces and villous structures were examined for the presence of parasitized cells. The slides were examined under 40x, or 100x magnification and parasite presence scored semi-quantitatively as follows: scanty = between 1-5 parasites in any high power field (HPF), moderate= 5-10 parasites in 2 or more HPF, heavy= >10-15 in 2 or more fields examined (Table 5:1) and absent if no parasites were detected in any of the 50 fields examined at 40x magnification.

Observation of brownish granular looking material that birefringence under polarized light was identified as pigment. Pigment deposition was semi-quantitatively assessed: mild if small amounts were present focally, moderate when pigment was seen distributed either as small spots in many locations or larger deposits, and severe when large amounts were distributed in the placental structures (Table 5:1). The structures examined for the presence of pigments was intervillous and perivillous fibrin, inflammatory cells, villous stroma, the syncytium and Hofbauer cells.

Inflammatory cells: The slides were scanned at 10x magnification and examined at 40x magnification for the presence of polymorphonuclear cells and monocytes and macrophages, henceforth referred as monocytes. The structures observed for presence of inflammatory cells were the villi and the intervillous spaces. The findings were recorded semi-quantitatively as mild if (<10 cells), moderate (10-25 cells), severe (>25 cells) per field (Table 5:1). Inflammatory cells were considered absent if none was observed in any of the 50 fields examined at 40x magnification.

TABLE 5: 1: SYSTEM FOR EVALUATION OF THE SEVERITY OF PLACENTAL PATHOLOGICAL CHANGES

	Pathological severity		
	mild	**moderate**	**severe**
Perivillous or intervillous fibrin	Present in 20% IVS	Present in 20-50% IVS	Present in ≥50% IVS
Fibrinoid necrosis	Focal: localised to few fibrin areas		Diffuse:>50% fibrin affected
Syncytial knots	Normal: <1/3 villi affected		>1/3 villi affected in total fields viewed
Pigment deposition	Focal presence in small amounts	Small spots or larger deposits in many locations	Large amount present widely
Polymorphonuclear cells	<10 cells/HPF	10-25 cells/HPF	>25cells /HPF
Mononuclear cells	<10 cells/HPF	10-25 cells/HPF	>25 cells/HPF
Parasite presence	**Scanty**: 1-5/in any HPF	5-10 in 2 or more HPF	**Heavy**: >10 in any 2 or more HPF

IVS= intervillous space, HPF=High power field

Placental structures: The placental features evaluated include the presence of intervillous and perivillous fibrin scored subjectively as mild if contained in less than 20% of the intervillous or perivillous space, moderate if present in 20-50% and severe if deposited in more than 50% of the spaces (Table 5:1). Syncytial knots were categorised as either normal, or increased if present in >1/3 of the villi in the total fields viewed. Fibrinoid necrosis and calcification was recorded as focal if localized in a few fibrin areas, and diffuse if greater than 50% were affected, or absent if none were viewed. Appearance of dark bluish purple spots in the placental structures that did not show birefringence on polarized light was recorded as calcifications. In addition to these structures, Hofbauer cells, fetal red blood cells and villous stroma were also evaluated for morphological changes.

5.3.3 Histological Classification of Infection

Malaria infection was classified using a combination of the criteria described by Bulmer et al (Bulmer, *et al*)and Ismail et al as follows (Table 5:2). Acute infection: parasites present in the maternal erythrocytes with or without pigmented monocytes. Very mild presence of pigment in fibrin (few pigment spots) if present were included. Chronic: presence of parasites with

pigmented monocytes either present or absent and moderate to severe presence of pigment in fibrin. Past infection: only pigment in the fibrin present (in any concentration). No-infection: absence of both parasites and pigment. Accordingly, all the cases with parasites were classed as active infections (acute and chronic) and those with only pigment as past infection.

TABLE 5: 2: HISTOLOGICAL CLASSIFICATION

Class	Description	Class	Description
1	**Acute Infection:** parasite + pigment in monocyte ± pigment in fibrin ±	3	**Past Infection:** Parasite = 0 pigment in fibrin+/ ++
2	**Chronic Infection:** parasite + pigment in monocytes ± pigment in fibrin or cells ++	4	**No Infection** parasite = 0 pigment = 0

5.3.4 PCR analysis for species confirmation

PCR was performed for species confirmation on a selected number of samples due to budget constraints. This consisted of the samples corresponding to histology positive cases and a similar number of negative samples chosen for quality assurance matched by gravidity and the study sites. In total 115 samples of placental red blood cell pellets collected at the time of delivery and stored at -40^{0}C were analysed for PCR using the process described in chapter 3. In brief, nested PCR was performed with the first round for genus specific analysis and the second round for species specific amplification to detect *P.falciparum* and *P.vivax*.

5.3.5 Statistical Analysis

Data was entered in Microsoft Access (2003) and analysed using SAS® 9.1 (Statistical Application Software, SAS Institute Inc. Cary NC). Changes to the placental lesions by infection class were evaluated using the χ^2 test. The impact of infection on birth outcomes was assessed using univariate and multivariate analyses. Univariate analysis was performed using Student's *t* test and analysis of variance (ANOVA) for continuous endpoints. Multivariate analysis was performed using General Linear Models (GLM) with birth weight, gestational age and haemoglobin as the continuous dependent variables and histology parameters as the independent predictors of interest. Separate models assessed the association between birth weight, gestational age and haemoglobin and the predictor variables consisting of different histologically classified

infections: acute, chronic, past and no infection. All the variables representing placental parameters of interest were included in all the initial models regardless of the strength of the association with the dependent variable in the univariate analysis. The socio-demographic parameters; socioeconomic status, education, residence and gravidity were also included in the initial model to adjust for potential confounding and to assess for effect modification. Backward elimination was used with a significance level of P=0.05 to retain variables in model. The interaction between the presence of parasites and pigmented monocytes was assessed. Pigmented monocyte was entered as a variable consisting of 3 categories: mild, moderate-severe and absent.

5.4 Results

A total 506 sections were examined for histology including 50 samples from women with evidence of malaria by standard microscopy or RDT and 456 negative controls. The proportion of low birth weight (<2500g) and preterm birth (<37 week by Ballard score) was 37% and 20%, which, as expected was higher than in the main sample (33.3% and 14.5% respectively) because the sample selection for this sub-study included a higher proportion of women with malaria than in the overall sample. The prevalence of moderate to severe anaemia (Hb<9g/dL) was 21%.

5.4.1 Malaria infection by histopathology

On histological examination, placental malaria was identified in 52 of the 506 sections; 38 (7.5%) sections were active infections defined by the presence of parasites and 14 (2.8%) were classified as past infections defined by the presence of only pigment in fibrin. Out of the 38 active infections, 20 (3.9%) fulfilled the classification criteria of acute infection and 18 (3.6%) were chronic infections (Table 5:3).

The overall distribution of parasitized erythrocytes in the intervillous space was sparse: 60% was identified as scanty, observed by the presence of 1-5 parasitized cells in any high power field (40x). Most of the infected erythrocytes were seen within the intervillous spaces and they were rarely seen adhering to the syncytial layer. No parasites were seen in fetal erythrocytes or within the villous structures. Monocyte infiltrate, when present, was generally mild (60%) or moderate (30%) with only 1 case identified as severe intervillous infiltrate. Overall 64% of infected sections had a ratio of inflammatory cells to parasitized cells of <10%.

TABLE 5: 3: MALARIA INFECTION BY DIFFERENT TEST METHODS CORRESPONDING TO HISTOLOGICALLY CLASSIFIED INFECTION IN 506 SAMPLES

Histopathology	Positive	Acute	Chronic	Past	Non-infected
	N	n (%)	n (%)	n (%)	n (%)
	52	20 (3.9)	18 (3.6)	14 (2.8)	454 (89.7)
Any species					
Peripheral blood microscopy, n (%)	39	20 (51.3)	5 (12.8)	2 (5.1)	12 (30.8)
Placental impression smear, n (%)	22	14 (63.6)	3 (13.6)	1 (4.6)	4 (18.2)
Placental incision smear, n (%)	32	18 (56.3)	5 (15.6)	2 (6.2)	7 (21.9)
PCR*, n (%)	62	19 (30.6)	13 (21.0)	6 (9.7)	24 (38.7)
P.falciparum					
Peripheral blood microscopy, n (%)	28	20 (100.0)	5 (27.8)	0	3 (0.7)
Placental impression smear, n (%)	19	14 (70.0)	3 (16.7)	0	2 (0.4)
Placental incision smear, n (%)	26	18 (90.0)	5 (29.4)	0	3 (0.7)
PCR, n (%)	45	18 (90.0)	12 (66.6)	3 (37.5)	12 (25.0)
P.vivax					
Peripheral blood microscopy, n (%)	11	0	0	2 (14.3)	9 (2.0)
Placental impression smear, n (%)	3	0	0	1 (7.1)	2 (0.4)
Placental incision smear, n (%)	6	0	0	2 (14.3)	4 (0.9)
PCR, n (%)	10	0	0	3 (37.5)	7 (14.5)

* PCR was performed only on a sub-set of 115 samples.

Correlation with microscopy: Table 5:3 shows the results of conventional microscopy of peripheral and placental smears corresponding to the histological class of infection; 20 out of the 28 cases with a *P.falciparum* positive peripheral smear corresponded to histology classed acute infection and 5 to chronic infection; i.e. 25/28 (89%) had an active infection. In contrast, none of the peripheral smear *P.vivax* positive cases had evidence of either acute or chronic infection by placental histology (0/11). Also none of the smear positive *P.falciparum* cases corresponded to past infection, whereas 2 smear positive *P.vivax* cases had a matched histologically classed past

TABLE 5: 4: CHANGES IN THE PLACENTA ACCORDING TO HISTOLOGICAL CLASSIFICATION OF INFECTION

Placental features	No† infection (n=454)	Acute infection (n=20)	Chronic infection (n=18)	Past†† Infection (n=14)	Active♣ Infection (n=38)	Active *vs* No infection *P-value*	Past *vs* No infection *P-value*
Syncytial Knots							
Normal, n (%)	414 (91.2)	12 (60.0)	6 (33.3)	8 (57.1)	18 (47.4)	<0.0001	<0.001
Increased (>1/3), n (%)	40 (8.8)	8 (40.0)	12 (66.7)	6 (42.9)	20 (52.6)		
Fibrinoid necrosis						0.003	0.0001
Absent, n (%)	393 (86.5)	20 (100.0)	7 (38.9)	7 (50.0)	27 (71.1)		
Focal, n (%)	58 (12.8)	0	9 (50.0)	6 (42.9)	9 (23.6)		
Diffuse, n (%)	3 (0.7)	0	2 (11.1)	1 (7.1)	2 (5.3)		
Calcification						0.008	
Absent, n (%)	416 (91.6)	20 (100.0)	9 (50.0)	9 (64.3)	29 (76.3)		0.002
Focal, n (%)	31 (6.8)	0	7 (38.9)	4 (28.6)	7 (18.4)		
Diffuse, n (%)	7 (1.6)	0	2 (11.1)	1 (7.1)	2 (5.3)		
IVS* Polymorphonuclear cell							
Absent, n (%)	340 (74.9)	14 (70.0)	12 (70.6)	13 (92.9)	26 (70.3)		
Mild, n (%)	80 (17.6)	4 (20.0)	5 (29.4)	1 (7.1)	9 (24.3)	0.75	0.47
Moderate, n (%)	33 (7.3)	2 (10.0)	0	0	2 (5.4)		
Severe, n (%)	1 (0.2)	0	0	0	0		
IVS* monocytes							
Absent, n (%)	358 (78.8)	1 (5.0)	2 (11.1)	11 (78.6)	3 (7.9)		
Mild, n (%)	74 (16.3)	12 (60.0)	12 (66.7)	3 (21.4)	24 (63.2)	<0.0001	0.63
Moderate, n (%)	22 (4.9)	6 (30.0)	4 (22.2)	0	10 (26.3)		
Severe, n (%)	0	1 (5.0)	0	0	1 (2.6)		
IVS* fibrin							
Absent, n (%)	134 (29.5)	4 (20.0)	0	0	4 (10.5)		
Mild, n (%)	268 (59.0)	15 (75.0)	10 (55.6)	5 (35.7)	25 (65.8)	0.0002	<0.001
Moderate,n (%)	52 (11.5)	1 (5.0)	7 (38.9)	9 (64.3)	8 (21.1)		
Severe, n (%)	0	0	1 (5.5)	0	1 (2.6)		

Note: all are column %; †histology classified no-infection, ††includes 2 cases corresponding to *P.vivax* infection; ♣includes histology classed acute & chronic cases *IVS = intervillous space

infection. Subsequent PCR analysis showed the smear positive results corresponding to the respective histological class to be positive.

5.4.2 Placental structures associated with Malaria Parasites and Pigment

P.falciparum: Table 5:4 shows the placental morphological changes stratified by their histological categories. Compared to histologically uninfected placentas, chronic infections were associated with an increased frequency of syncytial knotting (66.7%), focal fibrinoid necrosis (50%) and focal calcification (38.9%); whereas monocyte infiltrate was significantly associated with active infection. Intervillous polymorphonuclear cell infiltrates were not associated with acute, chronic, or past infections.

TABLE 5: 5: PLACENTAL CHANGES AMONG PERIPHERALLY PATENT CASES BY SPECIES

Placental feature	No† infection (n= 441)	*Pf* (n= 28)	*Pv* (n=11)	*Pf vs* no infection *P-value*	*Pv vs* no Infection *P-value*	*Pf vs Pv* *P-value*
Syncytial Knots						
Normal, n (%)	406 (92.1)	16 (57.1)	4 (36.4)	<0.0001	<0.0001	0.03
Increased (>1/3), n (%)	35 (7.9)	12 (42.9)	7 (63.6)			
Fibrinoid necrosis						
Absent, n (%)	380 (86.2)	24 (85.7)	9 (81.8)			0.68
Focal, n (%)	58 (13.2)	3 (10.7)	2 (18.2)	0.25	0.85	
Diffuse, n (%)	3 (0.6)	1 (3.6)	0			
Intravillous Calcification						
Absent, n (%)	403 (91.4)	25 (89.3)	11 (100.0)	0.73	0.59	0.52
Focal, n (%)	31 (7.0)	2 (7.1)	0			
Diffuse, n (%)	7 (1.6)	1 (3.6)	0			
Intervillous fibrin						
Absent, n (%)	125 (28.3)	6 (21.4)	7 (63.6)			
Mild, n (%)	265 (60.1)	20 (71.4)	4 (36.4)	0.48	0.03	0.03
Moderate,n (%)	51 (11.6)	2 (7.2)	0			
Severe, n (%)	0	0	0			
IVS* polymorphonuclear cells						
Absent, n (%)	335 (75.9)	19 (70.4)	3 (27.3)			
Mild, n (%)	77 (17.5)	6 (22.2)	3 (27.3)	0.91	<0.0001	0.01
Moderate, n (%)	28 (6.4)	2 (7.4)	5 (45.4)			
Severe, n (%)	1 (0.2)	0	0			
Intervillous monocytes						
Absent, n (%)	351 (79.6)	4 (14.3)	5 (45.5)			
Mild, n (%)	69 (15.7)	13 (46.4)	5 (45.5)	<0.0001	0.02	0.12
Moderate, n (%)	21 (4.7)	10 (35.7)	1 (9.0)			
Severe, n (%)	0	1 (3.6)	0			

Note: all are column %; †negative for parasites by smears & histology; *Pf = P.falciparum*; *Pv = P.vivax* *IVS =intervillous space

P.vivax: Table (5:5) shows placental morphological changes and inflammatory response in the placenta of women with peripheral blood *P.vivax* mono-infections. PCR confirmed *P.vivax* mono infections were associated with increased frequency of syncytial knots (63.6%), presence of intervillous polymorphonuclear cells ($P<0.0001$), and monocytes ($P=0.02$).

P.falciparum associated placental changes in PCR positive and smear negative women: 13 sections were negative by conventional smear microscopy, but positive by PCR. They corresponded to histologically classed chronic infections. These infections were associated with increased morphological changes in the placental structures compared with uninfected placenta, with the exception of changes in intervillous polymorphonuclear cells (Table 5:6). Sub-clinical placental infection was detected in samples in Katni and Maihar: 13/265 (4.9%) and 8/196 (4.1%) respectively. None was detected in Jabalpur, urban site samples.

TABLE 5: 6: PLACENTAL CHANGES ACCORDING TO SMEAR NEGATIVE, PCR POSITIVE ***P.FALCIPARUM*** INFECTIONS

Placental features	**No† Infection** (n= 441)	***Pf* placental only** (n= 13)	***Pf vs* no infection** *P-value*
Syncytial Knots			
Normal, n (%)	406 (92.1)	5 (38.5)	<0.0001
Increased >1/3, n (%)	35 (7.9)	8 (61.5)	
Fibrinoid necrosis			
Absent, n (%)	380 (86.2)	6 (46.2)	<0.0001
Focal, n (%)	58 (13.2)	6 (46.2)	
Diffuse, n (%)	3 (0.6)	1 (7.6)	
Calcification			
Absent, n (%)	403 (91.4)	7 (53.8)	<0.0001
Focal, n (%)	31 (7.0)	5 (38.5)	
Diffuse, n (%)	7 (1.6)	1 (7.7)	
IVS* polymorphonuclear cells			
Absent, n (%)	335 (75.9)	10 (76.9)	0.78
Mild, n (%)	77 (17.5)	3 (23.1)	
Moderate, n (%)	28 (6.4)	0	
Severe, n (%)	1 (0.2)	0	
IVS monocytes			
Absent, n (%)	351 (79.6)	2 (15.4)	<0.0001
Mild, n (%)	69 (15.7)	11 (84.6)	
Moderate, n (%)	21 (4.7)	0	
Severe, n (%)	0	0	
IVS fibrin			
Absent, n (%)	125 (28.3)	0	<0.0001
Mild, n (%)	265 (60.1)	6 (46.2)	
Moderate, n (%)	51 (11.6)	6 (46.2)	
Severe, n (%)	0	1 (7.6)	

Note: all are column % †negative for parasites by smears & histology; *Pf = P.falciparum*; *IVS =intervillous space;

5.4.3 Impact of histological infection (*P.falciparum*) on birth outcome & anaemia

Birth weight

Univariate analysis: Factors significantly associated with birth weight were the presence of parasites and pigmented monocytes. The presence of parasites was associated with a 218g lower mean birth weight (95%CI -379, -58) compared to no parasites and with a 378g difference (95%CI -602, -152) in the presence of pigmented monocytes compared to the absence of pigmented monocytes (Table 5:7). Pigment in fibrin (108g) and focal calcification (116 g) were also associated with reduced birth weights, but these associations were not statistically significant.

Multivariate analysis: The GLM analyses adjusted for gravidity, education, residence and socioeconomic status, with the interaction terms for pigmented monocytes and parasites showed that the presence of parasites in the absence of pigmented monocytes was associated with a non-significant reduction of -51g (95%CI -264, 161) in mean birth weight, but this reduction was 371g (95%CI -591, -151) in the presence of parasites and pigmented monocytes, when compared against the mean birth weight among women with neither malaria parasites or pigment (Table 5:7). Further stratification showed that the reduction of birth weight was greatest (623g) in women with moderate-severe infiltration of pigmented monocyte and parasites, compared to 315g in women with mild infiltration of pigmented monocyte and parasites. None of the other histological measures were significantly associated with birth weight independent of the presence of parasites and pigmented monocytes.

In other models that looked at the association between birth weight and the conventional histological class of placental malaria infection, only acute infections were associated with reductions in birth weight (390g; 95% CI -690, -169). Sub-patent infections (histology positive, peripheral and placental smear negative) were not associated with a significant reduction in birth weight in the presence of pigmented monocytes, but numbers were small (n=7) and the confidence interval wide (207g, 95%CI -534, 119).

Gestational Age

Univariate analysis: Table (5:8) shows factors significantly associated with gestational age. These were presence of parasites, pigmented monocytes, increased syncytial knots, and focal calcification.

Multivariate analysis: In the GLM adjusted for gravidity and socioeconomic covariates, reduction in mean gestation was significantly associated with the presence of parasites and pigmented monocytes, but not with the other factors (Table 5:8). The adjusted mean gestational age in uninfected cases was 37.2 weeks. The presence of parasite and pigmented monocytes reduced the mean gestational age by 0.6 weeks (95%CI -1.3, 0.01). The presence of parasite together with moderate-severe infiltration of monocyte shortened mean gestation by one week (95%CI -2.5, 0.3). With histologically classified acute infections the mean gestational age was associated with a reduction by 0.8 weeks (95%CI -1.4, -0.2) but the reduction of gestational age associated with either chronic or past infection was not statistically significant. In the smear negative, histology positive model

the mean gestational age was reduced by 0.5 weeks if parasite and pigment were both present, but this was not significant.

Maternal Anaemia

Univariate analysis: A significant reduction in mean haemoglobin was associated with the presence of parasite and with pigmented monocytes (Table 5:10). There was no association between other morphological changes and mean haemoglobin.

Multivariate analysis: In the GLM adjusted models the presence of parasite together with pigmented monocyte was associated with reduced mean haemoglobin [1.1g (95%CI -2.1, -0.1)] (Table 5:11), and this was worse in those with moderate-severe pigmented monocyte infiltration (1.3g). Histologically classed acute infection was also associated with a decrease of 1.5g in mean haemoglobin (95%CI 0.5, 2.5). With the smear negative placental infections, the combined presence of parasites and pigmented monocytes decreased the mean haemoglobin but this did not reach significance.

TABLE 5: 7: UNIVARIATE ANALYSIS OF PLACENTAL HISTOLOGICAL DETERMINANTS OF BIRTH WEIGHT

Factors	Mean birth weight (SD)	Mean difference (95% CI)	*P-value*
Placental parasite			
present (n=33)	2419 (535)	-218 (-379, -58)	0.01
absent (n = 427)	2637 (444)	reference	
Syncytial knots			
increased (n =53)	2546 (524)	-86 (-216, 44)	0.2
normal (n = 411)	2631 (442)	reference	
Fibrinoid necrosis			
present (n=73)	2620 (483)	-1 (-116, 111)	0.9
absent (n =391)	2622 (449)	reference	
Intervillous fibrin			
mild (n=277)	2615 (473)	-25 (-142, 92)	0.8
moderate-severe (n=68)	2616 (432)	-24 (-186, 139)	
absent (n=119)	2640 (424)	reference	
Calcification			
focal (n =51)	2519 (426)	-116 (-248, 16)	0.1
absent (n =413)	2635 (457)	reference	
Pigmented fibrin			
present (n=34)	2522 (465)	-108 (-266, 51)	0.2
absent (n = 430)	2630 (453)	reference	
Pigmented monocytes			
present (n = 16)	2257 (516)	-377 (-602, -152)	0.001
absent (n = 441)	2635 (447)	reference	
*IVS Polymorphonuclear cells			
mild (n=82)	2647 (440)	30 (-101, 161)	
moderate-severe (n=28)	2605 (448)	-12 (-222, 198)	0.8
absent (n=353)	2617 (459)	reference	
*IVS monocytes			
mild (n=90)	2565 (454)	-71 (-197, 55)	0.4
moderate-severe (n=30)	2634 (515)	-2 (-205, 201)	
absent (n=344)	2636 (444)	reference	

*IVS =intervillous space

TABLE 5: 8: UNIVARIATE & MULTIVARIATE ANALYSIS OF THE IMPACT OF HISTOLOGICAL PLACENTAL INFECTIONS ON BIRTH WEIGHT IN 506 PLACENTAL SAMPLES

Factors	N	Crude birth weight			Adjusted birth weight[♣]		
		Mean (SD)	Mean difference (95 % CI)	*P-value*	Mean (SD)	Mean difference (95% CI)	*P-value*
1. Placental infection							
parasite +pigmented monocyte	16	2257 (516)	-379 (-645, -111)	0.003	2286 (440)	-371 (-591, -151)	0.004
parasite only	17	2570 (523)	-66 (-324, 194)		2606 (437)	-51 (-264, 161)	
both absent	427	2636 (440)	reference		2657 (447)	reference	
2. Placental infection							
parasite + mod-severe monocyte	3	2010 (582)	-626 (-1293, 40)	0.01	2035 (439)	-623 (-1125, -120)	0.01
parasite + mild pigmented monocyte	13	2315 (507)	-321 (-645, 2.9)		2343 (436)	-315 (-557, -72)	
parasite only	17	2570 (523)	-66 (-324, 194)		2606 (437)	-52 (-264, 161)	
parasite, monocyte, absent	427	2636 (440)	reference		2658 (445)	reference	
3. Placental infection							
acute	16	2232 (541)	-399 (-683, -114)		2206 (460)	-390 (-609, -169)	0.001
chronic	17	2594 (481)	-37 (-312, 239)	0.002	2618 (432)	21 (-197, 241)	
past	14	2790 (484)	159 (-144, 462)		2819 (434)	223 (-11, 458)	
no infection	427	2636 (439)	reference		2596 (721)	reference	
Calcification							
focal	51	2519 (426)	-114 (-240, 11)	0.1	2493 (449)	-133 (-266, 0.6)	0.05
absent	423	2633 (452)	reference		2626 (431)	reference	
4. Smear negative infection (any)							
parasite + pigmented monocyte	7	2449 (481)	-189 (-578, 200)	0.5	2450 (436)	-207 (-534, 119)	0.4
parasite, no pigmented monocyte	13	2668 (490)	30 (-257, 317)		2688 (432)	31 (-210, 217)	
no infection	427	2636 (440)	reference		2657 (447)	reference	

[♣] Models adjusted for socioeconomic status, gravidity, education, residence and site; **Note:** serial number denotes a separate GLM model. Model 4 = infections detected on histology but negative on peripheral and placental impression smear examination.

TABLE 5: 9: UNIVARIATE ANALYSES OF PLACENTAL HISTOLOGICAL DETERMINANTS OF GESTATIONAL AGE

Factors	Mean gestational age (SD)	Mean difference 95% CI	*P-value*
Placental parasite			
present (n=33)	36.7 (1.2)	-0.5 (-1.04, 0.11)	0.02
absent (n=427)	37.1 (1.3)	reference	
Syncytial knots			
increased (n= 53)	36.8 (1.7)	-0.5 -0.87, -0.11)	0.01
absent (n = 410)	37.3 (1.2)	reference	
Fibrinoid necrosis			
present (n =73)	37.0 (1.4)	0	0.9
absent (n =390)	37.0 (1.3)		
Intervillous fibrin			
mild (n=276)	37.2 (1.3)	0	
moderate-severe (n=68)	37.1 (1.4)	-0.1 (-0.55, 0.39)	0.8
absent (n =119)	37.2 (1.2)	reference	
Calcification			
focal (n =51)	36.8 (1.3)	-0.5 (-0.88, -0.11)	0.01
absent (n = 412)	37.3 (1.3)	reference	
Pigmented fibrin			
present (n =34)	37.0 (1.2)	-0.2 (0.75, 0.17)	0.2
absent (n =429)	37.2 (1.3)	reference	
Pigmented monocytes			
present (n = 16)	36.5 (1.2)	-0.7 (-1.38, 0.10)	0.02
absent (n = 447)	37.2 (1.3)	reference	
*IVS Polymorphonuclear cells			
mild (n=81)	37.3 (1.3)	0.1 (-0.10, 0.56)	
moderate-severe (n=28)	37.5 (1.1)	0.3 (-0.25, 0.96)	0.2
absent (n=353)	37.2 (1.3)	reference	
*IVS monocytes			
mild (n=90)	37.1 (1.2)	-0.1 (-0.48, 0.25)	
moderate-severe (n=30)	37.4 (1.6)	0.2 (-0.43, 0.74)	0.6
absent (n=343)	37.2 (1.3)	reference	

*IVS = intervillous space

TABLE 5: 10: UNIVARIATE & MULTIVARIATE ANALYSES OF THE IMPACT OF HISTOLOGICAL PLACENTAL INFECTIONS ON GESTATIONAL AGE IN 506 PLACENTAL SAMPLES

Factors	N	Crude gestational age			Adjusted gestational age[♣]		
		Mean (SD)	Mean difference (95 % CI)	*P-value*	Mean (SD)	Mean difference (95% CI)	*P-value*
1. Placental Infection							
parasite +pigmented monocyte	16	36.6 (1.1)	-0.7 (-1.5, 0.04)	0.04	36.6 (1.2)	-0.6 (-1.3, 0.01)	0.03
parasite, no pigmented monocyte	17	36.8 (1.3)	-0.5 (-1.2, 0.3)		36.7 (1.2)	-0.5 (-1.2, 0.1)	
both absent	427	37.3 (1.3)	Reference		37.2 (2.0)	reference	
2. Placental Infection							
parasite + mod-severe monocyte	3	36.0 (1.0)	-1.3 (-3.1, 0.5)	0.06	36.1 (1.7)	-1.1 (-2.5, 0.3)	0.05
parasite + mild pigmented monocyte	13	36.7 (1.2)	-0.6 (-1.5, 0.3)		36.7 (1.0)	-0.5 (-1.2, 0.2)	
parasite, no pigmented monocyte	17	36.8 (1.3)	-0.5 (-1.2, 0.3)		36.7 (1.2)	-0.5 (-1.2, 0.1)	
both absent	427	37.3 (1.3)	Reference		37.2 (2.0)	reference	
3. Placental infection							
acute	16	36.5 (1.3)	-0.8 (-1.6, 0.03)	0.05	36.3 (1.2)	-0.8 (-1.4, -0.2)	0.05
chronic	17	36.8 (1.3)	-0.3 (-1.1, 0.4)		37.0 (1.2)	-0.1 (-0.7, 0.5)	
past	14	37.3 (1.0)	0		37.2 (1.1)	0.1 (-0.6	
no infection	427	37.3 (1.3)	Reference		37.1 (2.0)	reference	
Calcification							
focal	51	36.8 (1.3)	-0.5 (-0.8, -0.1)	0.01	36.6 (1.4)	-0.4 (-0.8, -0.1)	0.02
absent	423	37.3 (1.3)	reference		37.1 (2.0)	reference	
4. Smear negative infection (any)							
parasite + pigmented monocyte	7	36.8 (0.4)	-0.5 (-1.7, 0.5)	0.5	36.8 (1.3)	-0.5 (-1.5, 0.4)	0.4
parasite, no pigmented monocyte	13	37.2 (1.3)	-0.1 (-1.0, 0.7)		37.1 (1.4)	-0.1 (-0.8, 05)	
no infection	427	37.3 (1.3)	reference		37.3 (2.0)	reference	

[♣] Models adjusted for socioeconomic status, gravidity, education, residence and site; **Note:** serial number denotes a separate GLM model. Model 4 = infections detected on histology but negative on peripheral and placental impression smear examination.

TABLE 5: 11: UNIVARIATE ANALYSIS OF PLACENTAL HISTOLOGICAL DETERMINANTS ON HAEMOGLOBIN

Factors	Mean (SD)	Mean difference 95% CI	*P-value*
Placental parasite			
present (n=38)	9.6 (2.4)	-0.9 (-1.62, -0.16)	0.02
absent (n=427)	10.5 (2.1)	reference	
Syncytial knots			
increased (n =58)	10.3 (2.6)	-0.2 (-0.93, 0.27)	0.3
normal (n= 433)	10.5 (2.1)	reference	
Fibrinoid necrosis			
present (n=75)	10.6 (2.2)	0.2 (-0.32, 0.76)	0.4
absent (n=415)	10.4 (2.2)		
Intervillous fibrin			
mild (n=281)	10.4 (2.3)	-0.4 (-0.90, 0.18)	0.1
moderate-severe (n=68)	10.2 (1.8)	-0.6 (-1.34, 0.20)	
absent (n =125)	10.8 (2.1)	reference	
Calcification			
focal (n=51)	10.2 (2.8)	-0.3 (-1.02, 0.25)	0.2
absent (n=440)	10.5 (2.1)	reference	
Pigmented fibrin			
present (n =34)	10.2 (2.1)	-0.3 (-1.04, 0.43)	0.4
absent (n= 431)	10.5 (2.2)	reference	
Pigmented monocytes			
present (n =19)	9.1 (2.7)	-1.4 (-2.40, -0.38)	0.01
absent (n = 441)	10.5 (2.1)	reference	
*IVS Polymorphonuclear cells			
mild (n=87)	10.4 (2.3)	-0.1 (-0.73, 0.51)	
moderate-severe (n=31)	10.8 (2.5)	0.3 (-0.60, 1.34)	0.5
absent (n =356)	10.5 (2.1)	reference	
*IVS monocytes			
mild (n=95)	10.4 (2.4)	-0 .1(-0.70, 0.48)	
moderate-severe (n=32)	9.7 (2.7)	-0.8 (-1.87, 0.04)	0.1
absent (n =348)	10.5 (2.1)	reference	

*IVS= intervillous space

TABLE 5: 12: UNIVARIATE AND MULTIVARIATE ANALYSES OF THE IMPACT OF HISTOLOGICAL PLACENTAL INFECTIONS ON HAEMOGLOBIN IN 506 PALCENTAL SAMPLES

Factors	N	Crude Haemoglobin			Adjusted Haemoglobin*		
		Mean (SD)	Mean difference (95 % CI)	*P-value*	Mean (SD)	Mean difference (95% CI)	*P-value*
1. Placental Infection							
parasite +pigmented monocyte	19	9.1 (2.7)	-1.4 (-2.6, -0.2)	0.01	9.3 (1.7)	-1.1 (-2.1, -0.1)	0.05
parasite , no pigmented monocytes	19	10.1 (2.1)	-0.3 (-1.5, 0.8)		10.2 (1.7)	-0.2 (-1.2, 0.7)	
both absent	427	10.5 (2.1)	reference		10.4 (2.1)	reference	
2. Placental infection							
Parasite + mod-severe monocyte	4	8.9 (1.5)	-1.6 (-4.3, 1.7)	0.04	9.1 (2.0)	-1.3 (-3.4, -1.2)	0.1
Parasite + mild pigmented monocyte	15	9.2 (2.9)	-1.3 (-2.8, 0.1)		9.3 (1.9)	-1.1 (-2.2, 0.1)	
Parasite, no pigmented monocytes	19	10.1 (2.1)	-0.3 (-1.6, 0.9)		10.2 (1.7)	-0.2(-1.2, 0.7)	
both absent	427	10.5 (2.1)	Reference		10.4 (2.0)	reference	
3. Placental infection							
acute	20	8.8 (2.4)	-1.7 (-3.0, -0.4)	0.01	8.9 (1.7)	-1.5 (-2.5, -0.5)	0.02
chronic	18	10.6 (2.2)	0.1 (-1.3, 1.4)		10.6 (1.8)	0.2 (-0.8, 1.1)	
past	13	10.5 (2.1)	0.0		10.3 (1.8)	-0.1 (-1.3, 1.0)	
no infection	427	10.5 (2.1)	Reference		10.4 (2.0)	reference	
4. Smear negative infection (any)							
parasite + pigmented monocyte	7	9.9 (3.1)	-0.6 (-2.5, 1.3)	0.7	10.0 (2.1)	-0.5 (-2.1, 1.1)	0.7
parasite, no pigmented monocyte	14	10.3 (2.3)	-0.2 (-1.5, 1.1)		10.3 (1.9)	-0.2 (-1.3, 0.9)	
no infection	427	10.5 (2.1)	Reference		10.5 (2.1)	reference	

* All models adjusted for socioeconomic status, gravidity, education, residence; **Note:** serial number denotes a separate GLM model. Model 4 = infections detected on histology but negative on peripheral and placental impression smear examination

5.5 Discussion

Histological examination of a sub-set of 506 placental sections was performed to assess the placental changes associated with *P.falciparum* and *P.vivax* infections in a low transmission area and the associated impact on adverse birth outcome and maternal anaemia. Placental infections were classified according to the commonly accepted histological classification (Bulmer, *et al* 1993a, Ismail, *et al* 2000). Most of the peripherally patent *P.falciparum* infections corresponded with histologically classified active infections defined by the presence of parasites in the placenta. Accordingly, it was rare for peripherally patent infections to be negative by histological examination (0.7%) in this setting. In contrast, 13 women (3%) with negative conventional smear microscopy proved to have placental infections based on histology. These were chronic infections, indicating that parasites could remain sequestered within the infected placenta while being absent or present at sub-microscopic levels in the peripheral circulation. This highlights that placental histology is a more sensitive method to detect infection and conventional peripheral and placental microscopy smear results alone may underestimate the true burden in this region.

Histology is recognised as a useful method of characterizing the chronology of placental infections (Bulmer, *et al* 1993a, Ismail, *et al* 2000). In this study, acute infections were more frequent than past infections. An explanation for this might be that the low malaria exposure and low host immunity in this population triggers delivery early when the placenta becomes infected. The low exposure is also reflected by the sparse distribution of parasites seen within the placenta and the corresponding mild inflammatory response, which is in contrast to the massive monocyte infiltration reported from highly endemic areas in sub-Saharan Africa (Ordi, *et al* 1998a). Similar to the reports from elsewhere, parasites were rarely seen adhering to the syncytial layer, and none were observed within the villous structure (Ismail, *et al* 2000, McGready, *et al* 2004,Yamada, 1989 #1062)}.

Chronic *P.falciparum* infection was also associated with increased frequency of changes in syncytial knots, focal fibrinoid necrosis and calcification. Interestingly, these changes were also evident in past infections, suggesting they may persist for longer than previously appreciated (Ismail, *et al* 2000). Alternatively, there may have been insufficient time for their resolution as time between occurrence of infection and delivery might have been shorter in this study as was described in studies on the Thai-Burmese border and contrasts to studies in semi-immune women in malaria endemic Africa (McGready, *et al* 2004).

Despite the scarcity of parasites within the placenta, a striking finding was the impact of placental *P.falciparum* infection on the baby and maternal anaemia. Of all the histological measures, only the presence of parasites and pigmented monocytes were consistently associated

with maternal anaemia, reduced birth weight and shorter gestation in multivariate analysis. There was evidence of interaction between the two parameters that were significantly associated with birth weight, in that a much greater effect on birth weight was observed when parasites were present together with pigmented monocytes (-371 grams) rather than the presence of parasites alone (-51 grams). The reduction of birth weight was associated with the severity of pigmented monocytes. The reduction in birth weight may in part be explained by the average reduction of 8 days in the duration of pregnancy observed in the presence of parasites and moderate to severe levels of pigmented monocytes compared to pregnancies with uninfected placentas. The findings suggest that the inflammatory response to acute infections consisting mainly of monocyte-macrophage might induce premature labour. There is increasing evidence that mononuclear cells have a role in the pathophysiology of poor fetal growth in placental malaria (Abrams, *et al* 2003, Rogerson, *et al* 2003c). Moreover inflammation has been indicated as a source of cytokines which trigger premature delivery (Fried, *et al* 1998a, Moormann, *et al* 1999, Abrams, *et al*). The scanty level of infection of 1-5 parasites per field found in this study also suggests that mechanisms other than the presence of placental parasitaemia *per se* play a role. This is further supported by the fact that *P.vivax*, which is not known to sequester in the placenta also affects birth weight (Nosten, *et al* 1999, Poespoprodjo, *et al* 2008).

Placental malaria with its inflammatory response of monocytes was associated maternal anaemia. The reductions in haemoglobin ranged from 1.1g/dL in cases of parasite and mild monocytosis to 1.5g/dL (95%CI -2.5, -0.5) in cases of acute infection. This compares with a study from Malawi where an association with pigmented monocytes and anaemia was reported (Rogerson, *et al* 2003b). Our findings also implicate the role of inflammation with TNF and monocytes related to placental malaria and anaemia, as suggested by previous investigators (Fried *et al* 1998).

There was no evidence of *P.vivax* sequestration in the placenta. Pigment in the fibrin, corresponding to past infection was observed in two peripherally patent *P.vivax* cases, although previous infections with *P.falciparum* cannot be excluded. The results are consistent with previous studies from Thailand and Peru where both *P.falciparum* and *P.vivax* are co-endemic, and which demonstrated the presence of placental pigment associated with *P.vivax* infections (McGready, *et al* 2004, Parekh, *et al* 2010). A noteworthy finding in our study was the significantly higher presence of polymorphonuclear leukocytes and syncytial knots observed in *P.vivax* cases compared to *P.falciparum* infected placentas, possibly reflecting an inflammatory response within the placenta that may be different from that observed with *P.falciparum*. There was no association, however, between polymorphonuclear cells or increased syncytial knots and birth weight or preterm births.

The findings of this study differ from placental histological studies from high transmission regions of Africa. In the African studies, heavy parasitaemia and massive monocyte infiltrates were predominant and with it chronic infections were associated with low birth weight (Menendez, *et al* 2000, Galbraith, *et al* 1980, Ordi, *et al* 1998a). In contrast to previous studies from Malawi, there was no evidence that past infections, defined by the presence of haemozoin pigment in fibrin were associated with reduced birth weight and shorter duration of pregnancy (Rogerson, *et al* 2003b). Haemozoin is considered an inert substance which does not attract inflammatory cells in the absence of parasites (Leopardi, *et al* 1996, McGready, *et al* 2002, Menendez, *et al* 2000). However, there are similarities between our findings and those observed in Thai-Burmese border such as scanty parasitaemia, and the low rate of massive intervillositis and chronic infections (McGready, *et al* 2004). These together with the major impact of parasites and pigmented monocytes infiltrates on birth weight, is consistent with the notion that malaria associated low birth weight results primarily from a shortening of gestation in women with little protective immunity triggered by acute infections. The low proportion of chronic infections further suggest the occurrence of low birth weight related to fetal growth restriction associated with insidious placental inflammatory responses and placental insufficiency associated with chronic infection as observed in predominantly asymptomatic infections in Africa is less likely.

As expected, placental histology was more sensitive than standard placental or peripheral smear microscopy. Overall, 2.8% active infections (acute or chronic) were detected in women that were peripheral smear negative, and 4.6% in women that were placental smear negative. When these 'sub-patent' infections are taken into account and extrapolated to the overall sample of 2282 women enrolled, the prevalence of placental malaria increases from 1.0% to 5.5%, a five-fold increase compared with the prevalence estimates based on placental or peripheral blood microscopy only (see chapter 4), but consistent with the high rate of sub-microscopic infections detected by PCR in antenatal women (chapter 7). However unlike antenatal sub-microscopic infections no sub-clinical placental infections were detected in Jabalpur the urban site. Importantly, the sub-microscopic infections detected by histology were associated with reductions in birth weight and gestation although this did not reach statistical significance.

The findings of this study stress the importance of malaria control in this population. The fact that malaria association with negative birth outcomes were significantly greater in women with acute rather than chronic or past infections, suggest infections should be captured early through intermittent scheduled screening or should be prevented by use of intermittent preventive treatment together with ITNs.

The study was limited by its cross-sectional design, which did not allow assessing the impact of previous infections that may no longer be evident as 'past' infections. It also makes the

differentiation between the effects by species difficult. Since the investigations did not include immuno-histochemistry, we were unable to characterize the inflammatory and immune cells and had to rely on a description of the morphological changes only. Although a single reader examined the sections, and only a selected number of positive slides and those corresponding to peripherally patent *P.vivax* were reviewed by a second reader; (a senior placental histopathologist) the results were matching. Since the number of slides re-examined were few, a formal correlation testing between reader 1 and 2 using kappa was not done. However, histological detection and species were confirmed on all positive histological cases by PCR.

In conclusion, in this population of pregnant women, there was a higher rate of sub-clinical infections detected by placental histology among women with negative peripheral or placental smears. Overall the placental changes associated with *P.falciparum* infections were generally milder than those reported from studies in highly endemic areas in sub-Saharan Africa, yet the combined presence of *P.falciparum* parasites and moderate to severe levels of pigmented monocytes were consistently associated with maternal anaemia, and major reductions in mean birth weight. The shortened gestation is likely to be the main contributor to the observed decrease in birth weight rather than fetal growth restriction resulting from chronic placental infections. In women with PCR confirmed *P.vivax* infections, although mild placental changes were observed with infiltrates of polymorphonuclear cells, there was no evidence *P.vivax* sequestration.

Chapter 6

Risk factors associated with low birth weight in Madhya Pradesh

"low birth weight is essentially a manifestation of maternal nutrition"
quoted with reference to S.Asia by S.R.Osmani

6.1 Introduction

Low birth weight (<2500g) is a consequence of preterm birth and intrauterine growth retardation (IUGR) and a well recognised indirect determinant of child health. It is associated with poor growth and development, impaired cognition in children and an increased risk of chronic diseases in adulthood. Recent UNICEF estimates shows that 15% of all newborn babies are born with low birth weight (<2500g) and it is an underlying factor in 60-80 percent of neonatal deaths globally. Of these deaths an estimated 27% were due to preterm birth (<37 weeks gestation), while the other two main causes were severe infections and birth asphyxia (UNICEF 2009). However, a proportion of low birth weight can be averted by reducing the risk of specific causes such as infectious diseases.

Globally, 40% of low birth weight babies born in developing countries are born in India. The malaria in pregnancy survey we conducted also showed a high prevalence of low birth weight and preterm births, and was consistent with the NFHS-India estimates of 34% prevalence of low birth weight in Madhya Pradesh. Furthermore, Madhya Pradesh is a state that has a high neonatal (59 per 1000 live births) and infant mortality rate (1 in 14 children die in the first year of life) in India (NFHS-3 2005-2006). Despite this high prevalence, the risk factors of low birth weight and preterm births have been poorly studied in India. Malaria is a known determinant of low birth weight and preterm. But malaria prevalence is generally low in India, including the findings of the current study (chapter 4); in which malaria could only explain 4.6% of low birth weight in the population. To support efforts to reduce low birth weight in India, a sub-analysis was conducted to identify amendable determinants of preterm birth and IUGR-LBW.

6.2 Methods

The methods were discussed in detail in chapter 3. In brief, this sub-analysis included the mother-infant pairs which were enrolled at the delivery units from the rural and semi-rural sites Maihar and Katni during surveys 2 and 3 (October 2006-September 2007). The Jabalpur site was excluded from this analysis as it is a tertiary referral centre with more high risk and premature deliveries than the two rural sites and because the site was excluded in survey 3 due to the low malaria burden observed in surveys 1 and 2.

6.2.1 Procedures

Birth Weight: The babies were weighed to the nearest 10g on a digital weighing scale (Sansun model, Sansui Electronics Pvt Ltd, India) within 24 hours of delivery. The weighing scales were calibrated fortnightly with a standard weight.

Gestational Age Assessment: The gestational age of babies was estimated within 24 hours of delivery by trained staff using the modified Ballard assessment (Ballard, *et al* 1991). The Ballard assessment consists of a simplified scoring system for assessing fetal maturation. It uses six separate criteria of physical and neuromuscular maturity of the newborn baby with a score ranging from 0-5 for each criterion (Annexe 4). The method was chosen to determine gestational age, because the majority of women in this setting were uncertain of their last menstruation date. Additionally, antenatal ultrasonography was not a standard practice in the selected sites.

Definitions

Babies with a Ballard assessment of less than 37 weeks gestation were classified as preterm; those between 28-31 weeks as very preterm, 32-33 weeks as moderate and 34-36 weeks as mild preterm births (Moutquin 2003). Small-for-gestation-age (SGA) was defined by birth weight below the 10^{th} percentile on the Williams gender specific percentile curves. This is the WHO recommended growth reference for classifying newborns into different gestational categories. The other categories were appropriate for gestation (AGA) = birth weight between the 10^{th} and 90^{th} percentiles, large for gestation (LGA) = birth weight above the 90^{th} percentile (Williams 1975, Williams, *et al* 1982). Low birth weight was defined as a birth weight <2500g. Term babies (≥37 weeks gestation) with birth weight <2500g were classed as IUGR. This IUGR definition was used rather than the SGA definition because the definition of SGA includes term babies with birth weights >2500g as well as preterm babies. Therefore it does not suit this analysis which aimed to assess the risk factors of low birth weight by its two components; preterm-LBW and IUGR-LBW. MUAC was categorised as a binary variable with 23cm as the cut off value based on the criterion described in the WHO Collaborative studies, and considered as the criterion indicative of poor maternal nutrition (WHO. 1997). Short stature was defined as maternal height <150cm. This cut-off was used for uniformity with other studies from India and elsewhere, but it should be noted that the mean maternal height in this study sample was 151cm.

6.2.2 Statistical Analysis

Predictive Models: Data were analysed using SAS® version 9.1 (Statistical Application Software, SAS Institute Inc. Cary NC). A log binomial model using PROC GENMOD was applied to conduct the unadjusted (univariate) and adjusted (multivariate) determinants analysis to obtain prevalence ratios. Separate models were run with low birth weight, preterm birth and IUGR each as the dependent variable (i.e. outcome). If the models did not converge, the COPY-method was used as described in chapter 4. In brief, with the COPY-method the data set was expanded to 1000 original copies together with one copy of the original data set where the dependent variable is transposed (1s and 0s are reversed). The method gives a more accurate maximum likelihood ratio (MLE) by calculating the correct estimate of the standard error of the prevalence ratio after adjusting the standard error of the prevalence ratio of the large expanded dataset. Potential co-variates were eligible for entry into the initial models if the *P-value* for the association with the dependent variables was <0.1 in the univariate analyses. Using backward elimination, non-significant variables were deleted manually to obtain the final models. The final models of low birth weight, preterm birth and IUGR contain the variables that remained statistically associated with the dependent variable at *P-value* <0.05 based on the Wald type 3 statistic. A series of separate multivariate models were run for each outcome variable (i.e. low birth weight, preterm birth and IUGR). These included 1) models with all the variables of interest, 2) models to assess the association of malaria (exposure) in which the variable 'use of bednet' was excluded: when these two factors are in the model together, it could introduce bias and weaken the effect of malaria on LBW. 3) Models to assess the association of bednet in which morbidity factors such as history of fever, malaria and the use of drugs for fever were excluded. In model 3 the socio-demographic covariates: education, caste, socio-economic status, and residence were included to adjust for potential confounding and effect modification. Gravidity was categorised into 5 groups as primi, secundi, third, fourth and grandmultigravidae (≥5 pregnancies). The gravidae-3 group was used as the reference category to find out whether the association of LBW and preterm births increased in grandmultigravidae.

Percentile Curves: Growth curves of birth weight for gestational age and g-plot graphs were drawn with SAS software using the parametric quintile regression analysis to obtain the growth curves. This is an alternative approach to the conventional LMS growth modelling method and does not depend on a prior distribution assumption and is more suitable for use with small data sets (Chen 2005).

Seasonality analysis: The seasonal pattern of preterm births, IUGR and birth weight data was described using line and frequency bar charts by calendar month. The WIN-PEPI (PEPI acronym = 'Program for EPIdemiologists') program was used to clarify whether there was a statistically significant seasonal difference in the occurrence of preterm and IUGR babies. The Edwards' and Hewitt's sum-rank tests for harmonic analysis of seasonality which takes into account the order of monthly frequencies were used. The Edwards' test uses data on the frequency of events grouped into equal time intervals within a year or months (Edwards 1961, Reijneveld 1990). The year is represented as a circle with the rim divided into 12 parts representing the months. The model tests whether the events follow a harmonic curve of a single trough and peak within the 12-month period. The data is generated in a circular form (360^0) with time intervals divided into equal sectors, with the centre of gravity at the centre. The amplitude or the distance from the centre of the circle indicates the relative strength of the seasonal variation. The downside of the Edwards method is that it lacks power in samples with a low number of events. The Hewitt's sum-rank test ranks the monthly events from the lowest to the highest and assesses all the possible sequences for consecutive months. The Hewitt's test is applicable as long as the lower limit of monthly frequencies does not equal to zero which did not occur for low birth weight or preterm births (Hakko, *et al* 2002). Additional assessments with seasonality tests were carried out for mean birth weight and gestational age. Also seasonality tests were run to assess the seasonal significance of the proportion of MUAC <23cm.

Birth weight distribution: The frequency of the birth weight distribution was also assessed using a dedicated online programme (Analyze) provided by the National Institute of Environmental Health Sciences, USA (Wilcox 2002). The framework for the calculations in 'Analyze' programme are based on the Wilcox-Russell hypothesis which states that at population level birth weight is not on the causal pathway to mortality. The theoretical basis of their hypothesis is on the relation between optimum and mean birth weights and is founded on the controversial low birth weight paradox: low birth weight babies in high-risk populations usually have lower mortality than low birth weight babies in better-off populations (Hernandez-Diaz, *et al* 2008, Wilcox 2001). The parameters estimated by this programme are: 1) the predominant distribution (normal) defined by the mean and standard deviation and represents mainly term babies (≥37 weeks). 2) The residual distribution which is the tail of the curve that lies outside the predominant distribution, and is summarized as a percent of the whole population. Nearly all babies in the lower tail of the residual curve

represent preterm babies and correspond to the small preterm babies. Thus it estimates the percent of small and preterm babies who are also the babies at highest risk of mortality.

Attributable Fractions: The population attributable fraction (PAF) was calculated with the following formula: PAF = prevalence $_{(E)}$ (RR -1) / 1+ prevalence $_{(E)}$ (RR -1) where RR= relative risk and (E) = exposure. The formula (RR-1) / RR were used to calculate the attributable fraction (AF) in the exposed cases.

6.3 Results

6.3.1 Prevalence of Low Birth Weight, Preterm & IUGR

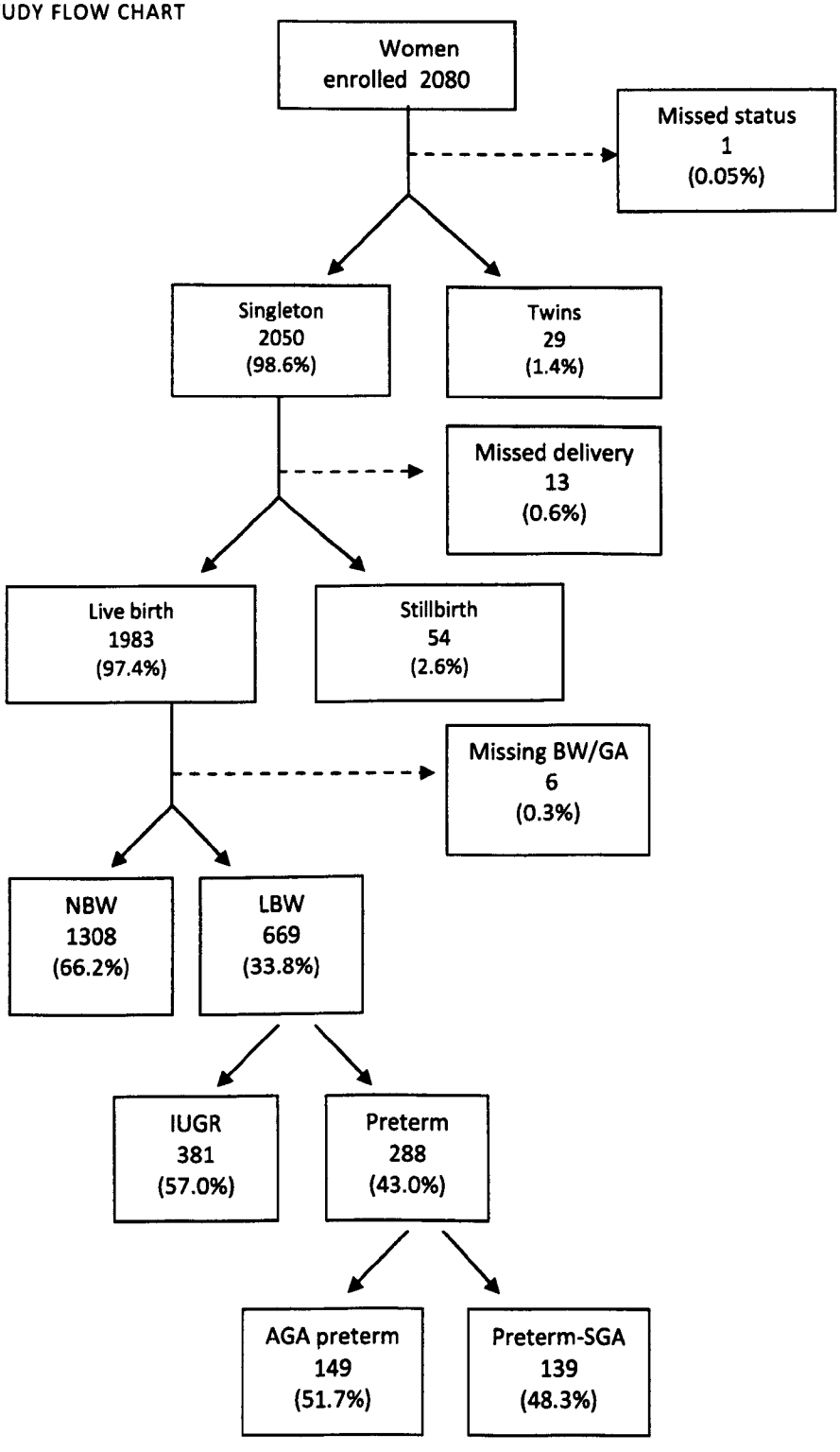
Between October 2006 and September 2007, 2080 women were enrolled from the delivery unit surveys conducted in Katni and Maihar. A flowchart is presented in Figure 6:1. There were 2050 singletons (98.6%) and 29 (1.4%) twin deliveries. Among the singleton births there were 1983 live births (97.4%) and 54 (2.6%) stillbirths. The data presented in this analysis includes 1977 singleton live babies for whom birth weights and gestational ages were available.

The overall prevalence of low birth weight was 33.8%. This was 14.5% for preterm and 19.2% for IUGR-LBW deliveries. Among the low birth weight babies, 381 (57.0%) were IUGR-LBW defined as term babies (≥37weeks) and weight <2500g, and 288 (43%) preterm-LBW (<37 weeks by Ballard score), of whom 48.3% were preterm-SGA and 57.7% preterm-AGA (Figure 6:2). Table 6:1 shows the mean and median birth weights in the

TABLE 6: 1: MEAN & MEDIAN BIRTH WEIGHT AMONG SINGLETON LIVE BIRTHS

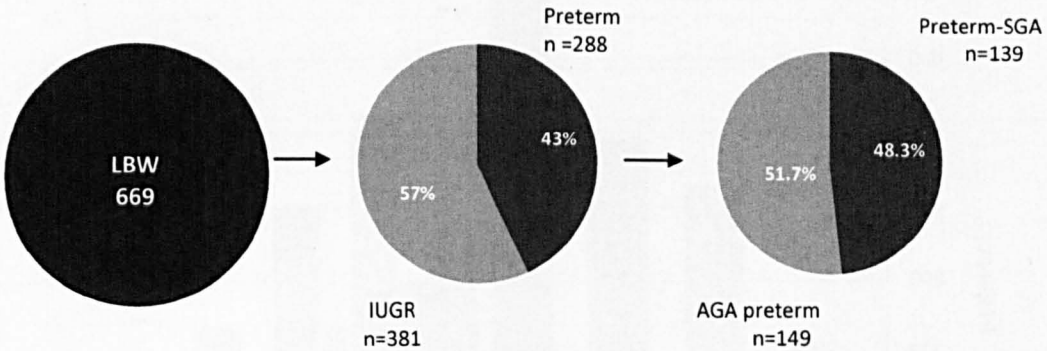
Category	n	Birth weight mean (SD)	Birth weight median (25 -75 percentiles)
Total babies	1977	2656 (417)	2650 (2404 -2917)
Term Babies	1689	2751 (355)	2728 (2515 - 2970)
IUGR	381	2312 (159)	2352 (2234 - 2434)
Preterm	288	2098 (297)	2169 (1980 - 2313)
Male babies	1055	2693 (432)	2714 (2416 - 2976)
Female babies	922	2612 (394)	2590 (2370 - 2844)

FIGURE 6: 1: STUDY FLOW CHART



***Note**: NBW=Normal birth weight, LBW =low birth weight, IUGR=intra uterine growth retardation GA=gestational age, AGA= appropriate for gestation, SGA= small for gestation*

FIGURE 6: 2: PROPORTION OF PRETERM & IUGR BABIES



***Note**: IUGR= intrauterine growth retardation, AGA= appropriate for gestational age, SGA=small for gestational age*

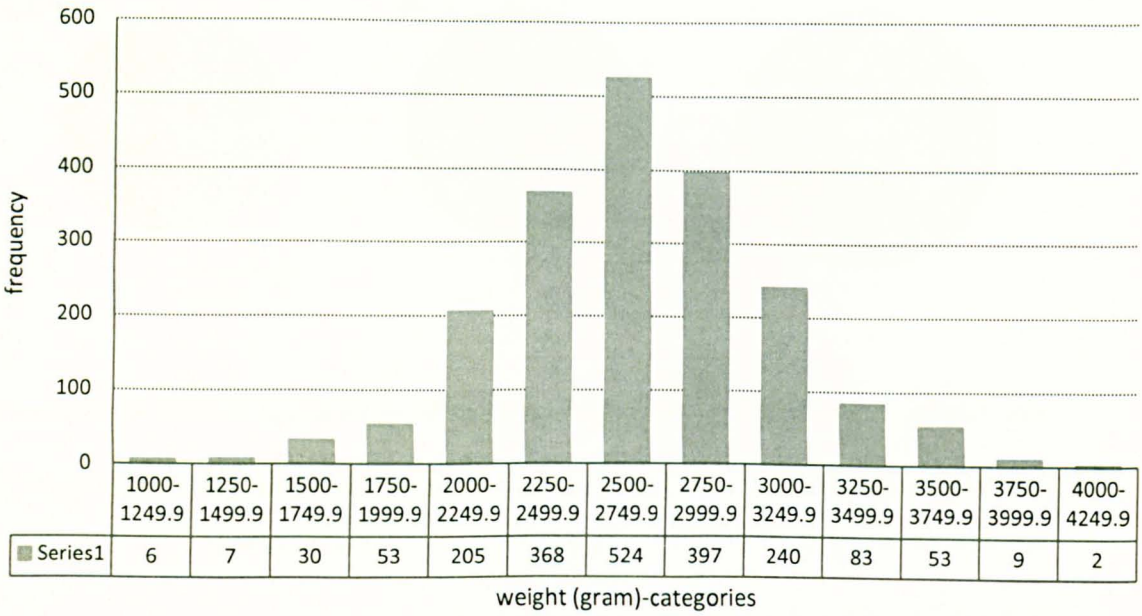
different sub categories. The mean birth weight (SD) of the overall sample was 2656g (417), with a mean difference of 81g (95% CI 44 – 117) between males and females.

6.3.2 Birth weight & gestational age distribution

The birth weight distribution in increments of 250g is shown in Figure 6:3 A, and as expected followed a Guassian (bell-shaped) distribution. This was confirmed by the birth weight distribution graph generated using the online 'Analyze' programme and shown in Figure 6:3 B. It provided additional information of the predominant (normal) and the residual (tail) distribution. The predominant curve mainly represents term births while the residual curve (lower left tail) in effect, consists of preterm births. The weight group between 2000-2500g comes under the predominant curve (Figures 6:3 A, and 6:3 B),

FIGURE 6: 3: BIRTH WEIGHT DISTRIBUTION IN SINGLETON LIVE BIRTHS

A: Distribution of Birth weight



B: Predominant and residual birth weight curve based on Wilcox-Russell Analyze program

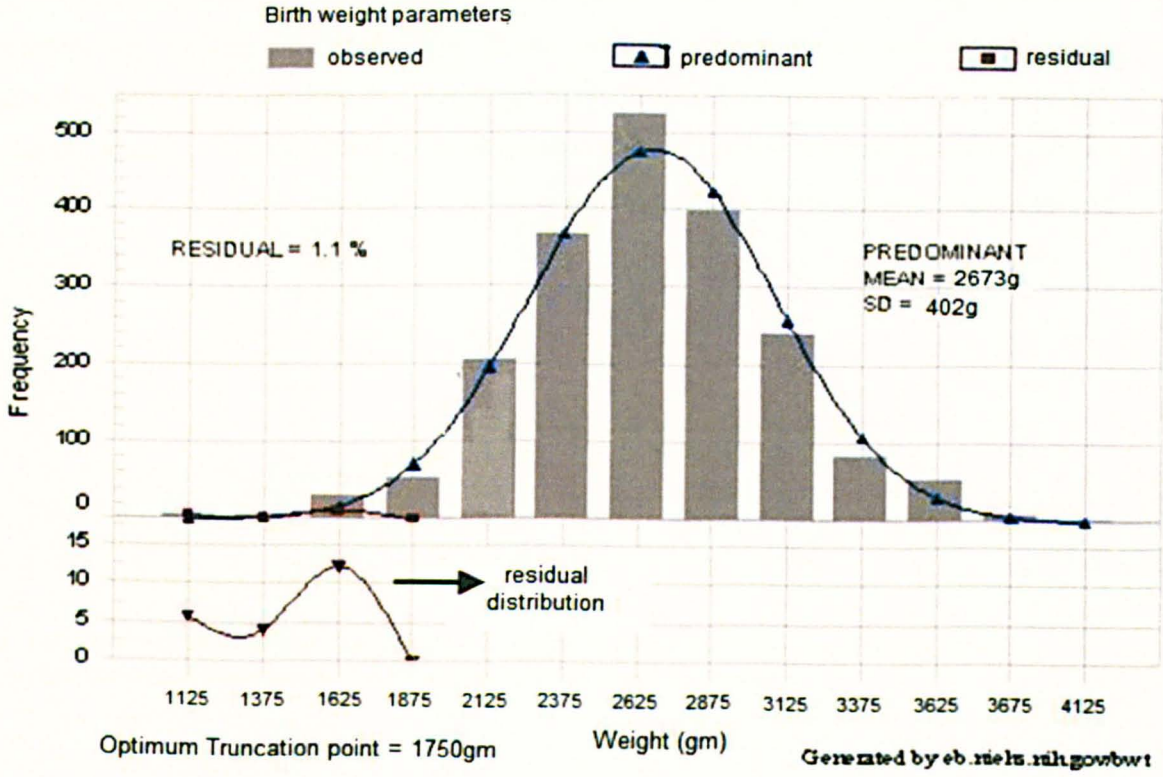


FIGURE 6: 4: DISTRIBUTION OF GESTATIONAL AGE AT BIRTH IN 1977 SINGLETON LIVE BABIES

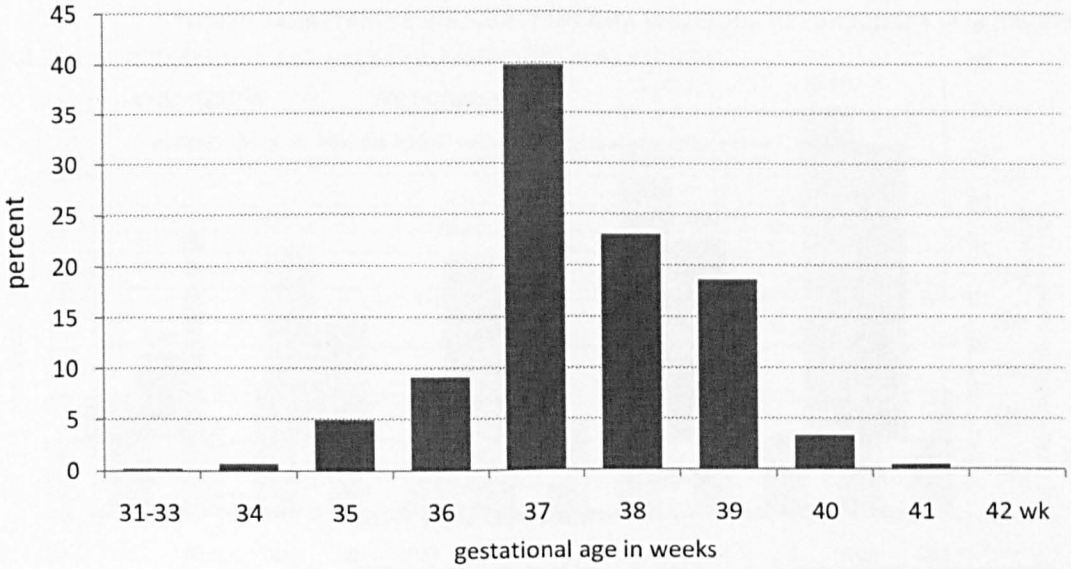


FIGURE 6: 5: FREQUENCIES OF PRETERM & IUGR BY GRAVIDITY

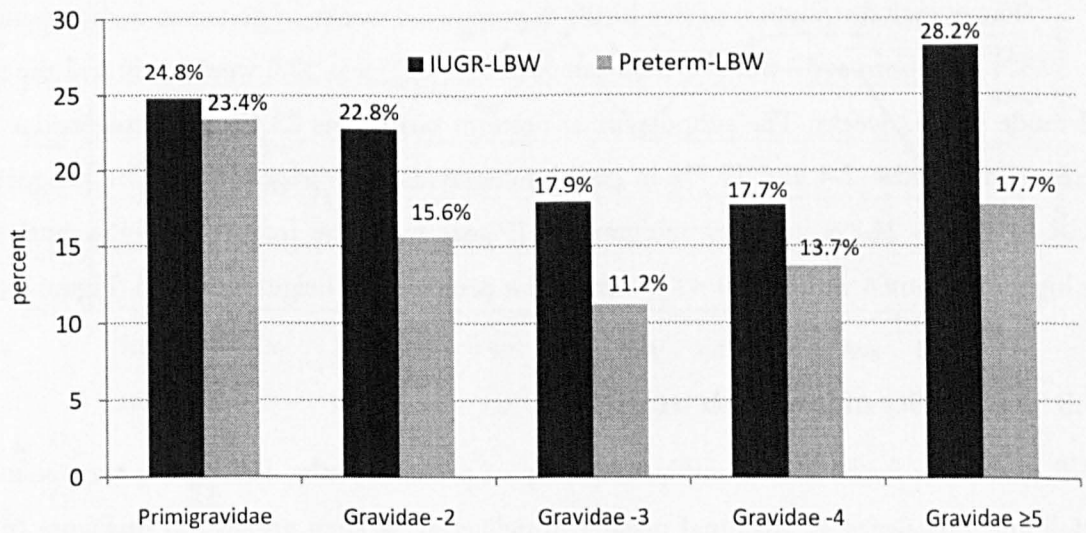
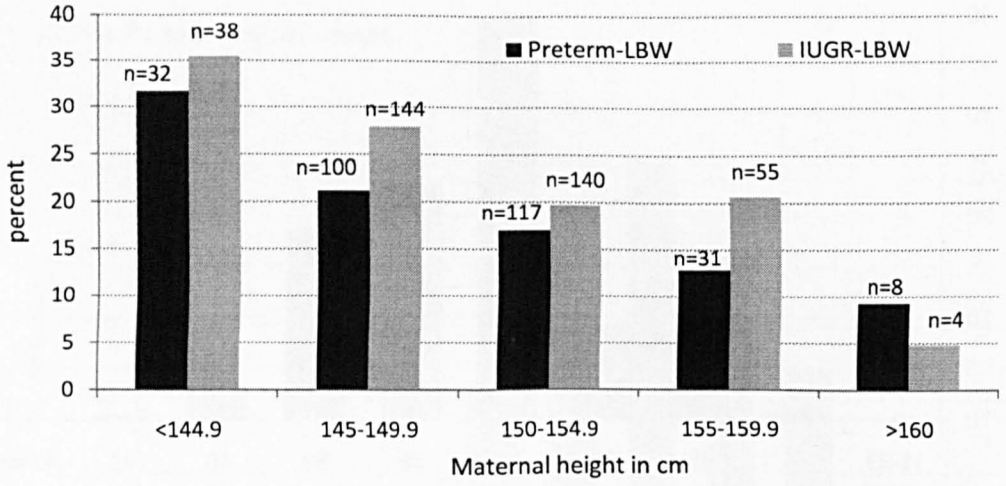


FIGURE 6: 6: FREQUENCY OF IUGR-LBW AND PRETERM-LBW BY MATERNAL HEIGHT



***Note**: maternal height data was available for 99.5% of LBW (1968/1977)*

whereas the lower tail starts at weight <1875g of the residual distribution (<2000g) .The optimum truncation point, which in biological systems infer the point beyond which death may occur, and it was at 1750g (Figure 6:3B; Annexe 8). The residual tail shows the small preterm babies who are the apparent high-risk group and was 1.1% in this study population.

The overall distribution of live births in completed weeks of gestation is shown in Figure 6.4: 39.8% were born at 37 weeks. The mean gestation (SD) was 37.2 weeks (1.5) and the median and mode was 37 weeks. The proportion of preterm births was 23.4% in primigravidae with a decrease in gravidae 2-4 and 17.7% in grandmultigravidae (≥5pregnancies). The proportion of IUGR-LBW was 24.8% in grandmultigravidae (Figure 6:5). The frequency of low birth weight was higher in women with height <149.9cm and it decreased as height increased (Figure 6:6).

6.3.3 Seasonality of low birth weight

IUGR & Preterm Births: The monthly frequency of preterm births, IUGR, the average monthly rainfall and prevalence of maternal malaria in delivering women are shown in Figure 6:7. The trough of preterm birth prevalence (2.7%) occurred in the dry season month of April, and the peak (25%) was observed in November, about 2 months after the peak of the rainy season. The significance of seasonality was confirmed by the Edwards test ($P < 0.000$) (Figure 6:9). 45.5% of all preterm births occurred in the months of October to December and this period was the statistically significant season ($P = 0.005$) by the seasonality tests. The estimated peak date calculated by the Edwards test was 1st November (Figure 6:9).

FIGURE 6: 7: PRETERM-LBW AND IUGR-LBW DISTRIBUTION BY MONTH

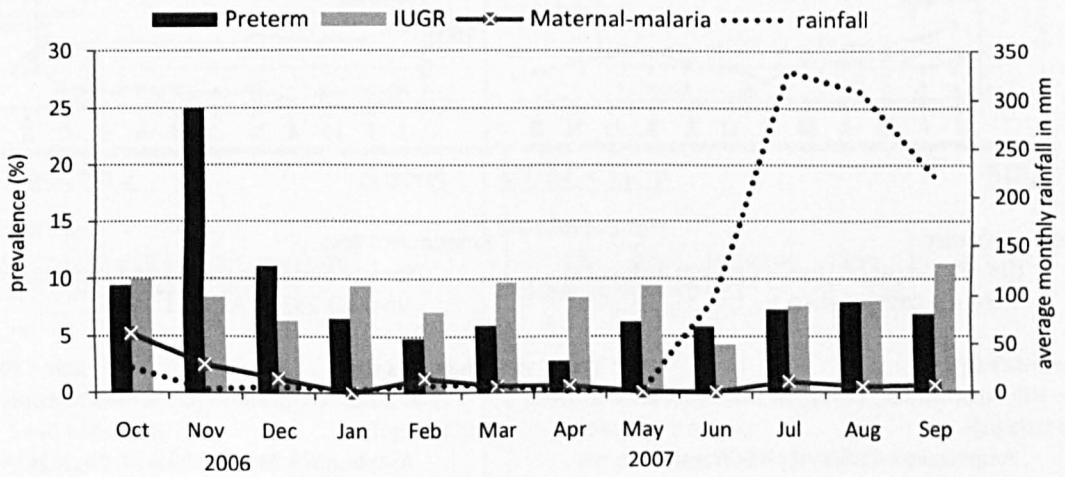
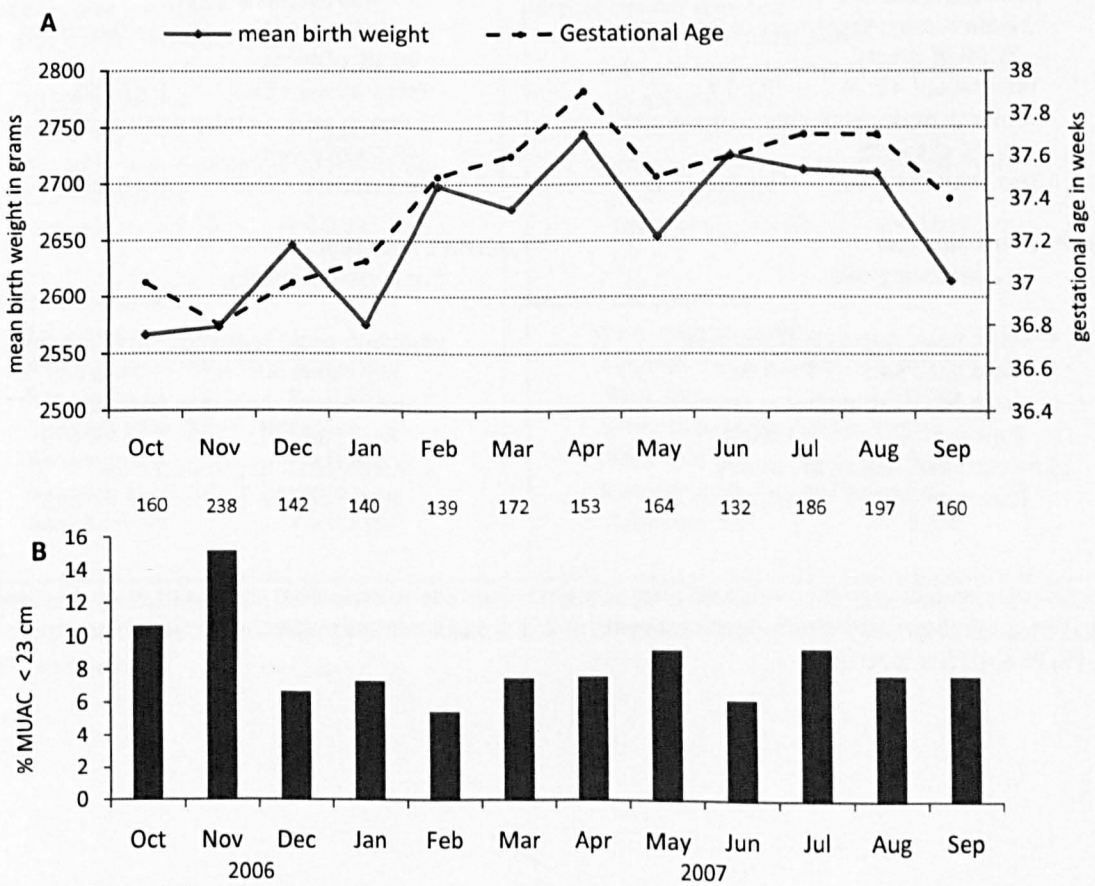
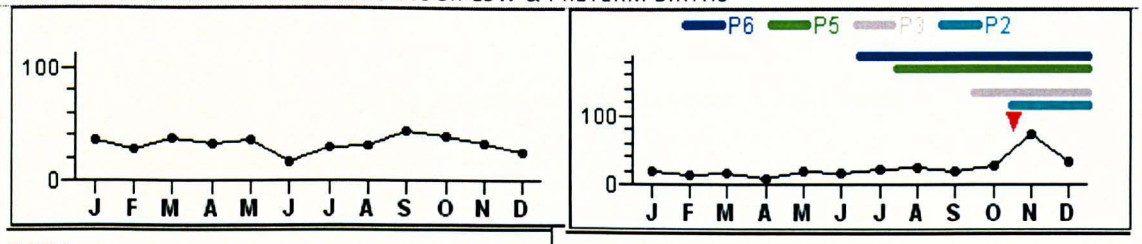


FIGURE 6: 8: MEAN BIRTH WEIGHT, GESTATIONAL AGE, BIRTH FREQUENCY & MUAC <23CM BY MONTH



***Note** :figures below the months in graph **A** represent number of singleton births per month*

FIGURE 6: 9: SEASONALITY TESTS FOR IUGR-LBW & PRETERM BIRTHS



IUGR

Freedman's test

(for any deviation from a uniform incidence):

V(N) = 0.052 P > 0.1

Edwards's test

(for a harmonic curve [6 months between peak and trough]):

Amplitude = 15.6% of peak frequency
Peak angle (year: 360°) = 312.8°
Peak date = November 13th
Chi-square = 1.15 (DF = 2} P = 0.564

Ratchet circular scan test

(for a short seasonal peak):

2-month peak: September to October
21.3% of events
Test statistic = 2.24 P > 0.1
3-month peak: September to November
29.7% of events
Test statistic = 2.01 P > 0.1

Hewitt's rank-sum test

(for a seasonal peak):

4-month peak: August to November
Rank sum: 34.5 P > 0.089
5-month peak: September to January
Rank sum: 40 P > 0.094
6-month peak: August to January
Rank sum: 45 P > 0.130

Preterm

Freedman's test

(for any deviation from a uniform incidence):

V(N) = 0.203 P < 0.01

Edwards's test

(for a harmonic curve [6 months between peak and trough]):

Amplitude = 71.8% of peak frequency
Peak angle (year: 360°) = 300.6°
Peak date = November 1st
Chi-square = 50.57 (DF = 2} P = 0.000 [1.0E-11]

Ratchet circular scan test

(for a short seasonal peak):

2-month peak: November to December
36.1% of events
Test statistic = 8.67 P < 0.005
3-month peak: October to December
45.5% of events
Test statistic = 7.79 P < 0.005

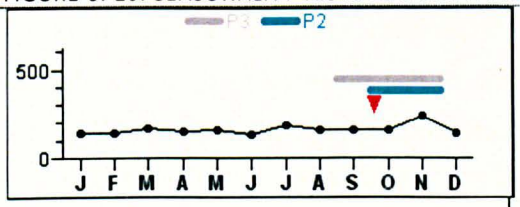
Hewitt's rank-sum test

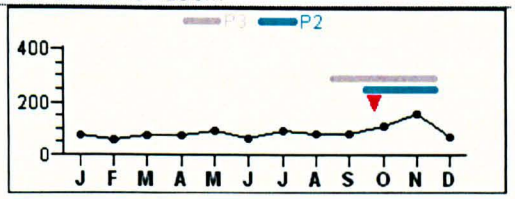
(for a seasonal peak):

4-month peak: September to December
Rank sum: 40 P = 0.089
5-month peak: August to December
Rank sum: 49 P = 0.029
6-month peak: July to December
Rank sum: 57 P = 0.013

The red triangle indicates the peak date at a 12 month sinusoidal curve within the positivity of the Edwards test. The graph shows statistically significant peaks of 2, 3, 5 and 6 months marked with colour bars denoted as P2, P3, P5 and P6 respectively.

FIGURE 6: 10: SEASONALITY TESTS FOR BIRTH FREQUENCY & MUAC <23CM





Birth Frequency

Freedman's test

(for any deviation from a uniform incidence): V(N) = 0.046 P < 0.01

Edwards's test

(for a harmonic curve [6 months between peak and trough]):
Amplitude = 14.0% of peak frequency
Peak angle (year: 360°) = 272.1°
Peak date = October 2nd
Chi-square = 6.13 (DF = 2} P = 0.047

Ratchet circular scan test

(for a short seasonal peak):
2-month peak: October to November
20.5% of events
Test statistic = 4.36 P < 0.005
3-month peak: September to November
28.7% of events
Test statistic = 3.76 P < 0.005

Hewitt's rank-sum test

(for a seasonal peak):
4-month peak: August to November
Rank sum: 34 P > 0.089
5-month peak: July to November
Rank sum: 45 P > 0.094
6-month peak: July to December
Rank sum: 48 P > 0.130

MUAC <23cm

Freedman's test

(for any deviation from a uniform incidence): V(N) = 0.091 P < 0.01

Edwards's test

(for a harmonic curve [6 months between peak and trough]):
Amplitude = 30.9% of peak frequency
Peak angle (year: 360°) = 277.1°
Peak date = October 7th
Chi-square = 19.66 (DF = 2} P = 0.000 [5.4E-5]

Ratchet circular scan test

(for a short seasonal peak):
2-month peak: October to November
25.8% of events
Test statistic = 7.69 P < 0.005
3-month peak: September to November
33.4% of events
Test statistic = 6.23 P < 0.005

Hewitt's rank-sum test

(for a seasonal peak):
4-month peak: August to November
Rank sum: 37 P > 0.089
5-month peak: July to November
Rank sum: 47 P = 0.094
6-month peak: July to December
Rank sum: 50 P > 0.130

The red triangle indicates the peak date at a 12 month sinusoidal curve within the positivity of the Edwards test. The graph shows statistically significant peaks of 2, and 3 months marked with colour bars denoted as P2, and P3, respectively.

The Hewitts sum-rank test showed the 5 month peak for preterm births occurred from August to November (P=0.029). In contrast, the occurrence of IUGR rate was more evenly distributed over the year, with the lowest percentage (4.2%) observed in June, and the highest (11.3%) in September. An absence of significant seasonality for IUGR was confirmed by the Edwards test (P= 0.56) and the other tests for seasonality (Figure 6:9).

Birth Frequency, Mean Birth Weight & Gestational Age: The highest 'means' for gestational age (SD) and birth weight (SD) [37.9 weeks (0.97) and 2743g (418g)] were observed in April while the lowest 'means' occurred in November [36.8 weeks (1.3) and 2573g (424)] (Figure 6:8). November was also the month with the peak frequency of births. A three month seasonal peak was observed from September to November (P<0.005) by the Ratchet circular seasonality test (Figure 6:9). June was the month with lowest frequency of births.

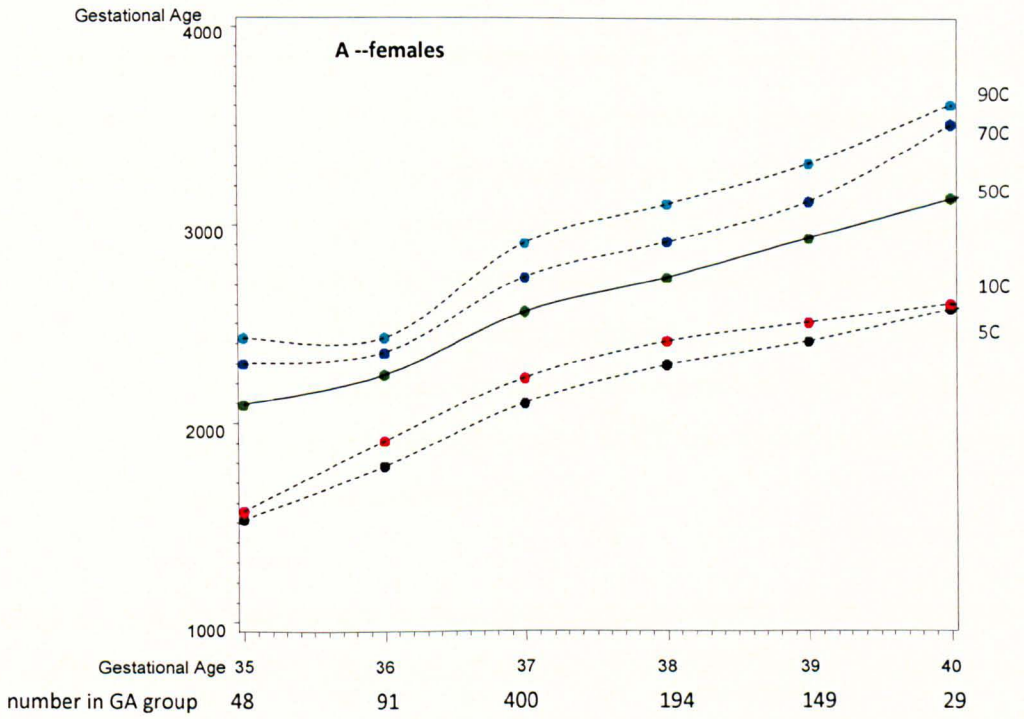
MUAC <23cm: Seasonality of MUAC <23 was examined and is shown in figure 6:8. The highest proportion of MUAC <23cm was observed in October and November and the lowest in February. The rest of the months it was fairly even. The seasonal significance in proportion of MUAC <23cm was also confirmed by the seasonality statistical tests and a 3 month seasonal peak was seen between September to November (P<0.005) (Figure 6:10).

In addition an association between MUAC <23cm and caste category was assessed: the risk of MUAC <23cm in women of scheduled caste was 1.5 (95%CI 1.27-1.78), in women of OBC RR1.2 (95%CI 1.07-1.45) and in women of Scheduled Tribe it was RR 1.29 (95%CI 1.06-1.56) compared with women of General Caste.

6.3.4 Percentile Curves

The Figures 6.11 & 12 shows the 5th, 10th, 50th, 75th and 90th percentiles of the birth weight-for-gestational age by gender and pooled growth curves. In the pooled growth curves the 50th percentile at 37 week falls on 2580g and the 90th percentile fall on 2950g.

FIGURE 6: 11: SELECTED PERCENTILES FOR BIRTH WEIGHT-FOR-GESTATIONAL-AGE GROWTH CURVES FOR FEMALES (A) & MALES (B)



Note: C=percentile, GA =gestational age

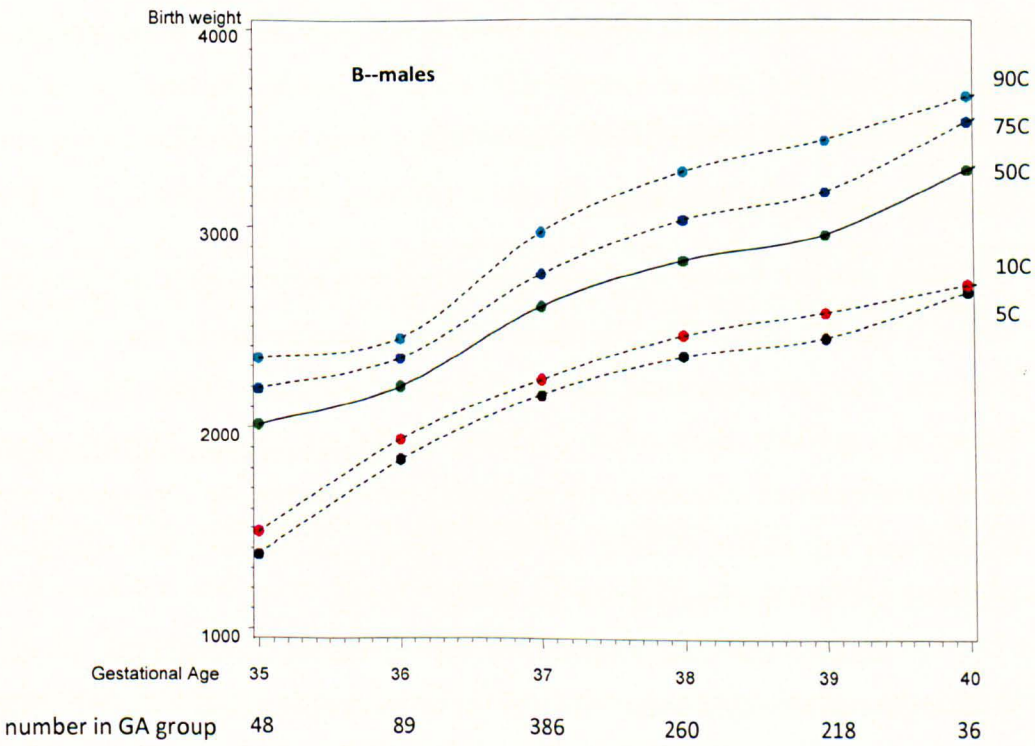
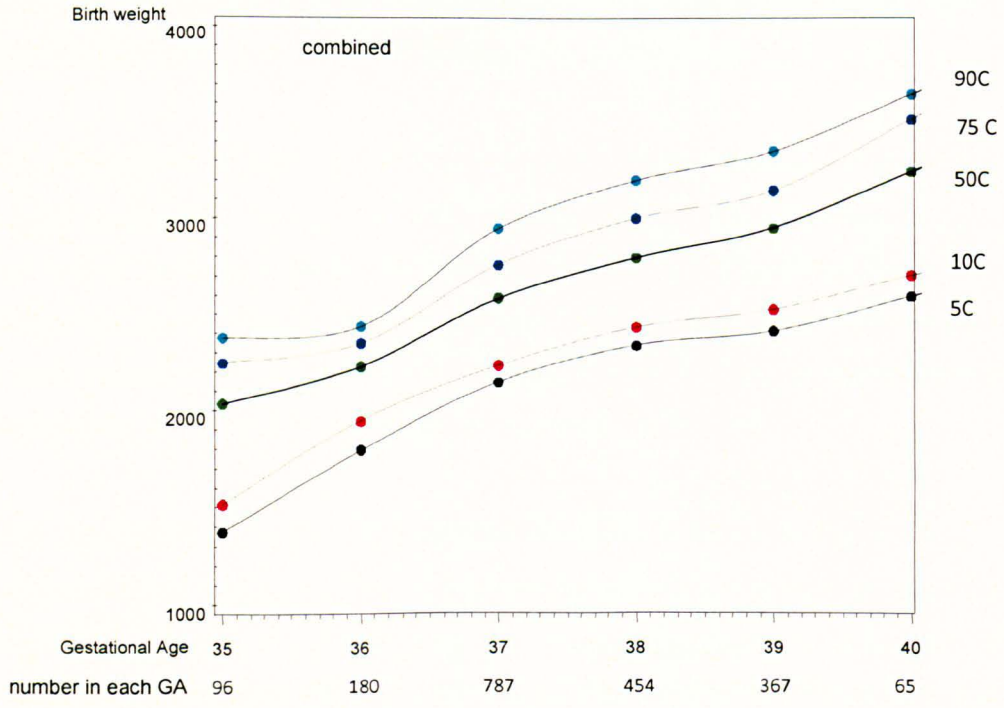


FIGURE 6: 12: SELECTED PERCENTILES FOR BIRTH WEIGHT-FOR-GESTATIONAL-AGE GROWTH CURVES COMBINED DATA FOR BOYS & GIRLS



Note: C= percentile, GA=gestational age

6.3.5 Predictive factors of Birth Outcomes

6.3.5.1Low Birth Weight & Preterm

Univariate analysis: Factors significantly associated with preterm birth and low birth weight was similar (Table 6:2 & 6:3). The significant socio-demographic factors were: caste category, rural residence, lower socioeconomic status and maternal education (Table 6:2). The maternal risk factors were gravidity, short stature (<150cm) and MUAC <23cm. The risk of preterm births significantly increased in women with moderate-severe anaemia, severe anaemia, history of fever in pregnancy and history of using any drug in pregnancy. Preterm birth was also significantly associated with placental malaria (RR 3.3, 95%CI 2.0-5.4).

A separate univariate analysis was also performed to assess the association of the optimum truncated weight point (<1750g) versus the weight group 1751-2500g with moderate-severe anaemia and placental malaria. In women with moderate-severe anaemia the risk of babies with birth weight <1750g was 1.7 (95%CI 1.18-2.62) times greater than the weight group 1751-2500g. In women with placental malaria this risk was 6.1(95%CI 1.66-23.13) compared to birth

weight group 1751-2500g. Similar results were observed for the residual tail (<1875g) in women with moderate-severe anaemia and placental malaria, with risk of 1.67 (95%CI 1.17-2.39) and 6.60(95%CI 1.91-22.7) respectively, compared with weight 1876-2500g (not shown as a table).

Other Factors: Preterm birth was associated with season and a significantly higher risk was observed in the cool months (RR 2.41, 95%CI 1.82-3.19) compared with the monsoon and dry season combined. The risk of preterm birth was also associated with number of antenatal visits and was increased 1.7 times in women with no antenatal visit (95%CI 1.26-2.37) compared with women who had 3 or more visits. Women who reported using haematinics or sleeping under a bednet during pregnancy had a decreased risk of preterm compared to women who did not use these measures (Table 6:3). All of these factors were also predictive of low birth weight (Table 6:2). In addition low birth weight was associated with gender and it was higher in female babies. The risk of gender was not associated with preterm birth.

Multivariate analysis: In the multivariate models lower socioeconomic status, cool season, primigravidae, and multigravidae (≥5 pregnancies), women with short stature (<150cm) and MUAC (<23cm) were significantly associated with low birth weight and preterm (Table 6:2 & 6:3). In addition baby gender was predictive of low birth weight, but not for preterm birth. Both low birth weight and preterm births were significantly associated with moderate-severe anaemia, history of fever in pregnancy and placental malaria. The number of ANC visits and maternal age were not factors associated with low birth weight or preterm birth. The multivariate models to assess the effect of bednet which included only the socio-demographic and maternal characteristic covariates, showed a positive association of between bednet and low birth weight and preterm births (RR 0.86, 95%CI 0.75-0.99) and (RR 0.78, 95%CI 0.61-0.99) respectively..

6.3.5.2 Intra-uterine Growth retardation

Univariate analysis: The societal factors significantly associated with IUGR were caste, education, rural residence, and socioeconomic status (Table 6:5). The maternal characteristics associated with the increased risk of IUGR-LBW were MUAC <23cm, short stature and less than 3 antenatal visits compared with ≥3 visits. Among the morbidity factors only history of fever in the past week was associated with IUGR-LBW. IUGR-LBW was not associated with placental malaria, moderate-severe anaemia and season. The risk of IUGR-LBW was higher in primi, secundi and multigravidae compared with gravidae-3, but did not reach statistical significance.

TABLE 6: 2: UNIVARIATE & MULTIVARIATE ANALYSIS OF FACTORS ASSOCIATED WITH LBW

	Univariate			Multivariate	
Demographic factors	**LBW** (n=669)	**RR** (95% CI)	***P-value***	**RR** (95%CI)	***P-value***
Age category					
<20 yr	20 | 66 (30.3)	1.09(0.71-1.56)	0.09		
20 -29yr	588 | 1690 (34.7)	1.25 (1.00-1.56)		---	---
≥ 30yr	61 | 220 (27.7)	reference			
Caste Category					
OBC	327 | 919 (35.6)	1.47 (1.21-1.78)			
SC	99 | 409 (24.2)	1.52 (1.23-1.88)	<0.0001		
ST	154 | 417 (36.9)	1.59 (1.25-2.02)			
GEN	84 | 218 (38.5)	reference			
Education					
No Schooling	254 | 665 (38.2)	2.00 (1.38-2.91)			
Primary	172 | 469 (36.7)	1.92 (1.32-2.81)	<0.0001		
Secondary	219 | 717 (30.5)	1.60 (1.10-2.34)			
Higher	24 | 126 (19.1)	reference			
Residence					
rural	504 | 1399 (36.0)	1.25 (1.08-1.44)	0.002		
urban	165 | 576 (28.7)	reference			
SES quintiles					
Poorest	145 | 396 (36.6)	1.77 (1.40-2.38)		1.56 (1.23-1.96)	
Second	162 | 413 (39.2)	1.89 (1.50-2.38)	<0.0001	1.62 (1.29-2.05)	0.0001
Third	170 | 424 (40.1)	1.93 (1.54-2.43)		1.70 (1.35-2.13)	
Fourth	113 | 358 (31.5)	1.52 (1.19-1.95)		1.40 (1.10-1.79)	
Richest	79 | 386 (20.5)	reference		reference	
Season					
Cool humid months	280 | 676 (41.4)	1.37 (1.18-1.60)	<0.0001	1.29 (1.15-1.45)	0.0002
Monsoon	200 | 673 (29.7)	0.98 (0.83-1.16)		}	
Hot dry months	189 | 628 (30.1)	reference		Reference[§]	
Maternal & Antenatal factors					
Gravidity					
Primigravidae	328 | 847 (38.7)	1.51 (1.23-1.86)		1.58 (1.30-1.92)	
Secundigravidae	192 | 594 (32.3)	1.26(1.02-1.57)	0.0001	1.29 (1.05-1.59)	0.0001
Gravidae-3	85 | 332 (25.6)	reference		reference	
Gravidae-4	33 | 122 (27.1)	1.05 (0.75-1.49)		1.02(0.72-1.44)	
Multigravidae (≥5)	31 | 82 (37.8)	1.47 (1.05-2.05)		1.49(1.08-2.07)	
MUAC (< 23cm)					
Yes	386 | 965 (40.0)	1.42 (1.26-1.62)	<0.0001	1.20 (1.06-1.37)	0.003
No	283 | 1011 (27.9)	reference		reference	
Maternal height					
Yes (<150cm)	315 | 762 (41.3)	1.36 (1.20-1.53)	<0.0001	1.24 (1.10-1.39)	0.0003
No	341 | 1122 (30.4)	reference		reference	
ANC visit number					
0 visit	76 | 196 (38.8)	1.28 (1.03-1.60)			
1 visit	67 | 174 (38.5)	1.28 (1.09-1.46)	0.004		
2 visit	261 | 691 (37.7)	1.28 (1.05-1.59)			
≥3 visit	217 | 728 (29.8)	reference			
Baby Gender					
female	351 | 921 (38.1)	1.26 (1.11-1.43)	0.002	1.21 (1.08-1.36)	0.001
male	318 | 1055 (30.1)	reference			

RR=Relative Risk; [§]monsoon & hot dry months combined as reference

Morbidity&malaria preventive factors	Univariate LBW (n=669)	RR (95% CI)	*P-value*	Multivariate RR (95%CI)	*P-value*
Anaemia†					
Yes	340 | 982 (34.6)	1.04 (0.92-1.18)	0.49		
No	329 | 992 (33.2)	reference			
Mod-severe anaemia ††					
Yes	157 | 389 (40.4)	1.24 (1.08-1.43)	0.003	1.18 (1.03-1.36)	0.01
No	512 | 1585 (32.3)	reference		reference	
Severe anaemia *					
Yes	55 | 110 (50.0)	1.51 (1.24-1.84)	0.002		
No	614 | 1864 (32.9)	reference			
H/o** fever in pregnancy					
Yes	150 | 351 (42.7)	1.34 (1.16-1.54)	<0.0001	1.14 (0.99-1.31)	0.05
No	517 | 1622 (31.8)	reference		reference	
Used any drug in pregnancy					
Yes	100 | 226 (44.2)	1.36 (1.16-1.60)	0.0004		
No	565 | 1743 (32.4)	reference			
H/o** fever past week					
Yes	99 | 239 (41.4)	1.26 (1.06-1.48)	0.01		
No	568 | 1730 (32.8)	reference			
Used any drug past week					
Yes	25 | 70 (35.7)	1.05 (0.76-1.45)	0.73		
No	641 | 1898 (33.8)	reference			
High BP (systolic ≥130mm Hg, diastolic ≥90mm Hg)					
Yes	112 | 313 (35.8)	0.96(0.81-1.14)	0.65		
No	440 | 1277 (34.5)	reference			
Placental Malaria (any)					
Yes	10|15 (66.6)	1.98 (1.38-2.85)	0.01	1.48 (1.23-1.77)	0.0002
No	655|1951 (33.5)	reference		reference	

†Hb <11g/dL; ††Hb <9/dL; *Hb <7g/dL; **history of fever

Multivariate Analysis: The factors predictive of IUGR-LBW were socioeconomic status, gravidity, maternal short stature, antenatal visits and baby's gender (Table 6:5). MUAC at cut-off <20cm was a significant predictive factor (RR1.5, 95%CI 1.2-2.0) of IUGR-LBW, but not at cut-off <23cm. A history of fever, moderate-severe anaemia and placental malaria was not predictive of IUGR-LBW. Use of bednet was positively associated with IUGR-LBW (RR 0.78, 95%CI 0.62-0.99).

TABLE 6: 3: UNIVARIATE & MULTIVARIATE ANALYSIS OF FACTORS ASSOCIATED WITH PRETERM-LBW

	Univariate			Multivariate	
Demographic Factors	**Preterm** (n= 288)	**RR (95%CI)**	***P-value***	**RR (95%CI)**	***P-value***
Age Category					
<20 yr	4 | 49 (8.2)	0.67 (0.24-1.85)	0.01		
20-30 yr	262 | 1359 (19.3)	1.58 (1.05-2.38)			
≥ 30yr	22 | 181 (12.2)	reference			
Caste Category					
OBC	140 | 730 (19.2)	1.71 (1.23-2.38)	0.002		
SC	69 | 329 (21.0)	1.87 (1.30-2.69)			
ST	37 | 170 (21.8)	1.94 (1.29-2.93)			
GEN	39 | 349 (11.2)	reference			
Education					
No schooling	118 | 526 (22.4)	1.57 (1.23-2.00)	0.001		
Primary	71 | 368 (19.3)	1.36 (1.03-1.79)			
Secondary +higher	99 | 696 (14.2)	reference			
Residence					
rural	217 | 1108 (19.6)	1.32 (1.03-1.69)	0.02		
urban	71 | 480 (14.8)	reference			
SES quintile					
Poorest	73 || 321 (22.7)	2.81 (1.85-4.25		2.35 (1.56-3.56)	
Second	67 | 316 (21.2)	2.62 (1.72-3.98)	<0.0001	2.03 (1.32-3.10)	0.0009
Third	72 | 325 (21.2)	2.74 (1.80-4.15)		2.24 (1.47-3.41)	
Fourth	49 | 294 (16.7)	2.06 (1.32-3.21)		1.80 (1.16-2.79)	
Richest	27 | 334 (8.1)	reference		reference	
Season					
Cool months	150 | 540 (27.8)	2.41 (1.82-3.19)	<0.0001	1.88 (1.53-2.31)	0.0000
Monsoon	81 | 554 (14.6)	1.27 (0.92-1.74)		reference[§]	
Hot dry months	57 | 496 (11.5)	reference			
Maternal & antenatal factors					
Gravidity					
Primigravidae	158 | 674 (23.4)	2.10 (1.46-3.00)		2.21 (1.55-3.14)	
Secundigravidae	74 | 474 (15.6)	1.40 (0.94-2.07)	<0.0001	1.42 (0.97-2.10)	<0.0000
Gravidae-3	31 | 278 (11.2)	reference		reference	
Gravidae-4	14 | 102 (13.7)	1.23 (0.68-2.21)		1.05 (0.57-1.93)	
Multigravidae (≥5)	11 | 62 (17.7)	1.59 (0.84-2.98		1.73 (0.93-3.27)	
MUAC (< 23cm)					
Yes	181 | 756 (23.9)	1.86 (1.49-2.31)	<0.0001	1.44 (1.15-1.79)	0.001
No	107 | 833 (12.9)	reference		reference	
Maternal height					
Yes (<150cm)	132 | 577 (22.8)	1.44 (1.16-1.77)	0.001	1.27 (1.04-1.56)	0.01
No	147 | 924 (15.9)	reference		reference	
ANC visit number					
0 visit	44 | 164 (26.8)	1.73 (1.26-2.37)			
1 visit	25 | 132 (18.9)	1.22 (0.82-1.82)	0.01		
2 visit	106 | 533 (19.9)	1.28 (0.99-1.65)			
≥3 visit	93 | 602 (15.4)	reference			
Baby Gender					
female	142 | 709 (20.0)	1.20 (0.97-1.48)	0.07		
male	146 | 880 (16.6)	reference			

RR= Relative Risk; [§]monsoon & hot dry months combined as reference;

Morbidity & malaria Preventive factors	Univariate			Multivariate	
	Preterm (n=288)	RR (95%CI)	*P-value*	RR (95%CI)	*P-value*
Anaemia †					
Yes	157 | 797 (19.7)	1.18 (0.96-1.46)	0.10		
No	131 | 790 (16.6)	reference			
Mod-severe anaemia ††					
Yes	82 | 314 (26.1)	1.61 (1.28-2.01)	<0.0001	1.40 (1.12-1.75)	0.003
No	206| 1273 (16.2)	reference		reference	
Severe anaemia *					
Yes	35 | 90 (38.8)	2.30 (1.73-3.05)	<0.0001		
No	253 | 1497 (16.9)	reference			
H/o**fever in pregnancy					
Yes	79 | 278 (28.4)	1.79 (1.43-2.24)	<0.0001	1.31 (1.04-1.65)	0.01
No	207 | 1308 (15.8)	reference		reference	
Used any drug in pregnancy					
Yes	56 | 180 (23.9)	1.91 (1.49-2.45)	<0.0001		
No	228 | 1402 (17.3)	reference			
H/o fever past week					
Yes	44 | 184 (23.9)	1.37 (1.03-1.82)	0.03		
No	243| 1399 (14.4)	reference			
Used any drug past week					
Yes	13 | 58 (22.4)	1.25 (0.76-2.04)	0.3		
No	273 | 1524 (17.9)	reference			
High BP (systolic ≥130mm Hg, diastolic ≥90mm Hg)					
Yes	44 | 244 (18.0)	0.95 (0.70-1.27)	0.6		
No	195 | 1029 (18.9)	reference			
Placental malaria (any)					
Yes	7|12 (58.3)	3.28 (2.01-5.35)	0.0003	1.96 (1.52-2.51)	0.0000
No	279|1569 (17.7)	reference		reference	

†Hb <11g/dL; ††Hb <9/dL; *Hb <7g/dL; * *history of fever

TABLE 6: 4: UNIVARIATE & MULTIVARIATE ANALYSIS OF FACTORS ASSOCIATED WITH IUGR-LBW

	univariate			multivariate	
Demographic factors	**IUGR** (n= 381)	**RR (95%CI)**	***P-value***	**RR (95%CI)**	***P-value***
Age category					
>20 yr	16|61 (26.2)	1.33 (0.80-2.20)	0.48		
20 -29 yr	325|1422 (22.8)	1.16 (0.86-1.56)			
<30 yr	39|198 (19.7)	reference			
Caste Category					
OBC	187|777 (24.1)	1.48 (1.14-1.93)			
SC	84|344 (24.4)	1.50 (1.11-2.02)	0.01		
ST	47|180 (26.1)	1.61 (1.14-2.25)			
GEN	60|370 (16.2)	reference			
Education					
No schooling	136|544 (25.0)	1.28 (1.04-1.58)			
Primary	101|398 (25.4)	1.30 (1.04-1.63)	0.01		
Secondary + higher	144|741 (19.4)	reference			
Residence					
rural	287|1178 (24.4)	1.30 (1.05-1.60)	0.01		
urban	94|503 (18.7)	reference			
SES quintiles					
Poorest	72|320 (22.5)	1.55 (1.12-2.14)		1.24 (0.87-1.76)	
Second	95|344 (27.6)	1.90 (1.40-2.58)	<0.0001	1.66 (1.20-2.30)	0.002
Third	98|351 (27.9)	1.92 (1.42-2.60)		1.66 (1.20-2.30)	
Fourth	64|309 (20.7)	1.42 (1.02-1.99)		1.20 (0.84-1.72)	
Richest	52|359 (14.5)	reference		Reference	
Season					
Cool months	130|520 (25.0)	1.08 (0.87-1.33)			
Monsoon	119|592 (20.1)	0.86 (0.69-1.08)	0.14		
Hot dry months	132|571 (23.1)	reference			
Maternal & antenatal factors					
Gravidity					
Primigravidae	170|686 (24.8)	1.38 (1.09-1.81)		1.49 (1.13-1.99)	
Secundigravidae	118|518 (22.8)	1.26 (0.95-1.69)	0.08	1.29 (0.98-1.74)	0.02
Gravidae-3	54|301 (17.9)	reference		reference	
Gravidae-4	19|107 (17.7)	0.98 (0.61-1.58)		0.95 (0.57-1.56)	
Multigravidae (≥5)	20|71 (28.2)	1.57 (1.00-2.44)		1.48 (1.06-1.91)	
MUAC (< 23cm)					
Yes	205|780 (26.3)	1.34 (1.12-1.60)	0.001		
No	176|902 (19.5)	reference			
Maternal height					
Yes (<150cm)	183|628 (29.1)	1.46(1.22-1.74)	<0.0001	1.38 (1.16-1.66)	0.0003
No	194|971 (19.9)	reference		Reference	
ANC visit number					
0 visit	32|152 (21.1)	1.07 (0.76-1.51)		1.03 (0.73-1.43)	
1 visit	42|149 (28.2)	1.43 (1.06-1.94)	0.01	1.42 (1.06-1.90)	0.04
2 visit	155|582 (26.6)	1.35 (1.10-1.67)		1.22 (1.01-1.50)	
≥3 visit	124|633 (19.6)	reference		Reference	
Baby Gender					
female	209|776 (26.9)	1.41 (1.81-1.69)	0.0001	1.35 (1.13-1.61)	0.001
male	172|906 (18.9)	Reference		Reference	

IUGR= Intrauterine growth retardation; RR=Relative Risk

Morbidity & malaria preventive factors	univariate			multivariate	
	IUGR (n=381)	RR (95%CI)	*P-value*	RR(95%CI)	*P-value*
Anaemia †					
Yes	182 | 822 (22.1)	0.95 (0.80-1.14)	0.63		
No	198 | 857 (23.1)	reference			
Mod-severe anaemia ††					
Yes	75|307 (24.4)	1.09 (0.88-1.36)	0.40		
No	305 |137 (22.2)	reference			
Severe anaemia*					
Yes	20|75 (26.7)	1.18 (0.80-1.74)	0.39		
No	360|1604 (22.4)	reference			
H/o fever in pregnancy					
Yes	71|270 (26.3)	1.19 (0.96-1.49)	0.11		
No	309|1410 (21.9)	reference			
Used any drug in pregnancy					
Yes	44|168 (26.2)	1.17 (0.89-1.54)	0.24		
No	336 |1509 (22.2)	reference			
H/o fever past week					
Yes	55|195 (28.2)	1.28 (1.00-1.64)	0.04		
No	324|1480 (21.8)	reference			
Used any drug in past week					
Yes	12|57 (21.7)	0.92 (0.55-1.54)	0.77		
No	367|1618 (22.7)	reference			
High BP (systolic ≥130mm Hg, diastolic ≥90mm Hg)					
Yes	67|267 (25.1)	1.11 (0.87-1.39)	0.40		
No	245|1078 (22.7)	reference			
Placental malaria (any)					
Yes	3|8 (37.5)	1.66 (0.67-4.00)	0.31		
No	376|1666 (22.5)	reference			

IUGR=Intrauterine growth retardation; †Hb <11g/dL; ††Hb <9g/dL; *Hb <7g/dL

TABLE 6: 5: UNIVARIATE ANALYSIS OF FACTORS ASSOCIATED WITH NON-ATTENDANCE AT ANC

	univariate		
Factors	No ANC attendance (n= 211)	RR (95%CI)	*P-value*
Caste Category			
OBC	92|962 (9.6)	1.49 (0.98 - 2.26)	
SC	48|436 (11.0)	1.72 (1.09 - 2.70)	<0.001
ST	42|234 (17.9)	2.80 (1.77 - 4.42)	
GEN	27|422 (6.4)	reference	
Education			
No schooling	101|705 (14.3)	3.66 (1.52 - 8.82)	<0.0001
Primary	51|486 (10.5)	2.68 (1.09 - 6.59)	
Secondary	54|750 (7.2)	1.84 (0.75 - 4.51)	
Higher	5|128 (3.9)	reference	
Residence			
rural	160|1473 (10.8)	1.29 (0.95 - 1.75)	0.09
urban	50|595 (8.4)	reference	
SES quintiles			
Poorest	60|423 (11.6)	3.37 (2.00 - 5.68)	
Second	46|421 (10.9)	2.60 (1.51 - 4.46)	<0.0001
Third	52|447 (11.6)	2.77 (1.62 - 4.71)	
Fourth	36|374 (9.6)	2.29 (1.31 - 4.01)	
Richest	17|405 (4.2)	reference	

OBC=other backward caste, SC= Schedule caste, ST= Schedule Tribe, GEN=General caste; SES= socioeconomic status

6.3.6 Antenatal Attendance

A separate analysis was performed to assess the association between women who had not attended antenatal care (before their enrolment at the time of delivery) and their socio-demographic status (Table 6:6). Non-attendance at antenatal care was significantly associated with level of education, socioeconomic status and caste. Women with no schooling were 3.6 times (95%CI 1.52-8.82) more likely not to attend antenatal care than women with higher education. The risk of non-attendance for antenatal care was highest in women of the Schedule Tribe compared with women of the General caste.

TABLE 6: 6: ATTRIBUTABLE FRACTIONS FOR SELECTED EXPOSURE VARIABLES

Outcome	Exposure	Exposure Prevalence	RR (95%CI)	Attributable Fractions	
				AF %	PAF %
LBW (<2500g)	*P.falciparum* placental malaria	0.9%	1.98 (1.38 - 2.85)	49.5	4.6
BW (<1750g)			6.19 (1.66 - 23.13)	83.8	8.2
BW (<1875g)			6.60 (1.91 - 22.70)	84.8	8.3
Preterm			3.28 (2.01 - 5.35)	69.5	6.6
LBW (<2500g)	Moderate-severe anaemia	20.0%	1.24 (1.08 - 1.43)	19.4	4.5
BW (<1750g)			1.76 (1.18 - 2.62)	43.1	13.2
BW (<1875g)			1.67 (1.17 - 2.39)	40.1	11.8
Preterm			1.61 (1.28 - 2.01)	37.9	10.9
LBW	Maternal height (<150cm)	40.3%	1.36 (1.20 - 1.53)	26.5	12.6
Preterm			1.44 (1.16 - 1.77)	30.5	15.1
IUGR-LBW			1.46 (1.22 - 1.74)	31.5	15.6
LBW	MUAC (<23cm)	49.5%	1.42 (1.26 -1.62)	29.5	17.2
Preterm			1.86 (1.49 - 2.31)	60.5	29.8
IUGR-LBW			1.34 (1.12 - 1.60)	25.3	14.4

AF= attributable fraction, PAF=population attributable fraction, LBW= low birth weight, BW=birth weight, IUGR=intrauterine growth retardation

6.3.7 Attributable Fractions

Both attributable fractions (AF) and population attributable fractions (PAF) were calculated. The attributable fraction (AF) indicates the prevalence risk in the exposed group, and the population attributable fraction (PAF) indicates the overall excess risk in the population and thus the reduction in the proportion of low birth weight in the population that could be achieved if the exposure is prevented (Table 6:7). Placental malaria accounted for 49.5% of low birth weight (<2500g) in women infected with placental malaria (AF). The AF of low birth weight (<1750g) was 83.8% in women with placental malaria, and 69.6% of preterm births in women with

placental malaria was attributable to malaria. The PAF of placental malaria was below 10% for low birth weight, preterm and IUGR-LBW. 19.4% of low birth weight in women with moderate-severe anaemia was attributable to moderate-severe anaemia, and this was 43.1% for birth weight <1750g and 37.9% for preterm births. With maternal stature (<150cm) as the exposure, 26.5% low birth weight, 30.5% preterm and 31.5% IUGR were attributable to short stature. The PAF was 12.6%, 15.1% and 15.6% of low birth weight, preterm and IUGR respectively in women of short stature. Similarly, 17.2% low birth weight, 29.8% preterm and 14.4% IUGR could be explained by maternal malnutrition as defined by a MUAC <23cm at population level.

6.4 Discussion

The study explored the prevalence and determinants of preterm birth and IUGR-LBW as two separate conditions in this population found to have a high level of low birth weight (chapter 4). Distinguishing the two types of low birth weight is important because of their different underlying mechanisms and prognoses. 33.8% low birth weight prevalence observed in this study is comparable to previous reports (28-30%) and exceeds the WHO recommended threshold of >15% for public health action (Baqui, *et al* 2006, de Onis, *et al* 1998, Mavalankar, *et al* 1992, UNICEF-WHO. 2004). Previous studies in India have rarely looked into preterm birth and IUGR-LBW as separate conditions. Using the Ballard method of gestational assessment we found that 1 out of every 7 babies born spontaneously was a preterm birth (14.7%) and they accounted for 43.0% of low birth weight. The rest were IUGR-LBW (57.0%). By separating low birth weight into two entities, a distinctly different determinants profile was observed with preterm births mainly associated with acute conditions whereas IUGR-LBW was associated with chronic conditions.

WHO recommends the use of one global reference growth curve for babies on the basis that populations across the world have the same intrauterine growth potential. However, the scatter plots of gestational age by birth weight of 1949 newborns in this study suggest that this is not the case. At 37 weeks gestation the median weight was 2580g: 420g lighter than the comparative value (3000g) of the WHO recommended reference curve (Fenton 2003)(Annexe 9). The 50th percentile at 37 weeks of the Fenton curve (3000g) in fact corresponds to the 90th percentile in this cohort, indicating that only 10% of babies had a birth weight of 3000g or more. Similarly, a lower median birth weight at 40 weeks compared with the WHO reference curve was observed. Although these findings are not surprising as a large proportion of the Indian population has small stature, it does suggest that the global definition of low birth weight at <2500g may not be the most appropriate threshold for this population. The low birth weight threshold (<2500g) was based on a 20 times increased risk of infant mortality rate in babies born

<2500g requiring special assistance in the neonatal nurseries in developed countries, and adopted by WHO in 1950 as a population measure (WHO 1950). Several investigators have since argued that this may not be representative of all populations (Buekens 2002, Chen, *et al* 1991, Hernandez-Diaz, *et al* 2008, Wilcox 2001).

As discussed above, the dichotomised definition of low birth weight assumes that all low birth weight babies could be pathological. However, on the basis of heterogeneity of birth weight in different populations, Wilcox and Russell's argument shows that mortality is independent of birth weight between populations (Wilcox 2002). Using their methodology of birth weight analysis, the birth weight distribution was separated into predominant (normal) and residual groups (pathological). Although the frequency of low birth weight was large, this analysis suggested that the residual (pathological) group was only a small proportion (1.1%) of newborns with a birth weight <1750g. The other interpretation of the birth weight distribution suggests that a large proportion of low birth weight babies that fall into the predominant group (normal) are likely to be constitutionally small babies. The 6 fold increased association with maternal moderate to severe anaemia and malaria observed in the small preterm with weight<1750g group compared with the 1751-2500g group also suggests that the large group of low birth weight babies <2500g may not all be pathological and contain distinct subgroups which require different intervention strategies. Although mortality data was not available in our study, these findings support suggestions that the universal definition of low birth weight at <2500g might not be a surrogate of infant mortality in this high low birth weight prevalent population.

Exploring the determinants of preterm-LBW and IUGR-LBW as two sub-groups clearly showed they represent two distinct determinants profiles, with few factors common to both conditions. Preterm birth was mainly associated with moderate-severe anaemia, history of fever in pregnancy, malaria, season and MUAC <23cm. In contrast, IUGR-LBW was more strongly associated with socioeconomic status, maternal height, gravidity, antenatal visits and female gender.

Some of the acute morbidity factors associated with preterm may explain the seasonality observed for preterm. The risk of preterm birth increased in October and November with a peak in November, as mean gestational age and mean birth weight reached their lowest annual level. Around this time of the year, women in this region are engaged in crop harvesting which involves strenuous physical activity. Although there is conflicting evidence, an increase in preterm birth has been associated with hard physical labour, particularly in women engaged in agriculture in developing countries (Launer, *et al* 1990, Rayco-Solon, *et al* 2005). Previous Indian studies have shown a decrease in birth weight associated with physical labour during the harvest season in women at around 28 weeks gestation and onwards, and an increase in birth weight with

decreased physical activity (Agarwal, *et al* 2001, Rao, *et al* 2009). In the present study the proportion of women with MUAC <23cm which reflects maternal malnutrition status was also highest in October and November suggesting that the high level of physical activity and energy expenditure during the harvest season is likely to contribute to low maternal nutrition. Furthermore, the association between preterm birth and moderate to severe anaemia also suggests an underlying nutritional effect in this population where iron deficiency anaemia is reported as the commonest cause of pregnancy anaemia (Agarwal 2005, Lone, *et al* 2004, Zhang, *et al* 2009).October and November is also the period corresponding to the peak malaria transmission season and malaria was a strong determinant of preterm delivery (chapter 4). As malaria transmission is relatively low this however could only explain a small proportion of all preterm deliveries in this population. Conversely, the lowest frequency of preterm birth was observed in April corresponding to the dry season when there is less agricultural work and malaria transmission is low. Thus, malaria together with physical activity is likely to influence the seasonality of preterm births and its contribution to low birth weight.

Maternal stature was a parameter associated with both preterm birth and IUGR-LBW. Although a biological explanation is unclear for preterm birth, shorter gestational duration has been observed among Asian women living in the United Kingdom (Patel, *et al* 2004). Other studies have also shown an increased risk of preterm birth in women with short stature of east and south-east Asian ethnicity and suggested an earlier filling of a smaller pelvis similar to the mechanism in adolescent pregnancies (Chan, *et al* 2009). Unlike preterm, the association between IUGR-LBW and maternal stature is well recognised (Kramer 1987, WHO. 1997). Maternal stature is linked to both genetic and environmental factors. Maternal stature also reflects a sustained impact of nutrition in early childhood on attained adult height, which contributes to the cycle of low birth weight. This association was evident in a recent study from the UK where the authors showed the persistence of lower birth weight in babies of second-generation women of Indian subcontinent origin, born and living in the UK (Margetts, *et al* 2002). The fact that with an increase in maternal height there was a decrease in low birth weight and the large proportion of women with short stature (40%) may also explain the large occurrence of IUGR-LBW in this population.

The other factors associated with IUGR-LBW that are of a public health interest were socioeconomic status and antenatal attendance. The exact biological mechanism by which socioeconomic status contributes to IUGR-LBW is unclear. Maternal under- nutrition linked to poverty, secondary to societal conditions has been suggested as an underlying basis for the high prevalence low birth weight India (Osmani 1997, Ramalingaswami, *et al* 1996). A similar possibility might also be explained by the association between MUAC <23cm and caste category

found in this study. We also found women with lower level of education, lower socioeconomic status, and belonging to the Schedule Tribe were less likely to attend antenatal care. This is consistent with the factors highlighted to have an association with low birth weight in a recent review of some hospital and community based Indian studies (Dharmalingam, *et al* 2010). In general, these are factors linked to poverty and as such are likely to be women who may not receive adequate nutrition during pregnancy. Thus the findings suggest that the non-antenatal attendance and under nourished women form a major sub-group associated with low birth weight.

This analysis highlights a number of determinants, however, because the main underlying burden study was not designed to assess the determinants of low birth weight, many known risk factors of low birth weight were not assessed. These include nutritional factors such as pre-pregnancy maternal weight and BMI, morbidity factors such as periodontal infections, bacterial vaginosis, STDs and HIV infections, as well as the use of tobacco smoking and or chewing, beetle nut and alcohol consumption. Although tobacco smoking is considered low among Indian women (2.4%), tobacco chewing is comparatively higher (12%) and has been shown to have an effect on low birth weight in India (Gupta, *et al* 2004). Furthermore, newborn length and head circumference was not measured and it was therefore not possible to separate symmetric and asymmetric IUGR-LBW to determine the proportion of pathological IUGR-LBW. The gestational age was estimated using the Ballard score which at best could have a variation of ±2 weeks and is considered less effective for assessing term babies. However, rigorous quality assurance by the researcher (paediatrician), made available reliable gestational age data, making it possible to distinguish low birth weight into preterm and IUGR.

The main risk factors identified for preterm-LBW; maternal malnutrition, maternal malaria, and maternal moderate-severe anaemia are potentially modifiable in the short term. The effect of each determinant is dependent on the prevalence and therefore its impact on the population could vary. For example 83% of 'small' preterm (weight <1750g, the residual group) and 69.5% preterm births in women with placental malaria could be attributed to malaria. Similarly about 40% of preterm births in moderate to severely anaemic women could be attributed to anaemia. An explanation for this might also be the fact that 20% women were moderate-severely anaemia and they could be anaemic early in pregnancy form iron and folate deficiency which is common in India (Agarwal 2005). Early to mid-pregnancy nutritional anaemia has been associated with preterm births (Scholl 2005, Zhang, *et al* 2009). However at population level the proportion of preterm births that could be reduced by eliminating these determinants is modest, because of the low prevalence of malaria, and of low exposure to these factors. Nevertheless, the positive impact observed with the use of bed nets and haematinics in

pregnancy suggest that these interventions could benefit when targeted at women at risk of malaria and anaemia. Maternal malnutrition as defined by a MUAC <23cm was an underlying factor for both preterm-LBW and IUGR-LBW suggesting 60% of preterm births and 25% of IUGR-LBW occurring in malnourished women could be attributable to maternal malnutrition (AF). Because of the high prevalence in this population, the prevention of this major contributing factor has the potential to reduce 30% of all preterm births and 14% of all IUGR-LBW in this population. If hard physical labour in the third trimester contributes to preterm births, as we suspect, efforts to reduce hard physical labour by pregnant women in the harvest season is likely to reduce spontaneous preterm births (Rao, *et al* 2009).

The main determinants of IUGR-LBW; maternal short stature and socioeconomic status are linked to poverty and as such are more long term modifiable general goals. Poverty reduction is the Millennium Development Goal number 1, and related efforts, together with the current economic growth in India is likely to improve conditions of the lower socioeconomic quartile which could show a positive impact on newborn weight in the long term. Since neonatal mortality contributes to 66% of under-5 mortality in India, a reduction in low birth weight would contribute to achieving the Millennium Development Goal for reducing child mortality (MDG-Report 2009). Reduction of preterm-LBW has the potential for decreasing neonatal mortality, whereas improvement of IUGR-LBW has long-term benefit of reducing the burden of chronic adulthood diseases and low birth weight in subsequent generations (Barker 1995). Through improved antenatal attendance and efforts directed to improve maternal nutrition there is the potential to improve birth weight in the IUGR-LBW group in the short-term as has been shown elsewhere (Ceesay, *et al* 1997, Lopez, *et al* 2009, Shrimpton 2003). Furthermore, the non-antenatal attending subgroup could be targeted through the currently implemented ASHA programme. The AHSA programme has successfully increased the number of women attending health facilities for deliveries and could be used to encourage antenatal visits and to educate the importance of antenatal care in improving newborn birth weight.

By examining the birth weight distribution and determinants profiles of preterm-LBW and IUGR-LBW as two separate entities the study highlights the heterogeneity of maternal factors and likely levels of newborn risk within this population. The two risk factor profiles suggest a need for different preventive strategies for the short and long-term improvements in low birth weight. A better understating of the non-pathological, constitutionally small low birth weight group is needed to provide long term public health benefit in this population. However, prevention of preterm births should be the key goal and an approach targeting the high risk small preterm group is likely to have the highest impact in reducing low birth weight in Madhya Pradesh.

Chapter 7

Accuracy of First Response Pf/Pv® *p*LDH-Rapid Diagnostic Test as a Screening Tool for Malaria as part of Antenatal Care

"In truth the 'gold standard' is already a barbarous relic"
-John Maynard Keynes-

7.1 Introduction

Microscopy remains the gold standard for the diagnosis of malaria. However, it is a time consuming technique and the accuracy is dependent on the smear quality, quality of the microscope, and the experience of the technician. Furthermore, the parasite detection threshold is limited to 20-50 parasites /μl when read by an expert examiner (Moody 2002). Malaria Rapid Diagnostic Tests (RDTs) are a relatively new tool, for the biological detection of malaria. RDTs are practical, and allow timely diagnosis in areas where good quality microscopic services are unavailable. Although the use of the RDT is more common for the diagnosis of patients with symptoms (e.g. in out-patient departments), they are increasingly being considered for screening of malaria in asymptomatic populations. One such potential application is for the control of malaria in pregnancy in regions that that do not have a strategy of intermittent preventive therapy in pregnancy (IPTp) as in many areas of low malaria transmission such as in most malaria endemic countries outside of Africa. Recent literature shows numerous malaria RDTs have been evaluated in areas of varying transmissions (Hopkins, *et al* 2008, WHO 2008b). However, few studies involved pregnant women, and even less have assessed the RDT as a screening tool for asymptomatic infections in pregnancy. With the growing interest in strategies for scheduled intermittent screening for malaria as part of focused antenatal care, there is a need to evaluate the performance of RDTs in screening asymptomatic pregnant women (Tagbor, *et al* 2010).

Most studies in pregnant women have focused on the performance of HRP-2 based RDTs in detecting *P.falciparum* in peripheral or placental samples (Leke, *et al* 1999, Mockenhaupt, *et al* 2002, Singer, *et al* 2004). Because the HRP-2 antigen is specific to *P.falciparum*, the HRP-2 based RDTs are not suited for the detection of *P.vivax*. Other RDTs based on the detection of parasite specific *Plasmodium* lactate dehydrogenase (pLDH) are currently available as *P.falciparum*-specific, PAN-species or *P.vivax*-specific and often manufactured as "Combo tests" in combination with HRP-2 strips (WHO-FIND 2009, Makler and Hinrichs 1993, Piper, *et al* 1999). A shortcoming of the HRP-2 based RDT is the potential for false positive results, because of the persistence of HRP-2 antigen up to several weeks after clearance of the initial infection (Laferi, *et al* 1997, Moody 2002). In contrast, pLDH based tests, although less sensitive, are more specific because pLDH rapidly declines to undetectable levels as parasites clear following successful antimalarial treatment.

This study aimed to evaluate the accuracy of the newly marketed (at the time the study commenced) pLDH based RDT, First Response Pf/Pv® (Premier Medical Corporation Ltd, Mumbai, India) in screening of asymptomatic and symptomatic pregnant women as part of antenatal care. PCR was used as the resolver test, to evaluate the agreement and disagreement between the RDT and microscopy results, and the performance of the RDT in detecting sub-

microscopic infections. The study also highlights the challenge of evaluating the RDT with an imperfect gold standard while using a highly sensitive resolver test.

7.2 Methods

7.2.1 Study population

Women of any gravidity above the age of 15 years attending routine antenatal care in 3 health facilities in urban and rural sites in Madhya Pradesh were enrolled in two 6-week cross-sectional surveys to determine the burden of malaria in pregnancy (detailed in chapter 3). All participants had a finger prick blood sample taken for RDT and malaria smears, and a sample stored for subsequent PCR analysis. The samples of the survey 2, conducted in October-November 2006, corresponding to the peak post-rainy transmission season were used for this analysis.

7.2.2 Reference and Resolver Tests

The RDT (index test) was evaluated using microscopy as the reference test. Microscopy, however, is an imperfect 'gold standard' (Ochola, *et al* 2006) and misclassification by microscopy introduces bias into the sensitivity and specificity estimates (usually downwards). PCR is superior to both microscopy and RDT in the detection of low density infection as it is capable of detecting parasites at densities of ≤5 parasites per μl (Snounou, *et al* 1993). PCR was the third test used as the resolver for the discrepant resolution of the cases for which there was disagreement between the results of the RDT and microscopy (discordant results) and to compare the results of a concordant sub-sample. The resolver test (PCR) should ideally be used on the entire sample, but this was not feasible because of budget constraints. Therefore, a sub-set of samples were processed for PCR analysis.

7.2.3 Modified discrepant resolution by PCR

The traditional approach to discrepant resolution consists of retesting only the discordant results with a third superior, resolver test (PCR in this study). However, resolver testing of only the discordant cases can bias estimates of test accuracy, inflating estimates of sensitivity and specificity (Hadgu 2001, McAdam 2000). Therefore Meier (1998) suggested a method of discrepant analysis which consisted of using a random sub-sample of the concordant and discordant results. This provides more consistent test estimates that are less prone to bias. A modified form of the Meier method was used in this analysis. Since the number of positive cases

detected by either RDT or microscopy was low, the number of concordant negative samples (RDT and microscopy negatives) was much higher than the number of concordant positives (positive by both RDT and microscopy) or discordant cases. Therefore, PCR analysis included all the concordant and discordant positive samples, but only a subset of the concordant negative samples. (Hawkins, *et al* 2001), had developed a method to allow the estimation of the confidence intervals of the test performance measures, and determination of the optimal sample size of the subsets to be tested when using the Meier method of discrepant resolution. This method was applied to determine the number of concordant negative samples needed to be tested by PCR using the precision of the final estimate of the sensitivity of the RDT as the endpoint as suggested by (Flahault, *et al* 2005). In these sample size considerations the precision of sensitivity was given higher priority than optimizing the precision of the specificity estimate. The calculation aimed at generating the minimum number of concordant negative samples required for PCR analysis that would result in a lower limit of the precision of the sensitivity of RDTs against PCR of at least 85% as outlined below.

A model was constructed in MicroSoft Excel (2003) based on the example provided by Hawkins, *et al* (2001). Using all the samples that had a result for the RDT and microscopy, the model took into account the following parameters: malaria prevalence detected by microscopy or RDT and the 'relative' sensitivity and specificity of the RDT obtained against microscopy as the reference test. Secondly, we assumed that the true sensitivity (using PCR) would be lower in the context of sub-microscopic infections (defined as PCR positive infections not detected by either microscopy or RDT). The number of sub-microscopic infections among the asymptomatic pregnant women was unknown at the time of the study design for this analysis. The assumption was it would be relatively rare because of the very low transmission and patent infections in the area and the corresponding low levels of malaria specific immunity among this population of pregnant women. Therefore, the assumption was that most infections would result in symptomatic malaria and that for every 5 patent infections at least 1 additional sub-microscopic infection would be present (as determined by PCR). E.g. we assumed that 1% of the concordant negatives could be positive by PCR if the prevalence of patent infection was 5%. Based on these assumptions, a sample size of 262 concordant negatives (plus all 52 concordant and discordant positives) was considered sufficient to detect a sensitivity of a lower 95% confidence limit of at least 85% (Annexe 10).

Selection of the concordant-negative samples: The 262 concordant negative test samples were selected proportional to the size of the numbers enrolled at each study facility during the second survey to minimize bias when extrapolating the result of the subset to the total study population.

The numbers required for each site were: 88, 107 and 67 for Jabalpur (33.4% of 262), Katni (41.0%) and Maihar (25.6%) respectively. The SAS software was used to randomly select the 262 samples from the main sample list. An additional spare list of 20 samples was generated for each site to replace missing samples or those with insufficient blood volume for which DNA could not be extracted.

7.2.4 Test Procedures

RDT procedures: The pLDH First Response® functions on the antigen detection principle differentiating between *P.falciparum* and PAN-species. A drop of blood was drawn up to the 5µl mark of the micro-pipette provided with the kit, and placed in the sample well of the test cassette. Two drops of buffer solution were added to the buffer well. Trained study staff performed the test. A single reader interpreted and recorded the results within 20 minutes, according to the manufacturer's instructions. The results were interpreted as positive or negative for *P.falciparum* and PAN-species according to the visibility of the line corresponding to the species mark. The test was considered invalid if the control line was not visible. The appearance of the PAN line only (with control line) was interpreted as *P.vivax* mono-infection. The appearance of *P.falciparum* line only was interpreted as *P.falciparum* while a combination of a positive PAN line and *P.falciparum* was considered a mixed species infection. The test applications by the staff were supervised regularly for quality assurance, and each batch of RDTs were tested on a known positive and negative sample as control, before using in the field. The results of the RDTs were recorded prior to the first reading of the malaria smears.

Microscopy: The procedure for smear preparation and microscopic examination was described earlier in chapter 3. Briefly, the field technician who was unaware of the RDT result performed the first microscopic examination. A senior technician at the central study laboratory in Jabalpur, blinded to the RDT and microscopy results from the field, performed the second reading. Any discrepant results (smear positive or negative) identified between the first and second technician were re-examined by a senior microscopist or the investigator and their decision taken as final.

PCR Analysis: The blood samples collected for PCR testing were spun and plasma and red cell pellets separated. The red cell pellets were stored at -20^{0}C until the time of PCR analysis. A two-step nested PCR assay was used as detailed in chapter 3. The first reaction was for plasmodium genus amplification, run on DNA extracted from the red cell pellets. The second reaction was for species amplification using rRNA primers for *P.falciparum* and *P.vivax*. The amplified DNA bands were visualized in the gel documentation system, after electrophoresis on 1.5% agarose gel and

staining with ethidium bromide. Staff unaware of the microscopy and the RDT results performed PCR analysis.

Operational quality control: The RDT packs were stored in an air-conditioned room at the central NIMR Field Station. All possible care was taken to maintain room temperature at the manufacturer's instructed level of 4^0C – 30^0C. During transport to the field sites, attention was paid to avoid exposure to direct sunlight. When the ambient temperature at the field sites became higher than 30^0C, the packs were stored in the refrigerator. Individual kits were opened only at the time of testing.

7.2.5 Data analysis

Statistical analysis: The data were double entered and checked using Epi-Info (version 3.5.1). SAS® version 9.1 (Statistical Application Software, SAS Institute Inc. Cary NC) software was used for analysis. The Standard of Reporting of Diagnostic Accuracy (STARD) was followed to report the results (Bossuyt, *et al* 2003a). The RDT accuracy for *P.falciparum* and *P.vivax* was analysed separately.

A two-step approach based on the method proposed by Hawkins *et al* (2001) was applied. In step one, the RDT (index test) was tested against microscopy as the reference test and the results summarized in the standard 2x2 table (Table 7:1). This first step of results therefore expressed the 'relative' sensitivity and specificity of the RDTs (the agreement and disagreement between the RDT and microscopy results). Second, the results of the PCR analysis were taken into account to obtain corrected estimates of the test performance measures, including the sub-sample of negative concordant results that was tested with PCR. Following discrepant resolution with PCR, the false negative result of the index test was calculated for the sub-sample. This proportion was extrapolated to the total number of concordant negatives to obtain the estimates for the total study sample. No such extrapolation was required for the other cells, as all their samples, were PCR assayed. The result showed the diagnostic accuracy of the RDT calculated from the total sample, reported as the sensitivity and specificity and predictive value pairs, and their corresponding 95% confidence intervals to indicate the precision of each estimate relative to the PCR results. Confidence intervals were generated using the "adjusted Wald method" because the usual 'Wald method' produces intervals that are too narrow with small samples. The Wald method is adjusted in that it requires the addition of two artificial positives to the numerator and two to the negatives (denominator) from the critical value of the normal distribution (1.96 = 2). After obtaining the probabilities as suggested in the Hawkins *et al* (2001) and other literature, the 'adjusted Wald method' was applied to generate the confidence intervals (Sauro, *et al* 2005). The

modified discrepant resolution concept using a resolver test is illustrated with the results of *P.falciparum* samples.

There were very few cases of *P.vivax* mono-infection in this study, and surprisingly the PCR cases did not match the RDT and microscopy concordant samples. This made it clear that the accuracy measures of the First Response® (index test) for *P.vivax* would be low, regardless of the analysis method. Nonetheless, the RDT results were assessed against microscopy and PCR. Since in the pLDH only RDT the combined PAN-species and *P.falciparum* lines cannot distinguish between *P.falciparum* and *P.vivax*, the few mixed species (5 samples) were not assessed separately.

TABLE 7: 1: STANDARD MEASURES OF TEST ACCURACY

	Reference test		
Index test	***Positive***	***Negative***	
Positive	True positive (a)	False positive (b) (Type 1 error)	Positive predictive value a /(a +b)
Negative	False negative (c) (Type 2 error)	True negative (d)	Negative predictive value d / (c +d)
	Sensitivity a /a+c	Specificity d /d+b	

7.3 Results

7.3.1 Study Population and samples

Between October-November 2006, 1000 women were enrolled from the 3 study sites, and both microscopy and RDT results were available for 989 women. Out of the 989 women, 76 were positive for malaria (any species) by microscopy or RDT. Of these 76 cases, 13 samples had inadequate volume for PCR analysis: 63 positive samples were available for PCR assay. PCR was performed on 314 samples, which included 262 concordant negative samples by both RDT and microscopic examination.

7.3.2 RDT Accuracy for *P.falciparum*

7.3.2.1 RDT compared against microscopy *(P.falciparum)*

The results of the RDT compared with microscopy for the 989 study samples are shown in Table 7:2. Out of the 57 *P.falciparum* cases detected by microscopy, 54 were positive by the RDT. Thus, the agreement of positive results between the RDT and microscopy expressed as the relative sensitivity of RDT for *P.falciparum* was 95% (54/57) (95% CI 84.5-98.6). The agreement

TABLE 7: 2: RDT PERFORMANCE AGAINST MICROSCOPY (*P.FALCIPARUM*)

	Reference test		
Index test	Microscopy +	Microscopy -	*Total*
RDT +	54	6	60
RDT -	3	926	929
Total	57	932	989

sensitivity	94.7% (95% CI 84.5-98.6%)
specificity	99.3% (95% CI 98.9-99.6%)
Positive predictive value	90.0% (95% CI 82.4-97.6%)
Negative predictive value	99.7% (95% CI 98.2-99.9%)
Prevalence	5.7%
Agreement between RDT and microscopy = 99.0%	

of the negative results between the RDT and microscopy expressed as the relative specificity was 99.3%, (95% CI 98.9-99.6). The overall agreement between RDT and microscopy was 99.0%.

7.3.2.2 PCR resolution of the original microscopy results

PCR results for P.falciparum

The microscopy and RDT samples tested by PCR are presented in table 7:3. Out of the 314 samples selected for PCR, there were 47 concordant positive cases (RDT and microscopy positive), 5 discordant and 262 concordant negative cases. The cases that tested positive for *P.falciparum* by PCR assay were 43 out of the 47 concordant positive cases (RDT and microscopy positive) (91.4%). Out of the 3 apparent false-positive cases (RDT positive, microscopy negative), 2 were positive for *P.falciparum* by PCR (66.6%). Of the 2 apparent false-negative cases (microscopy positive, RDT negative) 1 case was positive by PCR. 42 of the 262 concordant negatives (microscopy and RDT negative) cases tested positive for *P.falciparum* on PCR assay (16.0%). Thus, there were a total of 2+ 42 cases classified as microscopy negative that were PCR positive (false-negatives) and 4+1 cases that were microscopy positive and PCR negative (false-positives) (Table 7:4). Conceptually they can be considered "errors" of the reference test (microscopy). In this situation, 4 of the concordant positive (microscopy and RDT positive) cases

TABLE 7: 3: TABULATION OF RDT AND MICROSCOPY SAMPLES TESTED BY PCR

	Reference Test (microscopy)		
Index Test	Positive	Negative	**Total**
RDT +	47	3	50
RDT -	2	262	264
	49	265	314

TABLE 7: 4: RESULT OF DISCREPANT RESOLUTION WITH PCR

	PCR Positive (' true')			**PCR Negative ('true')**			
	1. Microscopy (reference test)			2. Microscopy (reference test)			Total
Index test	positive	negative	**Total**	positive	negative	**Total**	
RDT +	43	2	**45**	4	1	**5**	**50**
RDT --	1	42	**43**	1	220	**221**	**264**

Note: the no: 1 2x2 table shows PCR identified positive cases in each cell, and no: 2 2x2 table shows PCR negative cases.

TABLE 7: 5: REVISED RESULTS OF RDT WITH PCR AS THE REFERENCE TEST (***P.FALCIPARUM***)

	Reference test		
Index test	PCR +	PCR -	Total
RDT +	45	5	50
RDT -	43	221	264
Total	88	226	314

False positive = 0.1%

False negative = 16.2%

Agreement between RDT and PCR = 84.7%

were negative by PCR and 42 of the concordant negative (RDT/microscopy negative) cases were positive by PCR.

TABLE 7: 6: RESULTS EXTRAPOLATED TO THE TOTAL STUDY SAMPLE (***P.FALCIPARUM***)

RDT compared with PCR			
Reference test			
Index test	PCR +	PCR -	Total
RDT +	45	5	50
RDT -	148	791	939
Total	193	796	989

Accuracy measures of RDT compared with PCR	
Sensitivity	23.2% (95% CI 17.6-30.0%)
Specificity	99.4% (95% CI 98.4-99.8%)
Positive predictive value	90.0% (95% CI 77.4-96.2%)
Negative predictive value	84.2% (95% CI 81.7-86.4%)
Prevalence (PCR)	19.5 % (95% CI 17.1-22.1%)

RDT Accuracy estimation for the total study population (P.falciparum)

The tabulation after collapsing the results in Table 7:4 over the PCR results is presented in table 7:5. Based on the PCR results there were 5 false positive cases (0.1%) and 43 false negative cases (16.2%) detected by RDT (index test), while the percent agreement between PCR and the RDT was 84.7% for the 314 samples tested with PCR (resolver test).

The 42 *P.falciparum* positive cases detected by PCR (resolver test) from the concordant negatives were extrapolated to the total study sample. This resulted in 148 sub-microscopic cases among the 989 samples (Table 7:6). Reclassification of the PCR results provided the accuracy estimates of the RDT for the study population relative to PCR: a sensitivity of 23.2% and specificity of 99.4% respectively (Table 7:6).

7.3.3 RDT Accuracy for *P.vivax*

7.3.3.1 RDT compared with Microscopy as the Reference Test

Among the 989 cases with both RDT and microscopy results available, there were 7 mono-*P.vivax* cases detected by microscopy. Out of the 7 mono-infection, 6 matched the RDT

results (PAN line positive and interpreted for *P.vivax*). A further 5 cases were positive for *P.vivax* by RDT, but negative by microscopy (Table 7:7). Thus, the relative sensitivity against microscopy was 85.7% (95%CI 42.0-99.2), and specificity was 99.0% (95%CI98.8-99.2).

7.3.3.2 RDT compared with PCR as the Reference Test *(P.vivax)*

There were 6 concordant RDT and microscopy positive *P.vivax* cases and surprisingly all were negative by PCR. However, out of the 5 discordant RDT positive cases, 3 were positive for *P.vivax* by PCR. Among the concordant negative samples, there were 17 new sub-microscopic *P.vivax* cases detected by PCR in the sub-set of 314 (Table 7:8). Relative to PCR (resolver test), there were 3 false positive cases (50.0%).

TABLE 7: 7: RDT COMPARED WITH MICROSCOPY *(P.VIVAX)*

	Reference test		
Index test	microscopy +	microscopy -	*Total*
RDT +	6	5	11
RDT -	1	977	979
Total	7	982	989

Sensitivity	85.7% (95% CI 42.0-99.2%)
Specificity	99.5% (95% CI 98.8-99.8%)
Positive predictive value	54.5% (95% CI 24.5-81.8%)
Negative predictive value	99.8% (95% CI 99.0-99.9%)
Prevalence	0.7% (0.31-1.52%)
Agreement between RDT and microscopy = 99.3%	

Accuracy estimation for P.vivax on the total study sample

The results in Table 7:8, after collapsing and reclassifying are present in Table 7:9. The 17 cases detected by PCR from the concordant negative samples were extrapolated to the total study sample. This resulted in 64 *P.vivax* cases among the total sample of 989 cases. Reclassification of the PCR results estimated the RDT *P.vivax* results for the study population relative to PCR. (Table 7:9) In this situation the sensitivity of the RDT decreased to 4.7% and specificity was 99.6%. The new prevalence of *P.vivax* was 6.7% (PCR).

TABLE 7: 8: RDT TEST PERFORMANCE WITH PCR AS THE REFERENCE TEST (*P.VIVAX*)

	Reference test		
Index test	PCR +	PCR -	Total
RDT +	3	3	6
RDT -	17	244	261
Total	20	247	267

False positive	= 50% (95%CI13.9-86.0%)
False negative	= 6.5% (95%CI 4.0-10.0%)
Agreement between RDT and PCR =92.6%	

TABLE 7: 9: RESULTS EXTRAPOLATED TO THE TOTAL STUDY SAMPLE (*P.VIVAX*)

RDT compared with PCR

	Reference test		
Index test	PCR +	PCR -	Total
RDT +	3	3	6
RDT -	64	919	983
Total	67	922	989

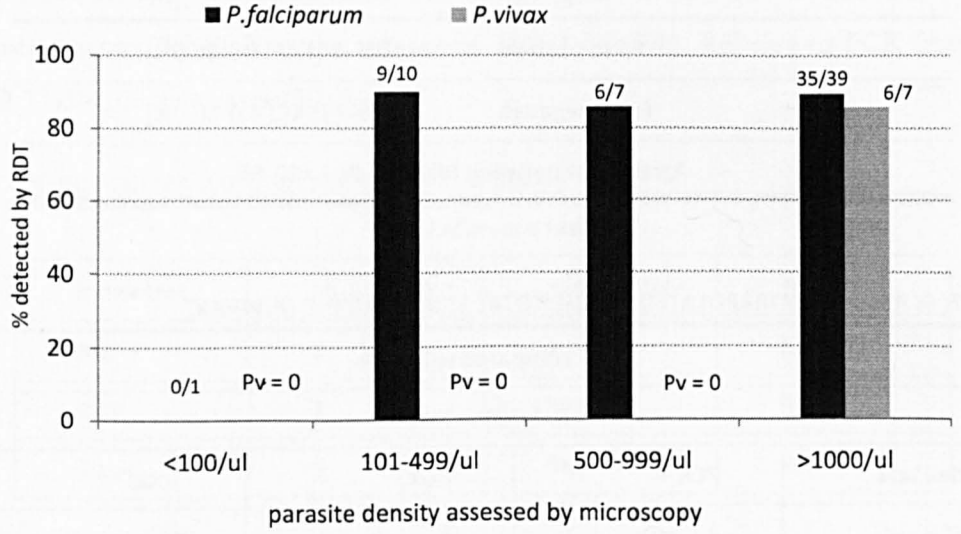
Accuracy measures of RDT compared with PCR	
Sensitivity	4.7% (95% CI 1.2-13.3%)
Specificity	99.6% (95% CI 98.9-99.9%)
Positive predictive value	50.0% (95% CI 14.0-86.0%)
Negative predictive value	93.4% (95% CI 91.7-94.9%)
Prevalence (PCR)	6.7 % (95% CI 5.3-8.5%)

7.3.4 RDT positivity at different ranges of parasite densities

The proportion of RDT detection (relative sensitivity) corresponding to different parasite densities assessed by microscopy is shown in Figure 7:1. There was 1 mono *P.falciparum* case with a parasite density of 80/µl (<100/µl) detected by microscopy and this case was not detected by RDT and was negative by PCR (Figure 7.2). The RDT sensitivity for microscopy positive cases with densities above 100/µl did not vary by density and was consistently between 80-90%. The

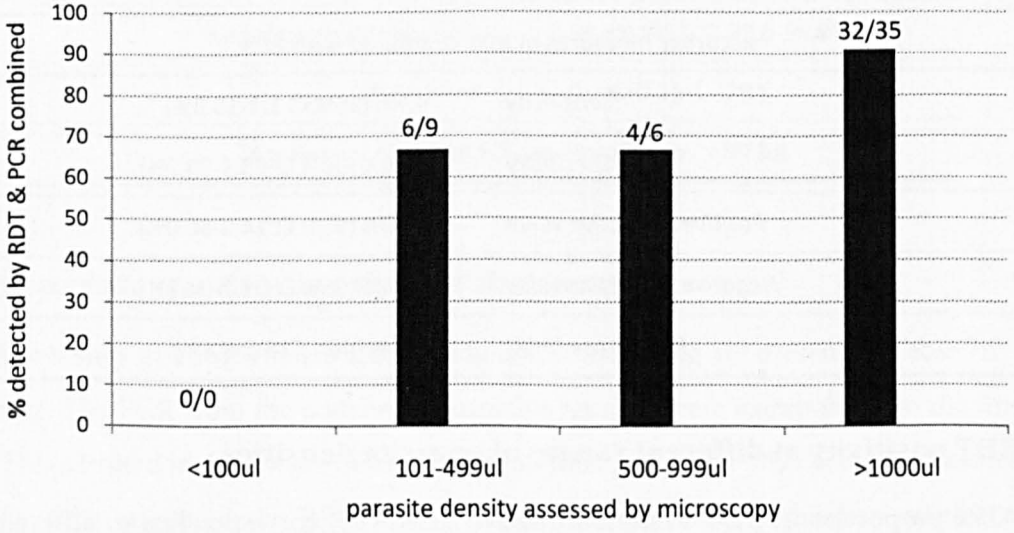
RDT sensitivity for *P.falciparum* cases that were positive for PCR showed a sensitivity of 66.0% at parasite densities between 100/µl- 1000/µl and 91.0% above 1000/µl. There were 7 cases of *P.vivax* detected by microscopy and all 7 cases had a parasite density >1000µl. 6 of the RDT *P.vivax* positive cases corresponded to density >1000ul. There were no microscopy positive *P.vivax* cases matching to PCR positive cases.

FIGURE 7: 1: INFECTION DETECTED BY THE RDT AT DIFFERENT PARASITE DENSITIES



***Note**: all P.vivax infections >1000/ul; the figures above the bars represent RDT positive numbers by microscopy detected cases for each category of parasite density*

FIGURE 7: 2: INFECTION DETECTED BY RDT & PCR AT DIFFERENT PARASITE DENSITIES (***P.FALCIPARUM***)



***Note**: there were no matching P.vivax cases. numerator numbers are cases positive for RDT& PCR, denominators are number of RDT positives in each parasite density category*

7.4 Discussion

The performance of the First Response Pf/Pv® pLDH based RDT to screen pregnant women attending antenatal care paralleled that of microscopy and had a high sensitivity (94.7%) and specificity (99.3%) for *P.falciparum* relative to expert microscopy. However, microscopy is an imperfect gold standard, and when accounted for PCR analysis, it was clear that there were more sub-microscopic infections (not detected by microscopy) than initially anticipated in this population of otherwise healthy and mostly asymptomatic pregnant women. Thus, although the RDT performance in detecting *P.falciparum* against microscopy was consistent with the manufacturer's report (sensitivity 95%, specificity 99.3%), the 'true' sensitivity of the First Response Pf/Pv® pLDH-based RDT relative to PCR was much lower (23.2%), whereas the specificity remained high (99.4%). This should not be interpreted as a failure of the RDT. The true sensitivity of microscopy when compared against PCR was equally low 22.9% (data not shown). These findings highlight the challenges in evaluating new diagnostics tests against an imperfect gold standard as the reference test when a much more sensitive test (PCR) is used as the resolver test.

The RDT sensitivity did not vary by parasite densities among the true PCR confirmed positives cases. The parasite (*P.falciparum*) detection rate of the pLDH based First Response Pf/Pv® at low parasite density (100-499/μl) was similar to that of microscopy as seen by the comparable numbers picked up by the RDT at densities between 101-499 parasites per μL. Although there was one case detected by microscopy at parasite density of 80/μl, this case was not positive by either PCR or RDT. This was important as we were interested to evaluate the RDT as a screening tool to detect malaria in asymptomatic women attending routine antenatal care. In addition the few apparent false positive tests (RDT against microscopy) when evaluated against PCR showed a high rate of agreement with the RDT (2/3, 66.6%), further suggesting the reliability of the First Response® pLDH based RDT for *P.falciparum* detection. The WHO-TDR/FIND has also evaluated the First Response (pLDH) RDT and found a sensitivity and specificity at higher parasite densities (2000 parasite/μL) of 100%. At low parasite densities of around 200 parasites per μL the sensitivity was 30% and specificity was 99.0% (WHO-FIND 2009).

In pregnant women there is the possibility that peripheral samples may miss parasites sequestered in the placenta. However this was a population where we found minimal difference between peripheral smears and placental malaria when detected by impression or incision smears (see also chapter 5); i.e. almost all women with evidence of peripheral infection also had

microscopic smear detectable placental infections consistent with previous observations from the Thai-Burmese border (McGready, *et al* 2004). It is therefore unlikely that there will be high level of circulating antigens from sequestered parasites that are missed by the RDT. Thus, our results suggest, that the pLDH-based First Response would be comparable to good quality microscopy as a screening test at antenatal care.

To the best of our knowledge, this is the first time an RDT (either pLDH or HRP-2 RDT) was assessed as a screening test for malaria in pregnancy in a low transmission setting outside of Africa. While the methodology was different, a pLDH based OptiMal® trial in pregnant Malawian women found 71% sensitivity for peripheral parasitaemia (Mankhambo, *et al* 2002). A recent study conducted among antenatal women in Ghana with the OptiMal® dipstick also reported a high accuracy compared with microscopy (Tagbor, *et al* 2008b). In non-pregnant population, the sensitivity reported for pLDH-RDTs has been lower than HRP-2 based RDTs (Hopkins, *et al* 2007). However in the present study the sensitivity of the pLDH-RDT for *P.falciparum* was within the WHO recommended level of sensitivity (95%) at parasite densities >100µl for an ideal RDT (94.7%) (WHO 2008b).

The presence of *P.vivax* was low in this population (0.7%). The sensitivity of the RDT for *P.vivax* relative to microscopy (85.7%) was much lower than for *P.falciparum* with wide confidence intervals which reflects the low numbers of *P.vivax* infections. Also, the low positive predictive value (40%) is a likely reflection of the very low *P.vivax* prevalence (0.7%). The true sensitivity when PCR was used as a resolver test was very low (5.0%), reflecting the relatively high prevalence of sub-microscopic infections detected with PCR as the resolver test. The RDT detected mixed species (5 cases) more readily than microscopy. An explanation for it might be that cross-species suppression may have affected *P.vivax* visualization by microscopy particularly of parasites in the ring-stage (Duffy 2001, Snounou and White 2004). A similar result was reported in a low transmission area in Philippines, where a combination HRP-2 Aldolase RDT picked up mixed infections undetected by microscopy (Bell, *et al* 2005).

The much higher than expected level of sub-patent infection for *P.falciparum* (16%) and *P.vivax* (6.4%) was a surprised finding. The study population has relatively low levels of background immunity to malaria and we anticipated that most infections would be patent and the majority (if left untreated) would eventually result in symptomatic malaria (Nosten, *et al* 2004). Although some sub-patent infections were expected, the rate cannot be explained solely in terms of an 'incubation period' before infections become patent. It is not clear whether these very low density infections matter in terms of adverse consequence to the developing fetus. In previous studies in Africa, no clinically relevant impact of sub-patent infections in pregnancy was found (Mockenhaupt, *et al* 2000, Saute, *et al* 2002). It is also not clear if left untreated the missed sub-

microscopic infections might contribute to transmission through the presence of gametocytes (Shekalaghe, *et al* 2007, Schneider, *et al* 2007). There is also very little known about the role of sub-patent infection and the development of premunition whereby it contains new infection. One experimental study, which used an inoculums of an ultra-low dose of infected red cells, showed that sub-patent infections induced immunity (Pombo, *et al* 2002). However it is uncertain whether the development of immunity is similar with naturally exposed sub-patent infection.

The higher sensitivity of PCR also raised an interesting challenge when the performance of a new diagnostic test (RDT) is evaluated against an imperfect gold-standard (microscopy) while using the highly sensitive resolver test (PCR). The lower detection threshold of parasitaemia by a field technician is 20-50 parasite/μl at best. The RDT detection also becomes less accurate at parasite levels below 100/μl (Moody 2002, Murray, *et al* 2008). PCR detects infection at much lower threshold including non-viable parasite debris. Because of it, and the analysis method used in this study, several negative cases were positive and reclassified to infected status by the resolver test (PCR). In comparison with the highly sensitive PCR, the RDT sensitivity decreased remarkably, and this was the case for microscopy as well. However had the study tested only the discrepant results, the RDT accuracy results would have been high. PCR is not the ideal field test for malaria detection, nor do manufacturer's report RDT performance against PCR. For these reasons and its high sensitivity, the suitability of evaluating RDT for field use against PCR becomes debatable at the current detection threshold of the RDTs.

The assessment approach with PCR as the resolver test showed that the accuracy of the RDT and microscopy is driven by sub-microscopic parasitaemia. A plausible option in burden studies could be to use to the PCR measured accuracy levels of the RDT and or microscopy to adjust the prevalence estimates. However, considering that sub-microscopic infections are likely to differ and the ratio of patent to sub-microscopic infection could vary between areas as observed in this work, the RDTs or microscopy accuracies tested in one region may not be applicable for adjusting MiP measurements in another region.

The study also enabled to assess the staff capability in performing the RDTs in the field. The staff employed for this study had no prior experience of using the RDT device. After a week of training and with minimal supervision, they managed to perform and interpret the test to a satisfactory standard. Another advantage of the RDT over microscopy was that the pregnant women could be shown the results, which made the test more acceptable, particularly in asymptomatic women and it encouraged them to comply with treatment (Tagbor, *et al* 2010). In contrast, microscopy examination took much longer to learn and required extended supervision to minimize diagnostic errors even for those who had some prior experience. Power failures in the field sites often disrupted microscopy readings adding to these inconveniences, there was a

longer waiting time to get the results, and unlike the RDT it was not possible for women to view their own test results.

As a diagnostic test the RDTs are used in symptomatic cases, which on average will have higher parasite densities, and thus infection is likely to be detected by the RDT. In contrast, for effective screening, the RDT should have an acceptable balance of high sensitivity and specificity and be able to 'rule out' or 'rule in' infection in asymptomatic cases that would typically have a lower parasite density. The pLDH First Response® demonstrated these qualities for *P.falciparum*, which was similar to microscopy in this low immune, low malaria prevalent population. The high sensitivity and negative predictive value of the First Response showed that it reliably identified cases and ruled out negative cases. Likewise, the specificity showed that it correctly indentified those not infected, which will help to avoid unnecessary treatment when screening pregnant women. Therefore, the First Response pLDH RDT performed as a suitable alternative to microscopy for screening pregnant women for *P.falciparum.*

The cross-sectional design of the study limited determining when sub-patent infection may become patent or whether there was spontaneous resolution of these infections. The First Response RDT used in this study is one of the new generation of RDTs with IgM antibodies, and therefore false positives are unlikely to be due to rheumatoid factor cross reactivity. This study environment, with dedicated research and support staff who were trained for the study and attention given to operational conditions, might not reflect the case in routine field settings. Further studies are required to evaluate the performance of the RDT and microscopy as a screening test under real life conditions in busy antenatal clinics.

The study found that the pLDH based First Response RDT was as good as microscopic examination in identifying *P.falciparum*, was convenient to use and well accepted by both staff and pregnant women. The RDT has ideal characteristics for use as a screening test and could be considered for use as in intermittent screening programmes. The resolver test approach used in this study identified a high prevalence of sub-microscopic infections and demonstrated the challenge of evaluating the test performance of a new diagnostic test against an imperfect reference tests with a more sensitive third test as the resolver test. Further studies are needed to evaluate RDTs for the screening of pregnant women with the new Combo RDTs consisting of Pan-pLDH and HRP-2 in areas where both *P.falciparum* and *P.vivax* are endemic.

Chapter 8

Comparison of different test methods for the diagnosis of placental malaria in a low transmission setting

"The obvious is sometimes false; the unexpected is sometimes true"
- Carl Sagan-

8.1 Introduction

The sequestration of *P.falciparum* in the intervillous spaces of the placenta is a characteristic feature of placental malaria and if left undiagnosed or untreated may lead to fetal growth restriction or preterm births. In chapters 4 and 5 we observed that this population was generally at low risk of malaria infection, yet the impact of each infection on birth weight and the duration of pregnancy were considerable, regardless of the presence of clinical disease. These findings stress the need for the early identification of malaria infected women for appropriate care. One option for early case detection during antenatal care is intermittent screening with either microscopy or RDT and treatment of infected women. It is not possible yet to diagnose the presence of placental infection during pregnancy. This means the detection of placental infections depends on diagnostic tests performed on peripheral blood and their level of agreement with placental infections. *P.falciparum* parasite densities in peripheral blood maybe at a lower density than those observed in the placental intervillous spaces, or may be absent while they sequester in the placenta (Ismail, *et al* 2000, Mockenhaupt *et al*, 2002). A better understanding of the accuracy of peripheral blood microscopy and RDT in detecting placental infections would aid the design of appropriate malaria control tools for malaria in pregnancy.

Placental histopathology is considered the 'gold standard' for microscopic detection of placental infections and provides information on the chronology of infection (Ismail, *et al* 2000, Rogerson, *et al* 2003a). But, it is a time consuming and expensive method requiring skilled expertise and is, therefore used less frequently. The other two direct placental sampling methods for microscopic detection of placental infections are incision smears and impression smears. Incision smears indicate parasites in 'free flowing' placental blood and consist of the rings and young trophozoite stages of the asexual cycle (Brabin, *et al* 2004). Impression smears by contrast are made from placental tissue and allow the identification of the presence of mature stages of the parasite sequestered in the placental tissue which can aid species diagnosis (Galbraith, *et al* 1980, Taylor 2009). Impression smears are relatively easy and quick to prepare, however, few studies have reported the use of impression smears and their performance has not yet been compared with the standard incision smears or histology.

Although the above mentioned methods are widely used for the diagnosis of malaria in pregnancy, information comparing these test's performance in determining placental malaria is very limited (Rogerson, *et al* 2003a). Here we report on the performance of two peripheral blood tests; maternal blood microscopy and a pLDH-based Pf/Pv RDT (First Response®) and of incision and impression placental smears, using placental histology as the "gold-standard" reference test.

8.2 Methods

Participants: Women enrolled at the time of delivery during surveys 2 and 3, (October 2006 to September 2007, see chapter 3) were included for this sub-study. A finger prick blood sample was collected during labour, prior to delivery for the preparation of thick and thin smears for microscopy and RDT. Placental incision smears, impression smears and histology samples for microscopic examination were obtained approximately within 3 hours of delivery.

8.2.1 Maternal and placental blood sampling

Peripheral Blood Microscopy: The details of microscopic examination were described in the methods chapter. In brief, the field technician, who was unaware of the results of the RDT, performed the first microscopic reading. A senior technician at the central laboratory in Jabalpur blinded to the field microscopy and the RDT results performed the second examination. The senior microscopist together with the investigator (RA) re-examined any discrepant results between the first and second reader and their decision was considered as final.

Placental Incision Smear: The placenta was placed in a tray with the maternal side facing upwards and the membranes were removed. Excess blood was cleaned by gently dabbing with filter paper. An incision was made with a sharp scalpel, about mid-way between the insertion of the umbilical cord and the edge of the placenta. Blood seeping into the incision was drawn into a Pastuer pipette or a 1 ml syringe. Three to four drops of this blood were placed on a clean, labelled, frosted slide and thick and thin smears prepared. The smears were stained with JSB stain, and examined using a similar methodology as for peripheral blood smear readings.

Placental Impression Smear: For preparing the impression smears, a 0.5x0.5x1cm placental tissue was cut half way between the maternal and fetal side, in an area free from infarcts. Excess blood of the cut piece was cleared by dabbing it on filter paper. The tissue was then gently pressed on the slide in 3-4 different spots to make imprints (Figure 8:1). The tissue was gently dragged alongside the imprints and additional impressions made. The impression smears were fixed and stained using a similar method to that described for thin peripheral blood smears.
The imprints allowed viewing of mature parasites and pigment (Figure 8:2). Percentage parasitaemia was calculated by counting 500 RBCs and dividing the infected RBCs by the total RBCs (infected + uninfected) and multiplying this by 100. If RBCs were less than 500, the total number present in the 50 high power fields examined was counted.

FIGURE 8: 1: IMPRESSION SMEARS TECHNIQUE

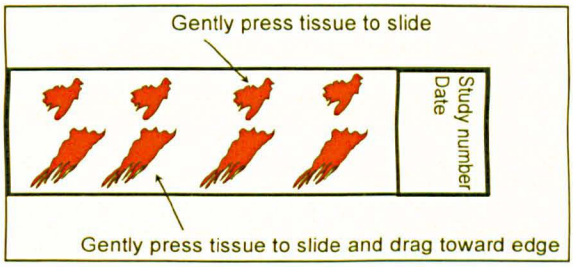
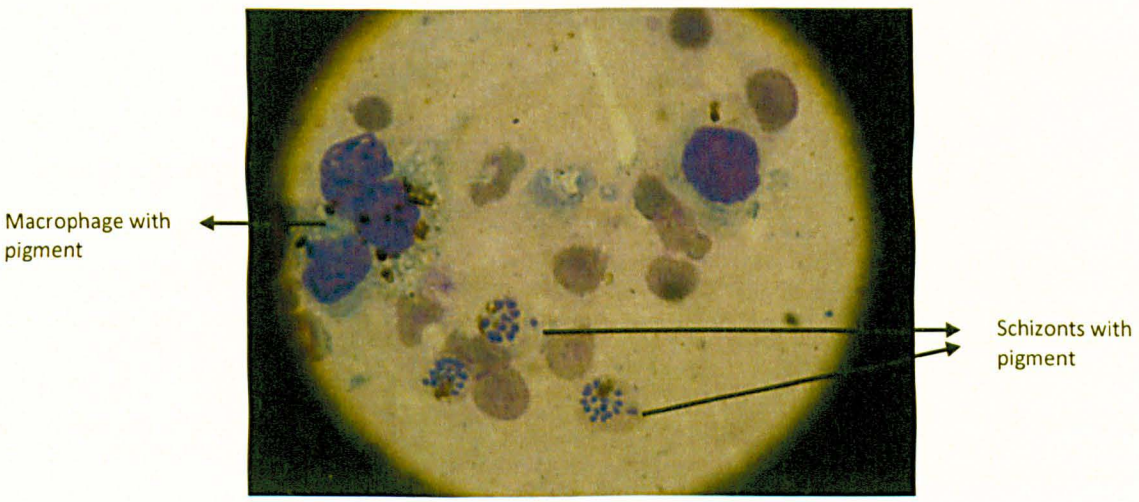


FIGURE 8: 2: MATURE PARASITES VIEWED ON IMPRESSION SMEAR MICROSCOPY



Placental Histology: Preparation of histology sections was described in chapter 5. In brief, two biopsy specimens were cut half-way between the placental edge and the insertion of the umbilical in opposite areas. The biopsied pieces were fixed overnight in phosphate buffered formalin. After fixation, they were embedded in paraffin, cut into 5μm sections, mounted on slides, and stained with haematoxylin and eosin (H&E). Examination was then performed using standard and polarised light microscopy described earlier in chapter 5.

8.2.2 Sample Selection for Analysis

2282 histology specimens were processed from the placenta of women enrolled from the delivery units during surveys 2 and 3. The samples in this sub-analysis include those chosen for the histopathology study and the selection process was described in chapter 5. In brief, 50 placental sections corresponding to the cases that tested positive for malaria either by peripheral

blood microscopy, RDT, placental incision or impression smears were chosen. The negative samples (negative by all the 4 test methods) were selected in proportion to women enrolled at each study site during the two surveys. These totalled to 456 placental sections. The random procedure in SAS software was used to select the 456 negative sections. A spare list of 10 sections was generated for each site per survey to replace any missing sample. A total of 506 samples were used in this analysis.

8.2.3 Statistical Analysis

All data was analysed using SAS (version) software. The analysis included two main categories of diagnostic comparisons:

1) Peripheral smear and RDT as index tests against placental histology as reference standard

2) Incision and impression smears against placental histopathology as reference standard.

For each comparison, the data obtained were summarized in the standard 2x2 table. The sensitivity and specificity, positive and negative predictive values were calculated by comparing the index tests with placental histopathology as the reference test. Histopathology was considered as the 'gold-standard' for the detection of placental malaria and represented the disease status. Likelihood ratios were calculated using an electronically available 'Diagnostic Test Calculator' provided by the University of Illinois, Chicago (mede.uic.edu) and, the posterior probability was read on the Bayes' nomogram also available at the same online site.

Definitions & test formulae: Sensitivity was defined as the true positive rate (TP) as the proportion of all women with evidence of placental malaria by histology (reference test) that tested positive by the index test. Similarly, specificity was defined as the proportion of negatives of the reference test that tested negative by index test (true negatives, TN). The positive predictive value (PPV) was estimated, with the TP divided by the sum of false-positive (FP) and the TP (Formula: TP/FP+TP). The negative predictive value (NPV) was estimated by dividing the TN by the false-negative (FN) plus TN (TN/FN+TN). The ratio of the true-positives to the false-positives defined the positive likelihood ratio, = TP /FP rate (sensitivity/1-specificity); in other words the probability of the positive test in those with disease compared to the probability of positive test in those without disease. The negative likelihood ratio was defined as FN / TN (i.e. 1-sensitivity/specificity).

The LR values >10 significantly increases the likelihood and < 0.1 significantly decreases the likelihood of disease (Akobeng 2006). Thus, in comparing the tests for the detection of placental malaria, the test showing a larger positive likelihood ratio was considered as the test

with a greater diagnostic ability. Likewise, the smaller the negative likelihood ratio less is the likelihood of disease which favours a negative test ability.

8.3 Results

8.3.1 Parasites in Peripheral and Placental Samples

Table 8:1 provides a summary of the species specific parasite numbers detected by the different test methods. The RDT detected 10 pure *P.falciparum,* 8 *P.vivax* cases, and 14 cases showing mixed species (both lines). The 14 mixed species were confirmed by microscopic examination as *P.falciparum*, and were considered as mono-*P.falciparum* cases (n=24) for this analysis. As expected there was an overlap in the number of positive cases detected by each test method against the peripheral blood results as the reference base: 29 incision smears, 20 impression smears and 29 RDT positives matched peripheral blood microscopy positive cases for any species.

Histological examination showed evidence of active infection in 38 sections, defined by the presence of parasites. In addition, 14 sections were classified as past infections, defined by the presence of pigment in fibrin in the absence of parasites. The histologically classed active infections corresponded to peripheral *P.falciparum* infections as described in chapter 5. There were no *P.vivax* cases corresponding to active infections detected by histological examination. All species corresponding to the histology infections were confirmed by PCR analysis (see chapter 5).

TABLE 8: 1: PARASITE SPECIES INDENTIFIED BY THE DIFFERENT TEST METHODS IN 506 SAMPLES

Test Method	*P.falciparum*	*P.vivax*	Total
Peripheral blood smear	29	11	40
*p*LDH-RDT (First Response®)*	24	8	32
Incision smear (placenta)	27	6	33
Impression smear (placenta)	20	3	23
Placental Histopathology	38 (active)	0	

*Performed on peripheral blood

8.3.2 Comparison of test methods with histology as reference test

Peripheral blood microscopy & RDT: The *P.falciparum* positive cases detected by peripheral blood microscopy and the RDT were compared against histology, because active infections indentified by histopathology corresponded only to *P.falciparum* cases. The results are summarised

in 2x2 sets in Table 8:2. Out of the 38 cases detected by histopathology, 25 were positive by microscopy and 17 by the RDT. The sensitivity of microscopy was 65.7% and of the RDT was 48.5%, whereas specificity was >99% for both tests. The false positivity was low with positive predictive values of 89% and 80.9% respectively for microscopy and the RDT. For a positive test the odds of a positive likelihood ratio were 8.3 and 4.3 for microscopy and RDT respectively. The odds of the negative likelihood ratio were similar for both tests (0.04).

Incision and Impression smears against histology: The results are presented in Table 8:3. Compared to histopathology the sensitivity of incision smears and impression smears was 62.2% and 44.7% respectively, and the specificity was >99% for both. The positive and negative predictive values of both tests were comparable. The odds of positive likelihood ratios were 7.6 and 8.5 for incision and impression smears respectively. The corresponding odds of a negative likelihood ratio were 0.03 and 0.05 respectively.

TABLE 8: 2: COMPARISON OF PERIPHERAL BLOOD MICROSCOPY AND PLDH-RDT WITH PLACENTAL HISTOLOGY IN DELIVERY UNIT WOMEN

Histology as Reference test							
Peripheral blood	**Histology**		**Test Performance**		**Likelihood Ratio & Posterior Probability**		
	Positive (n=38)	Negative (n=454)	Sensitivity (95%CI)	Specificity (95%CI)		Positive test	Negative test
Maternal Microscopy n = 482							
Positive	25	3	65.7 (48.5-79.8)	99.3 (97.8-99.8)	Likelihood ratio (95% CI)	**+ LR** 97 (31-305)	**- LR** 0.34 (0.22-0.53)
Negative	13	441	**PPV** 89.2 (70.6-97.2)	**NPV** 97.1 (95.0-98.4)	Posterior probability (odds) (95% CI)	89% (8.3) (73% -96%)	3% (0.04) (2% -4%)
RDT pLDH n = 485							
Positive	17	4	48.5 (31.7-65.7)	99.1 (97.5-99.7)	Likelihood ratio (95% CI)	**+ LR** 55 (19-154)	**- LR** 0.52 (0.38-0.72)
Negative	18	446	**PPV** 80.9 (57.4-93.7)	**NPV** 96.1 (93.8-97.6)	Posterior probability (odds) (95% CI)	81% (4.3) (60% -92%)	4% (0.04) (3% -5%)

PPV = positive predictive value; NPV = negative predictive value; LR = likelihood ratio; CI= Confidence Interval

TABLE 8: 3: COMPARISON OF PLACENTAL INCISION AND IMPRESSION SMEARS WITH PLACENTAL HISTOLOGY IN DELIVERY UNIT WOMEN

Histology as Reference test							
Smear method	**Histology**		**Test Performance**		**Likelihood Ratio & Posterior Probability**		
	Positive (n=38)	Negative (n=454)	Sensitivity (95% CI)	Specificity (95% CI)		Positive test	Negative test
Incision smear *n = 486*							
Positive	23	3	62.2 (44.7-77.1)	99.3 (97.8-99.8)	Likelihood ratio (95% CI)	**+ LR** 93 (29-295)	**- LR** 0.38 (0.25-0.580)
Negative	14	446	**PPV** 88.5 (68.7-96.9)	**NPV** 96.9 (94.8-98.2)	Posterior probability (odds) (95% CI)	88% (7.6) (70% - 96%)	3% (0.03) (2% - 5%)
Impression smear *n = 482*							
Positive	17	2	44.7 (28.9-61.5)	99.5 (98.2-99.9)	Likelihood ratio (95% CI)	**+ LR** 99 (24-414)	**- LR** 0.56 (0.42-0.74)
Negative	21	442	**PPV** 89.4 (65.4-98.1)	**NPV** 95.4 (93.0- 97.1)	Posterior probability (odds) (95% CI)	89% (8.5) (67%-97%)	5% (0.05) (3% -6%)

PPV = positive predictive value; NPV = negative predictive value; LR = likelihood ratio; CI= Confidence Interval

8.4 Discussion

The performance of peripheral and placental blood microscopy and the First Response® pLDH-based RDT in diagnosing *P.falciparum* placental malaria in delivering women were determined against placental histology as the "gold standard". Peripheral blood microscopy showed a low sensitivity and this was even lower for the pLDH-based RDT (48.5% [95%CI 31.7-65.7%]). The false negative results might be explained by the fact that placental sequestration of *P.falciparum* limits peripheral circulation of parasites. In contrast, both tests had a high specificity (>99%).

This is a setting where peripherally patent infections were rarely negative by placental smears or histopathology (see chapter 5). In this situation, even though the sensitivity and specificity are important attributes of a test, the findings of the predictive values reflect the presence of placental infection, conditional to the diagnosis by microscopy or RDT. The high positive and negative predictive values of maternal microscopy suggest that in a large proportion of cases microscopy results predicted placental infection in this setting. In comparison, the positive predictive value of the pLDH-RDT was lower (80.9%) than that of maternal microscopy. Although this implies that the probability of missing placental infections was greater with the RDT, perhaps this also reflects a lower sensitivity of the pan-pLDH antigen unlike that of HRP-2 based RDTs in detecting *P.falciparum* infections (Singer, *et al* 2004). Predictive values of a test are dependent on the prevalence of disease in the population and therefore the diagnostic ability of a test may differ from one region to another.

The likelihood ratios, which do not depend on the prevalence of a disease, indicated that the ability of the peripheral blood tests to diagnose placental infection was modest. For example, the 8.3 odds of a positive likelihood ratio of microscopy indicate that with a positive test the diagnostic likelihood of placental infection increased eight fold. Likewise, a woman with a negative microscopic result has 0.04 odds of having placental malaria. These findings suggest that although the sensitivity of the tests were low, with a positive microscopy the likelihood of placental malaria is increased, while with a negative microscopy the probability of placental infection was low.

Incision and impression smears were assessed as alternatives to placental histopathology in detecting placental malaria infection. As expected, both tests had a lower sensitivity relative to histopathology. Incision smear samples were drawn from a superficial cut made in the placenta and therefore might not have picked up parasitized cells sequestered deeply within the placental tissue (Brabin, *et al* 2004). Despite the low sensitivity, impression smears identify mature stages of different plasmodium species within the placenta (Taylor 2009) which is not the case with histopathology. In this study impression smear samples consisted of a small tissue taken from a

single area. This approach perhaps might have been deficient in capturing the scanty distribution of sequestered parasites seen in this setting (see chapter 5) and may explain the increased false negative results observed with impression smears. Nonetheless, the high specificity (99.5%) suggests that nearly nine out of ten times impression smears detected negative placental infection relative to histopathology. Likewise the positive predictive value results also showed that with a positive impression smear there was a high probability of placental infection being present. In addition, the positive odds (8.5) of the diagnostic likelihood ratio suggest that the infection being present in the placenta increased eight-fold with a positive impression smear. Translating this into a probability of infection, there was an 89% probability of placental infection given a positive impression smear, whilst with a negative result there was only a 5% probability of placental infection. Thus the likelihood ratio result suggests that detection of placental infections by impression smears is slightly higher than incision smears.

A deficiency of comparative studies on these different methods limits comparison of our findings. The sensitivity of incision smear microscopy relative to placental histology found in our study is comparable with results reported from the study in Malawi (Rogerson, *et al* 2003a). With regards to the pLDH-RDT, comparison is also limited because previous studies mainly focused on the ability of HRP-2 RDTs in detecting placental malaria (Mockenhaupt, *et al* 2002, Singer, *et al* 2004). However, the pLDH-RDT findings of 48.5% sensitivity for placental malaria and 71.7% for maternal malaria are comparable to those reported for Optimal (pLDH-RDT) in the study from Malawi, although transmission levels were different for the two regions (Mankhambo, *et al* 2002). A study from Kenya has also shown that histology was more sensitive than placental or peripheral smears in detection of placental malaria (Shulman, *et al* 2001).

In conclusion, this study demonstrated that despite a lower sensitivity, the diagnostic performance of impression smear in terms of likelihood ratios had a higher accuracy in detecting *P.falciparum* placental infection than incision smears. Future studies in low transmission settings should consider sampling of impression smears from multiple sites of the placenta to determine whether sensitivity can be improved. The study also showed that in this setting peripheral blood microscopy could be useful in predicting placental infections whereas the accuracy of pLDH-RDT (First Response®) was lower for the detection placental infections. Further studies testing pLDH based RDT on direct placental blood samples and in different epidemiological settings are needed to confirm its diagnostic accuracy of placental infections.

Chapter 9

General discussion and recommendations

"After great pain, a formal feeling comes"
-Emily Dickenson-

9.1 General Discussion

The work presented in this thesis addresses several important topics of malaria in pregnancy in a low transmission region in India. The main study systematically estimated the clinical burden of malaria in pregnancy in a sample of 4552 pregnant women in a rural and urban setting in the central Indian state of Madhya Pradesh. The large sample size allowed precise, site and species specific estimates of the prevalence of malaria by season. The burden estimates are strengthened by histological findings which showed that placental infection was associated with a considerable impact on maternal anaemia and low birth weight even though the malaria associated histological changes in the placenta were mild. The histological changes observed in this study contrast to the findings in stable high transmission areas and add to the limited data on placental histopathology from low transmission regions in Asia. Furthermore, the demonstration of a significant proportion of sub-clinical (asymptomatic) placental infections stresses the importance of placental histology in burden assessment studies. Another unexpected finding was the high prevalence of low-grade sub-microscopic infections detected by PCR in pregnant women attending antenatal care. This finding has important implications for the prevention of malaria in pregnancy in this population. An additional sub-study which assessed the accuracy of a pLDH based RDT (First Response®) found that this RDT was as good as microscopy in screening antenatal women for malaria and suggests it may be a practical test for use in the new intermittent screening and treatment strategy that is currently under evaluation in India. However, the secondary PCR evaluation also highlighted the challenges of assessing new RDTs for screening purposes when using microscopy as the 'gold-standard' at this low range of parasite densities. A secondary analysis of low birth weight showed that preterm-LBW and IUGR-LBW represent two conditions reflecting two different aetiologies, each with distinct amendable determinants highlighting the potential impact on low birth weight by targeting the amendable causes of both causes of low birth weight. This analytical approach has not been used widely in previous studies in India.

In the recently revised national malaria control policy in India there is no specific prevention guideline for malaria in pregnancy (NVBDCP 2010). Given the scarcity of data, this may reflect a lack of data or awareness of the burden of malaria pregnancy. The limited information about the appropriate intervention options for these low transmission areas are also seen by the fact that only 1 out of 16 drug-based prevention trials and 1 out of 5 ITN trials were conducted in Asia (**chapter 2**) (Gamble, *et al* 2006, Garner and Gulmezoglu 2006). This thesis contributes much needed knowledge to map the burden of malaria in pregnancy in India and in

the low transmission regions of south-east Asia, and to help formulate preventive strategies in the Asian region.

9.1.1 Malaria prevalence and risk groups

The overall prevalence of malaria in pregnancy in Madhya Pradesh when assessed by microscopy or RDT was low and highly seasonal (**chapter 4**). In antenatal women, the prevalence of these patent infections was 1.9 % in the dry season and 6.5% in the peak transmission season (post-monsoon). The corresponding prevalence of maternal malaria in women at delivery was 0.8% and 3.3% respectively. *P.falciparum* was the predominant species (89.0%) with transmission dominant for 3 months between October and December consistent with previous observations (Singh, *et al* 2000, N. Singh, *et al* 2004). However, unlike previous studies, *P.vivax* showed a low prevalence with little seasonal variation throughout the year suggesting possible relapses of hypnozoites rather than evidence of continued *P.vivax* transmission during the dry season. An increase in *P.vivax* relapses in pregnant women has been reported in the Thai-Burmese border (Nosten, *et al* 1999). Previous studies in Madhya Pradesh were based on data from symptomatic women presenting to hospitals (Singh, *et al* 1999a, Singh, *et al* 1995).

In contrast to previous observations from malaria endemic Africa, women of all gravidities and ages were equally affected. Differences with previously reported malaria rates from this region are more likely caused by differences in surveillance methodology. Malaria surveillance has generally focused on slide positivity rates ('prevalence') using passive screening of febrile patients who seek health care, whereas the current survey used active screening process in a random selection of (generally healthy) pregnant women. The transmission pattern observed in this study suggests that during the greater part of the year there is very low transmission for women to develop substantial malarial immunity prior to pregnancy and between pregnancies to acquire sufficient immunity to provide protection during subsequent pregnancies. This may also suggest the potential for outbreaks and epidemics which has been reported to occur in this and other regions that have shown an absence of gravidity specific immunity (Singh, *et al* 1988, Wickramasuriya 1937).

Malaria was five times more likely to occur in women from the rural areas than in urban areas and almost seven times more likely to occur in women of the Scheduled Tribe than other caste categories **(chapter 4)**. Although the prevalence variation seen between sites was greater than expected it also reflects the local pattern of transmission and the heterogeinity of the study population. The Scheduled Tribe lives mainly in the forested foothill regions within the

catchment area of the rural hospitals included in the study, which are areas where transmission is reported to be higher in India (Sharma, *et al* 2006, Singh, *et al* 2003).

Considering the overall low prevalence of infection, it is important to identify the women most at risk of malaria to guide targeted screening and prevention. The compilation of infection predictive index showed an increase with the concomitant presence of multiple predictive factors and was as high as 45% among women with all 5 factors present. Although they represented only a small proportion of the overall sample of infected women (23/51), some of these predictive factors may be useful if India would consider targeted screening programs for malaria as part of antenatal care instead of universal screening.

9.1.2 Effect of *P.falciparum* infection during pregnancy

Although the risk of malaria was low, the clinical implications of microscopic infection were substantial (**chapter 4**). Most, but not all, infections detected by microscopy during pregnancy or at delivery were symptomatic and associated with either a documented fever or history of fever in the previous week; *P.falciparum* (63.0%) and *P.vivax* (55.0%). The fever threshold was relatively low; 26.4% of infected women were symptomatic at parasite densities <499/μL. In contrast, observations in African adults describe that fever symptoms appear at parasite thresholds exceeding 10,000parasites/mm^3 (Delley, *et al* 2000). One study in the Democratic Republic of Congo showed that <14% of those above 15 years of age were febrile at parasite density <2000/μL (Ndounga, *et al* 2008). Thus, prevention or early detection of infection would be important to avoid infections progressing and becoming symptomatic as this is associated with preterm labour in the study population.

Anaemia (Hb <11.0g/dL) was common. Although the proportion of women who reported taking iron and folic acid supplement was high (86.8%), nearly one in five women had moderate to severe anaemia at delivery. The risk of moderate-severe anaemia was much greater in women with placental or peripheral *P.falciparum* infections and the mean haemoglobin was significantly lower in infected women (1.5g). Because of the high 'background' prevalence of moderate to severe anaemia in this population, this additional effect of malaria may result in clinically relevant reductions in absolute haemoglobin to levels that are likely to affect adversely the health of mother and newborn (Brabin, *et al* 2001a, Shulman, *et al* 2002). Nevertheless, the proportion associated with malaria and that could potentially be prevented by successful malaria control was modest (8.4%), reflecting clearly, aetiologies other than malaria such as chronic infections, geohelminths and nutritional causes predominate in this population (Agarwal 2005).

Few malaria studies in Asia have assessed the gestational age of babies and prematurity when reporting malaria associated low birth weight. In this study, most of the malaria associated

low birth weight was associated primarily with preterm birth rather than fetal growth restriction. *P.falciparum* was associated with an average shortening of the duration of gestation by 8 days and associated with a 3.4 fold increased risk of preterm births. Because the mean gestational age was only 37.2 weeks, the additional reduction of 8 days is likely to have an impact on the health of the newborn. The malaria associated reduction in mean birth weight was 487g (95% CI 250-722) among women with malaria, amounting to approximately 20% of the average birth weight (2656g), which is much larger than the observed effect in areas of stable transmission in Africa, where the average reduction in birth weight is 150g (Guyatt and Snow 2004). Acute infections associated with fever may in part explain the effect of *P.falciparum* associated preterm-LBW. We hypothesize that many infections, once they become patent, may be relatively short-lived because they become symptomatic and trigger preterm labour. Infections are thus less likely to become chronic. This is consistent with the observations that acute placental infections were more common than chronic or past infections and consistent with the relatively mild malaria associated histological changes observed in the placentas of infected women (**chapter 5**). These findings contrasts with observations in sub-Saharan Africa where almost all infections remain asymptomatic with chronic placental malaria associated fetal growth restriction being more common (Ismail, *et al* 2000, Menendez, *et al* 2000).

The high impact of placental infection is also illustrated in the high attributable fraction estimates for low birth weight (50%) and preterm birth (70%) among women with *P.falciparum* infected placentas. Nevertheless, even though the impact in the individual was large, patent malaria explained only a small fraction of the observed prevalence of low birth weight and preterm births at population level (PAF 4.6% and 6.6% respectively) (**chapter 4**). The development of control programmes that focus primarily on malaria control are therefore unlikely to have a major impact on these parameters in this population.

9.1.3 Effect of *P.vivax* infection

P.vivax was associated with a smaller impact on birth weight (126g), but this difference was not statistically significant. Although the small number of *P.vivax* mono-infections observed makes it difficult to draw firm conclusions about the impact, the findings are consistent with those from Thailand and Indonesia (Nosten, *et al* 1999, Poespoprodjo, *et al* 2008). *P.vivax* infection was significantly associated with a documented fever or history of fever in the previous week. These findings suggest that despite low level of transmission, *P.vivax* contributes to the burden of malaria associated morbidity.

9.1.4 Sub-microscopic infection

In areas of low transmission the frequency and impact of sub-microscopic infections is not clear. The general assumption is that asymptomatic sub-microscopic infections in pregnancy must be rare in Asia as individuals with low levels of acquired immunity may not be able to contain parasitaemia below patent levels. Contrary to this dogma, a surprise finding of this thesis work was a much higher than expected prevalence of sub-microscopic infection detected by PCR (**chapters 4 & 7**). One-in-five antenatal women with a negative RDT and malaria smear had a malaria infection detected by PCR. Likewise, placental histology also detected a high prevalence of sub-microscopic infections among the smear negative women, increasing the prevalence of malaria five-fold compared to that detected by standard microscopy alone (**chapter 5**). Most of these sub-microscopic infections were not (yet) associated with fever and whether they become patent later or they can persist undetected is uncertain. Our observations do suggest that sub-microscopic low density infections are much more common than generally appreciated in populations with low levels of malarial immunity. It is possible that this feature is unique to pregnancy, although this remains to be explored. It might suggest that the presence of the placenta allows accumulation of different infections over time.

Importantly, these sub-microscopic placental infections were associated with a (non-significant decrease) in haemoglobin in women attending ANC and in birth weight (207g) among women in the delivery module (**chapters 4 & 5**). In a recent, malaria in pregnancy burden study in Indonesia, we also found that both maternal and placental sub-microscopic infections detected by PCR were common and associated with maternal anaemia and low birth weight, although less marked than patent infections (work we conducted with Indonesian colleagues, unpublished). In the Indonesian study, a two-fold risk of low birth weight in women with sub-patent placental *P.falciparum* infection was observed compared with uninfected women (RR 1.9, 95%CI 0.7-4.7). Similarly, sub-patent *P.vivax* infection was also associated with low birth weight (RR 3.4, 95%CI 1.6-7.4). If the impact of sub-microscopic infections is accounted for, the burden of malaria in pregnancy maybe considerably higher than can be estimated from patent infections. Thus in future burden studies, it is important to investigate sub-microscopic infections in these low transmission areas. Furthermore, they should be taken into account in defining the optimal strategy for the control of malaria in pregnancy in this population and Asian region (see recommendations below).

9.1.5 Screening of pregnant women for malaria with RDTs

Because of the association between malaria infection, fever, and preterm delivery, it is likely that early detection of infection in pregnancy, prior to them becoming symptomatic, is going to be more effective than passive case detection (PCD) in preterm low birth weight. RDTs are a practical tool for screening pregnant women, although very few studies have assessed the performance of RDTs in the screening of mostly asymptomatic pregnant women (Tagbor, *et al* 2008b). Most of the previous studies were conducted in symptomatic patients who are likely to have significantly higher parasite densities. The evaluation of the First Response pLDH based RDT suggested that the RDT was as good as microscopy to detect low density *P.falciparum* infections (>100/μl) and therefore has great potential as an alternative to microscopy for screening of populations (**chapter 7**). However, the RDT sensitivity for the detection of *P.vivax* was much lower compared with microscopy. More studies are needed to assess the performance of the newer Pan-species based RDT developed by this and other manufacturers for the detection of *P.vivax*.

A constraint of screening for malaria using peripheral blood only is that placenta infections could be missed or that parasites may circulate below the detection threshold (Brabin, *et al* 2004). Although, RDTs were not inferior to microscopy in detecting *P.falciparum* infections, the sensitivity of both RDT and microscopy was very low when compared to PCR or histology (**chapter 8**). However, neither of these can be used for point of care. Until more sensitive methods are available to detect placental infections antenatally, the First Response pLDH based RDT (or equivalent alternative RDTs) is a practical alternative to microscopy. Thus, with the growing interest in the use of screening and treatment as a control option of malaria in pregnancy, RDTs are likely to become the preferred test in peripheral settings where good quality microscopy is difficult to maintain.

The 'discrepant resolution' approach used in this study highlighted the importance of the reference test and analytical method used to assess the accuracy of the RDT (**chapter 7**). Although WHO-TDR recommends testing both discordant and concordant samples with a third test only few studies have used this method (Bell, *et al* 2005, Hopkins, *et al* 2007). This study illustrated how informative a random sample of concordant negatives could be in diagnostic studies that use an imperfect gold standard such as microscopy. This is especially important in studies that are designed to evaluate new diagnostic tests for screening purposes rather than in the diagnosis of symptomatic patients, as many symptomatic infections may have densities around the threshold in the detection level for microscopy.

We also evaluated the performance of the less commonly used impression smears in the diagnosis of placental malaria. Impression smears were found to be useful to identify mature

stages in the placenta and for species diagnosis, which cannot be done with certainty by histopathology. However, the test sensitivity was low even though it had a high positive likelihood ratio (LR +99), (**chapter 8**). As expected placental histology was the superior microscopic method to diagnose placental infections (Rogerson, *et al* 2003a).

9.1.6 Determinants of low birth weight

Low birth weight was common (33.8%) and consistent with previously reported estimates in India (Lawn, *et al* 2005, UNICEF-WHO. 2004). The determinants of low birth weight were explored as two sub-groups; preterm-LBW and IUGR-LBW (**chapter 6**). This approach takes into account the etiological heterogeneity of low birth weight in recognition that low birth weight may have two distinct determinants profile associated with the aetiology of preterm-LBW versus IUGR-LBW. Preterm birth was mainly associated with acute morbidity conditions such as malaria, fever, moderate-severe anaemia and showed a significant seasonal variation. The physical labour exerted during the harvest season together with maternal malnutrition and malaria might explain the seasonality of preterm births. In contrast, IUGR-LBW was associated with chronic conditions such as socioeconomic status, maternal stature and non-antenatal attendance. These findings also implied that not all low birth weight reflects pathology. On further exploring the birth weight distribution it was seen that a large proportion of low birth weight babies were constitutionally small reflecting the small stature of the women in this population. This was further evident by the fact that a lower median birth weight was observed in term babies compared to the WHO reference curve. For example, the 50th percentile at 37 weeks of the Fenton curve (3000g) corresponded to the 90th percentile in this cohort, indicating that only 10% of babies had a birth weight of 3000g or more. Additionally, the excess of small preterm-LBW reflecting the pathological group (<1750g) observed in the left lower tail of the Wilcox method of birth weight distribution curve was small (1.1%). Nevertheless, from a public health perspective prevention of preterm birth is important. Since the main risk factors associated with preterm births are acute amendable conditions, prevention programmes targeting these conditions are likely to show the highest impact in the reduction of low birth weight.

9.2 Recommendations

9.2.1 Control of malaria in pregnancy

Despite an increasing global awareness of the public health impact of malaria in pregnancy in Asia, malaria control in pregnancy in this region has made little progress over the last few decades, including in India. At the time the study was conducted the national policy was chloroquine chemoprophylaxis in pregnancy (**chapter 1**). The newly revised national guideline still advises chemoprophylaxis in high *P.falciparum* endemic regions of India with a statement to encourage use of personal protection measures such as ITN and insect repellents (NVBDCP 2010). In India, case management strategies however have seen a significant improvement in the recent years, with the introduction of ACTs for first line treatment from second trimester onwards and quinine in first trimester, with chloroquine for *P.vivax* cases.

Designing a cost-effective country wide control strategy might be a challenge and not the optimal approach for India because of variability in transmission and existence of both *P.falciparum* and *P.vivax*. Madhya Pradesh is one of the highly malaria endemic states according to the Indian classification API >5. Similarly, Jharkand state which is also considered a high malaria endemic state found a prevalence of 1.7% and 2.4% among antenatal and delivery unit women (Hamer, *et al* 2009). These two studies suggest that in most of the malaria endemic belt in India a comparably low transmission could prevail with similar effects on pregnant women as observed in Madhya Pradesh and Jharkhand. At this point more burden studies may not be needed in India except in the known high transmission region of Assam and with a focus on further determining the ratio of sub-microscopic versus microscopic malaria. In future, burden studies will be needed after implementation of effective control measures to monitor the control efforts.

Determining a suitable intervention strategy however remains a challenge for India. The current practice in most Asian countries is the WHO recommended policy of passive case detection and management. The strong association between preterm birth and acute placental infection, combined with the surprisingly high prevalence of sub-microscopic infections, almost all of which and one third of the patent infections were asymptomatic, suggests that passive case detection strategies will not be sufficient to control malaria in this setting. Furthermore, the heterogeneity observed between sites and season suggests policy development should move away from a one size fit all frame work to develop different control strategies for different areas in Madhya Pradesh. For example, IPTp may be suitable for Maihar the rural site where the prevalence of malaria in antenatal women in transmission season was high (20.7%) whereas ISTp may be appropriate for Katni, while in Jabalpur, the urban site with 0.3% prevalence of malaria, passive case detection or ISTp could be an option.

9.2.2 Different Control Strategies

The two main intervention options involve IPTp or scheduled screening and treatment strategies involving RDTs and long acting ACT. Active screening strategies can be provided either as a single screening and treatment (SSTp) or as intermittent screening and treatment (ISTp).

Single Screening and Treatment: The SSTp was piloted and formally introduced in Indonesia in 2010. This strategy consists of all pregnant women being screened with either an RDT or microscopy at first antenatal visit and women found positive receive treatment with an ACT. On subsequent visits only symptomatic women get tested and if found positive receive malaria treatment. A limitation of this strategy is the lack of prevention of re-infections later in pregnancy.

Intermittent Screening and Treatment: The second screening strategy is the concept of ISTp to provide scheduled screening (3-4 times) for malaria, using an RDT and treating the RDT positive women with a long acting ACT, with the aim of clearing existing infections and providing additional post-treatment prophylaxis for three to six weeks. The screening events with RDT 4 to 8 weeks apart use the same schedule as focused antenatal care (FANC). Because only women with confirmed malaria receive ACTs, the potential advantage of this strategy is that drug exposure is minimized and restricted to parasitaemic individuals.

'Combo' RDTs that combine HRP2 and pLDH based antigen should be used in these screening programmes. Although they are more expensive than single antigen based RDTs, they have the potential advantage over pan-species pLDH-only RDTs in that the additional HRP2 component increases the test sensitivity needed to detect *P.falciparum* infections, while having all the benefits of the pLDH based tests.

The potential disadvantages of screening strategies are the non-adherence by health care providers to follow up or use test results and it is likely to be more expensive to implement than other intervention strategies. Another disadvantage of any screening strategy involving RDTs and microscopy is that with the current sensitivity of RDTs, low density infections will be missed.

Intermittent Preventive Treatment: Since the prevalence and adverse impact of sub-microscopic infections are much more common than previously appreciated, other intervention approaches using IPTp should also be considered. The concept of IPTp was first investigated and implemented on areas with moderate to high transmission, where most women develop significant levels of protective immunity and are able to control the infections. Low grade infections are common in high transmission settings, many of which can remain sub-patent (PCR

positive, but below the level of detection by microscopy) and may not be associated with adverse consequences, particularly in multigravidae. IPTp has not yet been evaluated in Asia which was evident in a recent systemic review of IPTp trials which illustrated that no IPTp studies were conducted outside of the African region (ter Kuile, *et al* 2007). It is unclear at what transmission level IPTp versus ISTp should be introduced. IPTp has not been recommended in low transmission areas because its potential benefits are likely to be more limited (Newman, *et al* 2003, Parise, *et al* 2003). Secondly, the only drug evaluated for IPTp is sulfadoxine-pyrimethamine and there is a perceived lack of effect of SP on *P.vivax* and lack of data on a suitable alternative. Thus, IPTp trials should be considered including women of all gravidity, preferably with a long acting drug that targets all species such as mefloquine, piperaquine (with or without artemisinin component, based on the availability of mono-therapy).

Seasonal Intermittent Preventive Treatment: Since Madhya Pradesh has a short seasonal transmission period, seasonal IPTp could be worth considering for the rural sites. Seasonal IPT has not been explored in pregnant women, however trials in Mali and Senegal has shown this strategy to be effective in children (Dicko, *et al* 2008, Sokhna, *et al* 2008). Since the start and the duration of transmission season is known, IPTp introduction could be timed to correspond with the rains. Nevertheless, the translation of such a seasonal strategy into practice may be complex, with significant logistical challenges.

Vector control measures: Other malaria control strategies to consider for this region are vector control measures with ITNs and IRS. ITN distribution is a choice likely to be acceptable by the women since there is already a high use of non-treated nets among women in this region (41.0%). However, ITN alone may not be as effective as observed in Africa because the predominant vectors (*A.fluviatilis* and *A.culicifacies*) in this region are mainly dusk biters and are exophilic and zoophilic. IRS is currently one of the three main interventions of the WHO Global Malaria Programme and is suited for focal endemic and epidemic areas. As transmission in Madhya Pradesh is low and is prone to outbreaks, this suits IRS. However, the two predominant vectors in Madhya Pradesh are reported to be resistant to all insecticides including DDT and pose a real challenge to the use of IRS in this region (NIMR 2010a).

9.2.2 Maternal health measures to improve low birth weight

Prevention of low birth weight is crucial to achieve an impact on the health of the baby. Antenatal care provides an opportunity to incorporate low birth weight prevention programmes. These should include efforts to improve maternal malnutrition which was a major determinant of

both preterm-LBW and IUGR-LBW. Programmes targeted to improve pre-pregnancy weight and weight gain during pregnancy is likely to show an impact. Antenatal care could be used for multi-pronged approaches with activities such as screening for anaemia and malaria, provision of haematinic supplementation, advice on maternal nutrition and benefit of good nutrition, malaria protection measures and distribution of LLITN integrated into the current focused antenatal care. Since preterm births were also higher in the harvest season when there is more physical activity, adequate rest and less exertion is likely to show an impact (Rao, *et al* 2009). Advice for change of such behavioural activities and nutrition improvement could also be given utilising the existing ASHA programme. To have a greater effect, a community based approach could be employed for specific food based strategies and infection control measures such as benefit of prevention and treatment of malaria in pregnancy. These should also include provision of micronutrients, iron and folate to the pregnant which has been shown to have an impact on the weight of the baby (Christian 2010).

9.3 Future Research

The findings in this thesis have helped to identify the interventions that should be assessed to control malaria in pregnancy in this setting, and have highlighted knowledge gaps which need to be addressed. Information on the potential role of all three interventions; SSTp, ISTp and IPTp discussed above are needed in India. Trials are ongoing in parts of India comparing passive case detection with ISTp using RDTs and SP-artesunate under the umbrella of Malaria in Pregnancy Consortium (MiPc 2010). A one size fit all policy may not be appropriate and all three interventions may have role for different regions in Madhya Pradesh. The research priority for Madhya Pradesh should be to determine if IPTp with a long acting ACT versus ISTp with RDT provided as part of focused antenatal care to women of all gravidity is more effective than the current PCD strategy in decreasing the impact of malaria in pregnancy. The type of RDT used is also important and therefore HRP2- Pan pLDH combination RDT which has the capability to capture both *P.falciparum* and *P.vivax* should be considered. If the RDTs are more sensitive and capable of detecting infections at lower parasite threshold, it could influence the decision on whether IPTp or ISTp would be required. Alongside this trial a study to evaluate the acceptability, feasibility and cost effectiveness of IPTp and ISTp should be conducted.

The scarcity of data on sub-microscopic infections in pregnancy warrants further studies to determine the impact of these infections. Additional studies are also needed on *P.vivax* to help define prevention strategies of *P.vivax* relapse in particular. It would also be of benefit to follow up preterm-LBW and IUGR-LBW to determine their outcome after discharge.

9.4 Conclusion

The overall prevalence of microscopically detectable malaria in Madhya Pradesh was low with wide variation between urban and rural sites and markedly seasonal with a predominance of *P.falciparum*. The persistence of *P.vivax* throughout the year probably reflects the relapse of the liver stages rather than year round transmission. In contrast to malaria endemic Africa, many, but not all infections were associated with fever. Maternal anaemia and low birth weight, primarily due to preterm births were the main adverse consequences. Severe parasite sequestration and associated changes with *P.falciparum* infections in the placenta as observed in Africa were not seen, yet, there were placental sub-clinical infections. An unexpectedly high prevalence of sub-microscopic infections was also detected by PCR in antenatal women and this was associated with increased risk of anaemia. This suggests that the risk of exposure and the burden of malaria in pregnancy in Madhya Pradesh may be much higher than previously appreciated and the presence of sub-microscopic infections should be taken into account in planning appropriate control measures in this region.

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Annexes

Annexe1: Consent forms

Consent form - antenatal clinic study

Readability level = (Flesch-Kincaid = 7.6).

Informed consent form for women in antenatal clinic

Assessment of Malaria in Pregnancy

Introduction

The Malaria Research Center in Jabalpur is doing a study in this area to find out how many pregnant women in this District have malaria. If you have malaria when pregnant it may cause some harm to you and to your baby. Malaria causes fever, it makes you feel sick and you may have less blood. Malaria in pregnant women can also cause the baby to grow less well. Sometimes pregnant women may have malaria without feeling sick. We can find out if you have malaria or less blood by doing simple blood tests. We will study the problem of malaria in about 1300 women in antenatal clinics in this District.

Purpose of the Research

We plan to check women's blood for malaria to know how many women in the District are infected. This will help us plan ways to lower the harmful effects of malaria in pregnant women and their babies.

Procedures

If you agree to take part we will ask you about yourself, your home and your health during pregnancy. We also would like to know about medicines you take/ took during pregnancy and what you do to stop mosquitoes from biting. We will note some information about your pregnancies from your clinic card. We will then check your temperature, and take finger prick blood to test for malaria and less blood. A team member will take few drops of blood from your finger tip. The staff will first clean your finger and use a sterile pricker to get blood. The first drop of blood will be wiped off. The next few drops will be used to check if you have less blood and to test for malaria. These tests will help us to learn more about how to best treat malaria in India in the future. We will not test your blood for other diseases. This will take about 20 minutes.

All your answers and test results will remain private and safe to the extent the law allows. If you agree to take part but do not want blood taken you may refuse it. You can also refuse to answer any question that you do not want to. Even if you refuse to take part today, you will have your usual clinic check and will be able to get the available health care now and in the future. As part of that standard available care you would only be screened and treated for malaria if you have symptoms.

Benefits

If we find that you have malaria we will treat you with a medicine called Fansidar. If you have less blood we will give you iron and folic acid tablets. If we think you may need other treatment we will refer you to the clinic staff. They will be able to give you the needed treatment. All the tests and medicines given by us for malaria and less blood will be free.

Risks or discomforts

We are always concerned about you and your baby's health. We therefore would like to make you aware of any bad effects that might happen. With the finger prick you will feel a pinch that may hurt for a few seconds. Rarely a small bruise may appear on the point from where we take blood. This usually goes away without any treatment. If you are shown to have malaria or less blood, we will give you medicines. The medicines we give you in pregnancy are proven safe and should not cause any harm to you or to the baby. However, if you feel any discomfort while taking the drugs you should contact the hospital. They can advise you on what to do.

If you have any questions about the study, please feel free to ask us now or later. If you have a query later about any aspect of this study, you may contact Dr. AP Shukla at MRC, Field Station, Jabalpur by telephone no: xxx. You may also contact Dr SB Awadhia (for Maihar), Dr.Shashi Khare (for Medical College in Jabalpur), or Dr.YS Parihar (for Katni) for questions about the study and Mr/Mrs. xxx with the human subjects department if you feel you have been harmed by the study.

Thank you very much for your time. Would you like to join in the study?

Participant's statement (signature or thumb print needed on line):
The above has been explained to me and:
I agree to join
Participant's name: _____ Date: __/__/

For women who cannot sign their names:
Witness' statement:
I verify that the participant was read the consent regarding the study and that any questions were answered. The participant agreed to be in the study:

Witness

Consent form - delivery unit study

Readability level = (Flesch-Kincaid = 7.6).

Informed consent form for women who attend the delivery unit

Assessment of Malaria in Pregnancy

Introduction

The Malaria Research Center in Jabalpur is doing a study in this area to find out how many pregnant women in this District have malaria. If you have malaria when pregnant it may cause some harm to you and to your baby. Malaria causes fever, it makes you feel sick and you may have less blood. Sometimes pregnant women may have malaria without feeling sick. When you are pregnant and you have malaria, the baby can be born small and weak even if you have not felt sick. The only way for us to know how many women may have this problem is to check a woman's blood when she delivers and the blood from the placenta after a woman gives birth. We plan to study the problem of malaria in about 750 women in this District.

Purpose of the research

We plan to check blood from you and the placenta to know how many women in the District are infected. This may help us plan ways to lower the harmful effects of malaria in pregnant women and their babies.

Procedures

If you agree to take part we will first ask you about yourself, your home and your health during pregnancy. We also would like to know about medicines you take/ took during pregnancy and what you do to stop mosquitoes from biting. We will note some information about your pregnancies from your clinic card. We will then check your temperature and your height. If you agree, we will take a few drops of blood from a finger stick to check your blood for malaria. After you give birth, we will weigh and examine your baby. We will also be looking for malaria in the placenta after your delivery. Before you have the baby, it will take about 10 minutes for us to do a finger prick to take blood. After you have the baby, it will take about one half hour for us to ask you questions, check your temperature, and measure your height. It will take about 10 minutes to weigh and examine the baby. If you are shown to have malaria or less blood, we will give you medicines.

All your answers and test results will remain private and safe to the extent the law allows. If you agree to take part but do not want blood taken you may refuse it. You can also refuse to answer any question that you do not want to. Even if you refuse to take part today, you will have your usual clinic check and will be able to get the available health care now and in the future. You would only be screened and treated for malaria if you have symptoms.

Benefits

You will be treated if you have malaria after birth. If you have less blood we will give you iron and folic acid tablets. If we think you may need other treatment we will refer you to the clinic staff. They will be able

to give you the needed treatment. All the tests and medicines given by us for malaria and less blood will be free.

Risks or discomforts

We are always concerned about you and your baby's health. We therefore would like to make you aware of any bad effects that might happen. With the finger prick you will feel a pinch that may hurt for a few seconds. Rarely a small bruise may appear on the point from where we take blood. This usually goes away without any treatment. If you are shown to have malaria or less blood, we will give you medicines. The medicines we give you in pregnancy are proven safe and should not cause any harm to you or to the baby. However, if you feel any discomfort while taking the drugs you should contact the hospital. They can advise you on what to do.

If you have any questions about the study, please feel free to ask us now or later. If you have a query later about any aspect of this study, you may contact Dr. A.P. Shukla at MRC, Field Station, Jabalpur by telephone no: ######. You may also contact Dr SB Awadhia (for Maihar), Dr.Shashi Khare (for Medical College in Jabalpur), or Dr.YS Parihar (for Katni) for questions about the study and Mr/Mrs. _____ with the human subjects department if you feel you have been harmed by the study.

Thank you very much for your time. Would you like to join in the study?

Participant's statement (signature or thumb print required):
The above has been explained to me and:
I agree for myself as well as my newborn to participate in the study
Participant's name: _____ Date: _/_/

For women who cannot sign their names:
Witness= statement:
I verify that the participant was read the consent regarding the study and that any questions were answered. The participant agreed to be in the study:
Witness

Consent form IPD component
Readability level = (Flesch-Kincaid = 7.3).

Informed Consent: Hospital ward, consent to access to Patient Record and Data Abstraction

Introduction: The MRC in Jabalpur is doing a study in pregnant women in this area. To do this, we need to study both pregnant and non-pregnant women. We want to know how many pregnant and non-pregnant women have malaria. We also want to learn about the harmful effects of malaria in pregnant and non-pregnant women. Pregnant women are more likely to get malaria than those who are not pregnant. As you may know malaria causes fever, makes you sick and you may have less blood. Having malaria during pregnancy may also affect the unborn baby.

Purpose of the research: If you allow us we would like to look at your hospital record. From this, we will collect facts about your illness. We also look at the results of tests done by your doctor. We may ask you a few questions about your illness. This is only needed when we can not find the facts from your record. We will not collect blood from you. We will not do extra tests.

Procedures: Knowing about your illness and test results will help us. It will show us if malaria causes more harm to pregnant women than to non-pregnant women. This study will help us to plan ways to lower the harmful effects of malaria in pregnant women and their babies.
All your test results and facts noted by us will remain private and safe to the extent the law allows. Even if you refuse us to check your record your care in hospital will not be affected.

Benefits and risks: As we only look at your hospital file, there are no known risks or benefits to you. This study is done to help plan ways to aid all Indian pregnant women in the future.

If you have any questions about the study, please feel free to ask us now or later. If you have a query later about any aspect of this study, you may contact Dr. Neeru Singh, O.I.C, at MRC, Field Station, Jabalpur by telephone no: 2370900 or 2371521. You may also contact Dr SB Awadhia (for Maihar), Dr. Shashi Khare (for Medical College in Jabalpur), or Dr. YS Parihar (for Katni) for questions about the study and Mr/Mrs. _____ with the human subjects department if you feel you have been harmed by the study.

Thank you very much for your time. Would you like to join in the study?

Participants's statement (signature or thumb print required):
The above has been explained to me and:

I agree to participate in the study

Mother's name: _____ Date: __/__/__

For women who cannot sign their names:

Witness= statement:

I verify that the participant was read the consent regarding the study and that any questions were answered. The participant agreed to be in the study:

Witness

Consent Form in Hindhi

सहमति फार्म :- प्रसवपूर्व क्लीनिक अध्ययन

महिलाओं के लिये प्रसवपूर्व क्लीनिक में सूचित सहमति फार्म

गर्भावस्था में मलेरिया का मूल्यांकन

परिचय

जबलपुर का मलेरिया अनुसंधान केन्द्र इस जिले में कितनी गर्भवती महिलाओं में मलेरिया है इस पर अध्ययन कर रहा है । यदि आपको मलेरिया है तो यह आपको और आपके होने वाले बच्चे का कुछ नुकसान कर सकता है । मलेरिया से बुखार होता है यह आपको अस्वास्थ कर देता है और आपके रक्त में अल्पता हो सकती है । गर्भावस्था में मलेरिया बच्चे के विकास में बाधा डाल सकता है । कभी-कभी गर्भवती महिलाओं में मलेरिया होते हुये भी वे बीमारी का अनुभव नहीं करती । हम आपके मलेरिया या रक्त की कमी के बारे में साधारण से परीक्षण करके जान सकते हैं । हम इस जिले में प्रसवपूर्व क्लीनिक में लगभग १३०० महिलाओं के उपर अध्ययन करेंगे ।

अनुसंधान का उद्देश्य

हम योजना के तहत जिले में गर्भवती महिलाओं का रक्त मलेरिया जाँच के लिये एकत्रित करेंगे । जिससे जानकारी मिलेगी कि कितनी गर्भवती महिलायें जिले में मलेरिया से पीड़ित हैं ।

कार्य पद्धति

यदि आप अध्ययन में भाग लेने को तैयार है तो हम आपके बारे में, आपके घर के बारे में, और आपके गर्भावस्था के दौरान स्वास्थ्य के बारे में पूछेंगे । हम यह जानना चाहेंगे कि कौन सी दवाईयाँ आपने गर्भावस्था के दौरान ग्रहण की । और मच्छरों के काटने से बचने के लिये क्या किया । हम लोग आपके क्लीनिक कार्ड से गर्भावस्था की कुछ सूचना एकत्र करेंगे । हम लोग आपका तापमान लेगें और मलेरिया परीक्षण हेतु आपकी उंगली को प्रिक (छेदन) करके रक्त लेंगे और रक्त अल्पता का परीक्षण भी करेंगे । एक टीम का सदस्य आपकी उंगली के उपरी हिस्से से कुछ रक्त लेगा । कर्मचारी पहले आपकी उंगली को साफ करेगा फिर जीवाणु रहित लिकर से रक्त प्राप्त करेंगा । पहली रक्त की बूंद को साफ कर देगा । दूसरी रक्त की बूंदों को मलेरिया परीक्षण व रक्त अल्पता के लिये जाँचा जायेगा । यह परीक्षण भारत में मलेरिया के उत्तम निदान हेतु सीखने में सहायक सिद्ध होगा । हम आपके रक्त दूसरे रोग हेतु परीक्षण नहीं करेंगे । इसमें लगभग बीस मिनट लगेंगे । आपके सारे उत्तर और परीक्षण परिणाम कानून की सीमा तक गुप्त व सुरक्षित रखें जायेंगे । यदि आप अध्ययन में सहमति देते हैं और रक्त को निकलवाना नहीं चाहते तो आप इन्कार कर सकते हैं । यहाँ तक कि आप अध्ययन में भाग लेने से मना कर सकते है तो भी आपकी

सामान्य क्लीनिक देखरेख होगी और जो भी उपलब्ध स्वास्थ्य सुविधायें अभी और भविष्य में प्राप्त होती रहेगी ।

लाभ

यदि हमें पता चलेगा कि आपको मलेरिया है तो हम आपको फेनसिडार नामक दवाई से इलाज करेंगे । यदि आपको रक्त अल्पता है तो हम आपको आयरन व फोलिक एसिड की गोलियाँ देंगे । यदि हम सोचेंगे कि आपको दूसरे इलाज की आवश्यकता है तो हम आपको क्लीनिक के कर्मचारियों को उल्लेखित कर देंगे वे आपको आवश्यक उपचार देने में समर्थ होंगे। हमारे द्वारा किये गये सारे परीक्षण एवं दवाईयां जो मलेरिया व रक्त अल्पता हेतु दी गई हैं नि:शुल्क होंगी ।

जोखिम और असुविधायें

हम हमेशा आप और आपके बच्चे के बारे में चिंतित हैं इसलिये कोई भी दुष्प्रभाव इन पर जो हो सकता है हम आपको सूचना देने हेतु तत्पर रहेंगे । उंगली छेदन के समय आपको चिकोटी जैसा अनुभव होगा जो कुछ समय तक रहेगा । एक हल्की खरोंच जहाँ से रक्त लिया गया है आ सकती है । यह सामान्यतः बिना उपचार के समाप्त हो जाती है । यदि आपको मलेरिया या रक्त अल्पता हुई तो हम आपकी दवाईयाँ देंगे । गर्भावस्था के दौरान हम जो दवाई आपको देंगे वह प्रमाणिक रूप से सुरक्षित हैं । यह आप या आपके होने वाले बच्चे को कोई हानि नहीं पहुँचायेगी । यदि दवाई ग्रहण करने के उपरान्त आपको कोइ असुविधा होती है तो आप अस्पताल से संपर्क कर सकते हैं । वे आपको क्या करना चाहिये इसकी सलाह दे सकते हैं । यदि आप अध्ययन से संबंधित कोई प्रश्न पूछना चाहते है तो आप स्वंतत्र रूप से अभी या बाद में पूछ सकते है । यदि आप अध्ययन से संबंधित कोई पूछताछ बाद में करना चाहे तो आप डॉ. एम.एम शुक्ला मलेरिया अनुसंधान केन्द्र टेलीफोन नंबर २६७२५०६ पर संपर्क कर सकते हैं, आप डॉ. एस.बी अवधिया (मैडर) डॉ शशि खरे (मेडीकल कालेज जबलपुर) या डॉ. आय.एस. परिहार (कटनी) से अध्ययन संबंधी प्रश्न पूछ सकते है । और यदि आपको लगता है कि आपको अध्ययन से कोई हानि हुई है तो और आप अध्यक्ष नैतिक समिती मलेरिया अनुसंधान केन्द्र दिल्ली से संपर्क कर सकते है और अपने अधिकारों के बारे में जान सकते हैं । आपने जो हमें समय दिया उसके लिये धन्यवाद । क्या आप अध्ययन में शामिल होने को तैयार है ।

भाग लेने वाले का कथन – (हस्ताक्षर या अंगूठे का निशान)

उपरोक्त विवरण मुझे समझा दिया गया है कि मै अध्ययन में शामिल होने हेतु स्वीकृति देता हूँ ।

भाग लेने वाले का नाम _____

दिनांक _____

भाग लेने वाले के हस्ताक्षर _____

अंगूठा निशान
(उनके लिये जो हस्ताक्षर नहीं कर सकते) _____

गवाह का कथन:

मै प्रमाणित करता हूँ कि अध्ययन में भाग लेने वाले ने सहमति फार्म देखकर अध्ययन में भाग लेने हेतु स्वीकृती दे दी है उसके सारे प्रश्नो के उत्तर दे दिये गये हैं । अध्ययन में भाग लेने हेतु वह सहमत है।

गवाह के हस्ताक्षर _____

दिनांक _____

Annexe 2: Antenatal Questionnaire

RAPID ASSESSMENT MALARIA IN PREGNANCY

ANTENATAL CLINIC QUESTIONNAIRE

गर्भावस्था में मलेरिया का त्वरित ऑकलन

प्रसव पूर्व देखभाल प्रश्नावली

1. Identification पहचान		
1.01	Date दिनांक	(dd/mm/yy)☐☐/ ☐☐☐/☐☐
1.02	Participant ID No: प्रतिभागी का पहचान कमांक *Facility/unit=A/ womans'no:* *सुविधा/इकाई=ए/महिला संख्या*	☐/☐/☐☐☐
1.03	Full Name पूरा नाम	.....
2. Personal Details Pregnant Woman गर्भवती महिला का व्यक्तिक विवरण		
2.01	What is your caste / tribe आपकी जाति क्या है?/जनजाति	SC ☐ ST ☐ GEN ☐ OBC ☐
2.02	Age (in years), *if age not known fill 99* उम्र (वर्ष में) यदि उम्र पता न हो तो 99 लिखें	☐☐
2.03	Age Group *(if precise age is not known)* आयु समूह (यदि सही उम्र पता न हो)	*15-19* ☐ *35-39* ☐ *20-24* ☐ *40-44* ☐ *25-29* ☐ *>44* ☐ *30-34* ☐
2.04	Village / Town Name गाँव / कसवा का नाम	..... rural ☐ urban ☐ ग्रामीण शहरी
2.05	Marital Status वैवाहिक स्थिति	married ☐ divorced ☐ widow☐ विवाहित तलाकशुदा विधवा
2.06	What language do you speak at home आप घर पर कौन सी भाषा बोलते हैं	hindi ☐ Other ☐ ..... हिन्दी अन्य
3. Socio-economic Indicators सामाजिक आर्थिक सूचकॉक		
3.01	Can you read? क्या आप पढ़ सकती हैं?	yes ☐ no☐ हाँ नहीं
3.02	What is the highest level of education that you finished? आपकी उच्चतम योग्यता क्या हैं? जहाँ से आपने पढ़ना बंद किया	no schooling ☐ vocational ☐ स्कूल नहीं गये व्यवहारिक primary ☐ higher ☐ प्राथमिक उच्चतर secondary ☐ माध्यमिक
3.03	What work does your husband do? आपके पति क्या करते हैं?	farming ☐ salaried job ☐ कृषि वेतनभोगी craftsman ☐ other ☐ दस्तकार अन्य
3.04	Does your household own farming land? क्या आपके मुखिया की स्वंय की कृज़ि भूमि है	yes ☐ no ☐ हाँ नहीं

3.05	Do any member of your household work on their own or family farmland क्या आपके घर का कोई व्यक्ति अपनी अथवा पारिवारिक कृजि भूम्य पर कार्य करता है	yes ☐ हाँ	no ☐ नहीं
3.06	In your household is there any of the following items *(If yes, mark boxes ✓)* क्या आपके घर में निम्न में से कोई वस्तु है? यदि हाँ, तो ✓ का निष्ठान लगायें	bicycle ☐ सायकल scooter ☐ स्कूटर moped/ luna ☐ मोपेड/लूना motorcycle ☐ मोटरसायकल room cooler ☐ रूम कूलर	radio ☐ रेडियो TV colour ☐ रंगीन टेलिविजन TV black/white ☐ स्वेत/श्याम टी वी electric fan ☐ बिद्युत पंखा bullock cart ☐ बैल गाड़ी
3.07	Does your household own any animal क्या आपके घर में काई जानवर है?	bullock ☐ बैल buffalo ☐ भैंस	cow ☐ गाय others ☐ अन्य
3.08	Does your household animal(s) sleep inside the house क्या आपके घर के जानवर घर के अन्दर सोते हैं?	yes ☐ हाँ	no ☐ नहीं
3.09	What is the roof your house made of? *(Mark only 1 of the following options ✓)* आपके घर की छत कैसी है? (सामने दिये विकल्प में से कोई एक ✓ करें)	tiles ☐ टाइल्स cement/concrete ☐ सीमेन्ट mud ☐ मिट्टी	thatch or grass ☐ कच्ची/ घास की Plastic sheet ☐ प्लास्टिक शीट other ☐ अन्य
3.10	What is floor of your house made of? *(Mark only 1 of the following options ✓)* आपके घर का फर्श कैसा है? (सामने दिये विकल्प में से कोई एक ✓ करें)	mud or sand ☐ मिट्टी या रेता cement ☐ सीमेन्ट tiles or carpet ☐ टाइल्स या कारपेट	dung and earth ☐ गोबर या पीला wood planks ☐ लकड़ी के तख्ते others ☐ अन्य
3.11	What is main cooking fuel in your household? *(Mark only 1 of the following options ✓)* आपके घर का खाना पकाने का मुख्य ईधंन क्या है? (सामने दिये विकल्प में से कोई एक ✓ करें)	kerosene ☐ मिट्टी का तेल biogas ☐ बायोगैस charcoal ☐ चारकोल	LPG ☐ रसोई गैस wood ☐ लकड़ी dung ☐ गोबर कण्डे
3.12	What is the main household source of lighting? *(Mark only 1 of the following options ✓)* उजाले का मुख्य श्रोत क्या है? (सामने दिये विकल्प में से कोई एक ✓ करें)	kerosene ☐ मिट्टी का तेल gas ☐ गैस others ☐ अन्य	electricity ☐ बिजली oil ☐ तेल .....

4. Reproductive History: Previous Pregnancy ('s)
प्रजनन इतिहास : पूर्व की गर्भावस्थाओं की

4.01	Number of pregnancies with live born child जीवित बच्चों सहित गर्भावस्थाओं की संख्या	☐☐
4.02	Number of pregnancies with loss of fetus गर्भपात की गर्भावस्था संख्या	☐☐
4.03	Total number of previous pregnancies *(add above three)* If total = 0, skip to Q5.01 कुल गर्भावस्थाओं की संख्या (उपरोक्त दोनों को जोड़कर) (यदि कुल = 0, तो प्रश्न 5.01 पर जाये)	☐☐

5. History current pregnancy वर्तमान की गर्भावस्था का इतिहास			
Malaria Prevention measures मलेरिया रोकथाम के उपाय			
5.01	Do you sleep under a bed net? *(if no skip to 5.06)* क्या आप मच्छरदानी के अन्दर सोती हैं? (यदि नही तो 5.06 पर जायें)	yes ☐ हाँ	no ☐ नहीं ? ☐
5.02	How many nights do you sleep under the net? कितनी रातों में आप मच्छरदानी में सोई हैं?	every night ☐ हर रात sometimes ☐ कभी कभी	only rain season ☐ केवल बरसात में rarely ☐ यदा कदा
5.03	Did you sleep under the net last night? क्या आप पिछली रात मच्छरदानी में सोई थीं?	yes ☐ हाँ	no ☐ नहीं ? ☐
5.04	Has your net been treated with insecticide? क्या आपकी मच्छरदानी में कीटनाशक लगा है?	yes ☐ हाँ	no ☐ नहीं ? ☐
5.05	Has the net been treated in past year? क्या आपकी मच्छरदानी में पिछले साल कीटनाशक लगाया गया था?	yes ☐ हाँ	no ☐ नहीं ? ☐
5.06	Did you take medicine to prevent malaria? *(if yes, fill 5.07)* क्या आपने मलेरिया रोकथाम हेतु कोई दवाई ली थी? यदि हाँ, तो 5.07 भरें ।	yes ☐ हाँ	no ☐ नहीं ? ☐
5.07	If yes, type of medicine taken यदि हाँ, तो ली गयी दवाई का प्रकार	malaria medicine ☐ मलेरिया की दवाई fever medicine ☐ बुखार की दवाई	☐..... unknown ☐ मालूम नहीं traditional med. ☐ देशी दवाई
6. Malaria morbidity history (present pregnancy) मलेरिया से बीमारी का इतिहास (वर्तमान गर्भावस्था)			
6.01	Have you had a fever or malaria *(If no skip to 6.06)* क्या आपको बुखार या मलेरिया था यदि नही, तो 6.06 पर जायें	yes ☐ हाँ	no ☐ नहीं ? ☐
6.02	Did you get a convulsion with fever / malaria क्या आपको बुखार अथवा मलेरिया से कपकपी लगी थी	yes ☐ हाँ	no ☐ नहीं ? ☐
6.03	Did you take medicine to treat your fever / malaria क्या आपने बुखार या मलेरिया ठीक करने हेतु दवाई ली थी	yes ☐ हाँ	no ☐ नहीं ? ☐
6.04	Type of medicine taken किस प्रकार की दवाई ली गयी थी	chloroquine ☐ क्लोरोक्वीन SP/Fansidar ☐ एस. पी./ फैन्सीडार quinine ☐ क्वीनीन antibiotics ☐ एन्टीबायोटिक्स	fever medicine ☐ बुखारी दवाई trad/herbal med ☐ देशी/ जड़ी बूटी unknown ☐ अन्जान

6.05	Did you stay in hospital overnight for treatment of fever / malaria क्या आप बुखार या मलेरिया उपचार हेतु अस्पताल में पूरी रात रूकी थी	yes ☐ हाँ no ☐ नहीं ? ☐
6.06	Are you taking iron and folic acid tablets (Fefol) क्या आपने लोह अथवा फोलिक एसिड की गोलियाँ ली हैं	yes ☐ हाँ no ☐ नहीं ? ☐
6.07	Did you get a blood transfusion क्या आपको खून चढ़ा था	yes ☐ हाँ no ☐ नहीं ? ☐
7. Recent malaria morbidity history (Past week) तत्कालिक मलेरिया का इतिहास (पिछले सप्ताह)		
7.01	Did you have a fever in the past week क्या आपको पिछले सप्ताह बुखार था	yes ☐ हाँ no ☐ नहीं ? ☐
7.02	Did you take medicine to treat your fever क्या आपने बुखार के इलाज हेतु दवाई ली थी (यदि हाँ तो प्रश्न संख्या 7.03 भरें)	yes ☐ हाँ no ☐ नहीं ? ☐
7.03	Type of medicine दवाई का प्रकार	malaria medicine ☐ मलेरिया की दवाई ..... fever medicine ☐ बुखार की दवाई unknown ☐ मालुम नहीं

8. ANC clinic visits and Examination प्रसव पूर्व देखभाल हेतु क्लीनिक का दौरा तथा परिक्षण				
8.01	Total number of antenatal clinic visits *(record from ANC clinic card) include todays visit* कुल कितनी बार क्लीनिक का दौरा किया (ए एन सी कार्ड से देखें) आज का दौरा भी शामिल करें			☐☐
		Date दिनांक	Fundal Height (in cm) भ्रूण की लम्बाई (से.मी.)	Gestational age (wk) if available गर्भ की आयु (सप्ताह में)
	First visit प्रथम दौरा	☐☐/☐☐☐/☐☐	☐☐	☐☐
	This visit (today) आज का दौरा	☐☐/☐☐☐/☐☐	☐☐	☐☐
8.02	Last menstrual period (LMP) *(if recorded)* अंतिम महावारी (एल एम पी) (यदि रिकार्ड में हो)			☐☐/☐☐☐/☐☐
8.03	Axillary temperature शरीर का तापमान (यदि तापमान ≥ 37.5 C तो तुरंत आर डी टी लगायें)			☐☐.☐
8.03b	Mid Upper Arm Circumference (MUAC)			☐☐.☐
8.04	Haemoglobin (Hb) g/dl *If Hb less than 7g/dl refer to ANC staff* हीमोग्लोबिन (एच बी) g/dl यदि एच बी < 7 g/dl तो ए एन सी स्टाफ को सूचित करें			☐☐.☐

8.05	Rapid Diagnostic test त्वरित जाँच किट (आर डी टी) परीक्षड़	Pf pure ☐ other malaria sp ☐ शुद्ध पी एफ अन्य मलेरिया प्रजाति negative ☐ नकारात्मक
8.06	Blood Smear (thick & thin) *(if febrile, or history of fever in past 7 days with negative rapid test read slide in clinic)* रक्त नमुना पट्टी (थिक / थिन) यदि बुखार है अथवा पिछले 7 दिन में बुखार आया था तथा आर डी टी किट निगेटिव थी तो स्लाइड जाँच लें	Positive ☐ धनात्मक Negative ☐ नकारात्मक Undetermined ☐ अज्ञात
8.07	Microtainer Blood sample taken माइक्रोटेनर रक्त नमुना पट्टी लिया गया	yes ☐ no ☐ हाँ नहीं
8.08	Blood smear taken रक्त नमुना पट्टी बनाई गयी	yes ☐ no ☐ हाँ नहीं
8.09	Antimalarial Drug (sulphadoxine-pyrimethamine) Given मलेरिया रोधी दवाई (सल्फाडाक्सीन-पायरामिथामिन) दी गयी	yes ☐ no ☐ हाँ नहीं
	Referred for further evaluation and treatment पुन: जाँच एवं उपचार हेतु भेजा गया	yes ☐ no ☐ हाँ नहीं
	Treatment given for anaemia? (dose + no days given) रक्ताल्पता का उपचार किया गया (खुराक + दिनों कि संख्या)	yes ☐ no ☐ हाँ नहीं
	Check all questions are answered & initial जाँच ले कि सारे प्रश्नों के उत्तर मिल गये हैं तथा हस्ताक्षर	

Thank the woman for her time
गर्भवती महिला को धन्यवाद बोलें

Quality Control
गुणवत्ता नियत्रंण

	Person व्यक्ति	Name or Signature नाम / हस्ताक्षर	Date दिनांक
10.01	Site Supervisor क्षेत्र पर्यवेक्षक		
10.02	Data Entry Clerk 1 डाटा इन्ट्री क्लर्क 1		
	Data Entry Clerk 2 डाटा इन्ट्री क्लर्क 2		
10.03	Program Coordinator (manager) प्रोग्राम समनवयक (प्रबधंक)		

Annexe 3: Delivery Unit Questionnaire

RAPID ASSESSMENT MALARIA IN PREGNANCY
DELIVERY UNIT QUESTIONNAIRE
गर्भावस्था में मलेरिया का त्वरित आँकलन
प्रसव इकाई प्रश्नावली

1. Identification पहचान		
1.01	Date दिनांक	(dd/mm/yy)☐☐/ ☐☐☐/☐☐
1.02	Participant ID No: प्रतिभागी का पहचान कमांक *Facility/unit=A/ womans'no:* *सुविधा/इकाई=ए/महिलासंख्या*	☐/☐/☐☐☐
1.03	Full Name पूरा नाम	.....
2. Personal Details Pregnant Woman गर्भवती महिला का व्यक्तिक विवरण		
2.01	What is your caste / tribe आपकी जाति क्या है? / जनजाति	SC ☐ ST ☐ GEN ☐ OBC ☐
2.02	Age (in years), *if age not known fill 99* उम्र (वर्ष में) यदि उम्र पता न हो तो 99 लिखें	☐☐
2.03	Age Group *(if precise age is not known)* आयु समूह (यदि सही उम्र पता न हो)	*15-19*☐ *35-39*☐ *20-24*☐ *40-44*☐ *25-29*☐ *>44*☐ *30-34*☐
2.04	Village / Town Name गाँव / कसवा का नाम	..... ग्रामीण rural ☐ शहरी urban ☐
2.05	Marital Status वैवाहिक स्थिति	married ☐ विवाहित divorced ☐ तलाकशुदा widow☐ विधवा
2.06	What language do you speak at home आप घर पर कौन सी भाषा बोलते हैं	hindi ☐ हिन्दी Other ☐ ..... अन्य
3. Socio-economic Indicators सामाजिक आर्थिक सूचकाँक		
3.01	Can you read? क्या आप पढ़ सकती हैं?	yes ☐ हाँ no☐ नहीं
3.02	What is the highest level of education that you finished? आपकी उच्चतम योग्यता क्या हैं? जहाँ से आपने पढ़ना बंद किया	no schooling ☐ स्कूल नहीं गये vocational ☐ व्यवहारिक primary ☐ प्राथमिक higher ☐ उच्चतर secondary ☐ माध्यमिक
3.03	What work does your husband do? आपके पति क्या करते हैं?	farming ☐ कृषि salaried job ☐ वेतनभोगी craftsman ☐ दस्तकार other ☐ अन्य
3.04	Does your household own farming land? क्या आपके मुखिया की स्वयं की कृषि भूमि है	yes ☐ हाँ no ☐ नहीं
3.05	Do any member of your household work on their own or family farmland क्या आपके घर का कोई व्यक्ति अपनी अथवा पारिवारिक कृषि भूम्य पर कार्य करता है	yes ☐ हाँ no ☐ नहीं
3.06	In your household is there any of the following items *(If yes, mark boxes ✓)* क्या आपके घर में निम्न में से कोई वस्तु है? यदि हाँ, तो ✓ का निशान लगायें	bicycle ☐ सायकल radio ☐ रेडियो scooter ☐ स्कूटर TV colour ☐ रंगीन टेलिविजन moped/ luna ☐ मोपेड/लूना TV black/white☐ स्वेत/श्याम टी वी

		motorcycle ☐ मोटरसायकल	electric fan☐ बिद्युत पंखा
		room cooler ☐ रूम कूलर	bullock cart☐ बैल गाड़ी
3.07	Does your household own any animal क्या आपके घर में काई जानवर है?	bullock☐ बैल	cow ☐ गाय
		buffalo☐ भैंस	others☐ अन्य
3.08	Does your household animal(s) sleep inside the house क्या आपके घर के जानवर घर के अन्दर सोते हैं?	yes ☐ हाँ	no ☐ नहीं
3.09	What is the roof your house made of? *(Mark only 1 of the following options ✓)* आपके घर की छत कैसी है? (सामने दिये विकल्प में से कोई एक ✓ करें)	tiles ☐ खपड़ा	thatch or grass ☐ कच्ची/ घास की
		cement/concrete ☐ सीमेन्ट	Plastic sheet ☐ प्लास्टिक शीट
		mud ☐ मिट्टी	other ☐ अन्य
3.10	What is floor of your house made of? *(Mark only 1 of the following options ✓)* आपके घर का फर्श कैसा है? (सामने दिये विकल्प में से कोई एक ✓ करें)	mud or sand ☐ मिट्टी या रेता	dung and earth ☐ गोबर या पीला
		cement ☐ सीमेन्ट	wood planks ☐ लकड़ी के तख्ते
		tiles or carpet ☐ टाइल्स या कारपेट	others ☐ अन्य
3.11	What is main cooking fuel in your household? *(Mark only 1 of the following options ✓)* आपके घर का खाना पकाने का मुख्य ईधंन क्या है? (सामने दिये विकल्प में से कोई एक ✓ करें)	kerosene ☐ मिट्टी का तेल	LPG ☐ रसोई गैस
		biogas ☐ बायोगैस	wood ☐ लकड़ी
		charcoal ☐ कोयला	dung ☐ गोबर कण्डे
3.12	What is the main household source of lighting? *(Mark only 1 of the following options ✓)* आपके घर में उजाले का मुख्य श्रोत क्या है? (सामने दिये विकल्प में से कोई एक ✓ करें)	kerosene ☐ मिट्टी का तेल	electricity ☐ बिजली
		gas ☐ गैस	oil ☐ तेल
		others ☐ अन्य	.....

4. Reproductive History: Previous Pregnancy ('s) प्रजनन इतिहास : पूर्व की गर्भावस्थाओं की		
4.01	Number of pregnancies with live born child जीवित बच्चों सहित गर्भावस्थाओं की संख्या	☐☐
4.02	Number of pregnancies with loss of fetus गर्भपात की गर्भावस्था संख्या	☐☐
4.03	Total number of previous pregnancies *(add above three)* If total = 0, skip to Q5.01 कुल गर्भावस्थाओं की संख्या (उपरोक्त दोनों को जोड़कर) (यदि कुल = 0, तो प्रश्न 5.01 पर जाये)	☐☐

5. History current pregnancy वर्तमान की गर्भावस्था का इतिहास				
Malaria Prevention measures मलेरिया रोकथाम के उपाय				
5.01	Do you sleep under a bed net? *(if no skip to 5.06)* कया आप मच्छरदानी के अन्दर सोती हैं? (यदि नही तो 5.06 पर जायें)	yes☐ हाँ	no☐ नहीं	?☐
5.02	How many nights do you sleep under the net? आप कितनी रातों में मच्छरदानी में सोर्ती हैं?	every night ☐ हर रात	only rain season ☐ केवल बरसात में	
		sometimes ☐ कभी कभी	rarely ☐ यदा कदा	
5.03	Did you sleep under the net last night? क्या आप पिछली रात मच्छरदानी में सोई थीं?	yes☐ हाँ	no ☐ नहीं	?☐
5.04	Has your net been treated with insecticide? क्या आपकी मच्छरदानी में कीटनाशक लगा है?	yes ☐ हाँ	no ☐ नहीं	?☐
5.05	Has the net been treated in past year? क्या आपकी मच्छरदानी में पिछले साल कीटनाशक लगाया गया था?	yes ☐ हाँ	no ☐ नहीं	?☐
5.06	Did you take medicine to prevent malaria? *(if yes, fill 5.07)*	yes ☐	no ☐	?☐

	क्या आपने मलेरिया रोकथाम हेतु कोई दवाई ली थी? यदि हाँ, तो 5.07 भरें ।	हाँ नहीं
5.07	If yes, type of medicine taken यदि हाँ, तो ली गयी दवाई का प्रकार	malaria medicine ☐ ☐..... मलेरिया की दवाई fever medicine ☐ unknown ☐ बुखार की दवाई मालूम नहीं traditional med. ☐ देशी दवाई

6. Malaria morbidity history (present pregnancy)
मलेरिया से बीमारी का इतिहास (वर्तमान गर्भावस्था)

6.01	Have you had a fever or malaria *(If no skip to 6.06)* क्या आपको बुखार या मलेरिया था यदि नही, तो 6.06 पर जायें	yes ☐ no ☐ ? ☐ हाँ नहीं
6.02	Did you get a convulsion with fever / malaria क्या आपको बुखार अथवा मलेरिया से कपकपी लगी थी	yes ☐ no ☐ ? ☐ हाँ नहीं
6.03	Did you take medicine to treat your fever / malaria क्या आपने बुखार या मलेरिया ठीक करने हेतु दवाई ली थी	yes ☐ no ☐ ? ☐ हाँ नहीं
6.04	Type of medicine taken किस प्रकार की दवाई ली गयी थी	chloroquine ☐ fever medicine ☐ क्लोरोक्वीन बुखारी दवाई SP/Fansidar ☐ trad/herbal med ☐ एस. पी./ फैन्सीडार देशी/ जड़ी बूटी quinine ☐ unknown ☐ क्वीनीन मालुम नहीं antibiotics ☐ एन्टीबायोटिक्स
6.05	Did you stay in hospital overnight for treatment of fever / malaria क्या आप बुखार या मलेरिया उपचार हेतु अस्पताल में पूरी रात रूकी थी	yes ☐ no ☐ ? ☐ हाँ नहीं
6.06	Are you taking iron and folic acid tablets (Fefol) क्या आपने लोह अथवा फोलिक एसिड की गोलियाँ ली थी	yes ☐ no ☐ ? ☐ हाँ नहीं
6.07	Did you get a blood transfusion क्या आपको खून चढ़ा था	yes ☐ no ☐ ? ☐ हाँ नहीं

7. ANC clinic visits and ANC card details प्रसव पूर्व क्लीनिक दौरा एवं ए एन सी कार्ड विवरण

7.01	Did you attend ANC during this pregnancy? *If no, go to Q8.01* क्या आप गर्भावस्था के दौरान ए एन सी क्लीनिक गई थीं? यदि नही तो प्रश्न संख्या 8.01 पर जायें	yes ☐ हाँ	no ☐ नहीं
7.02	Did you bring your ANC card today. *If no card, go to Q 8.01* क्या आज आप अपना ए एन सी कार्ड लाईं हैं? यदि नही तो प्रश्न संख्या 8.01 पर जायें	yes ☐ हाँ	no ☐ नहीं
7.03	Total number of antenatal clinic visits *(record from ANC clinic card)* आप कुल कितनी बार ए एन सी क्लीनिक गई? (ए एन सी कार्ड से रिकार्ड देखें)	☐☐	

		Date दिनांक	Fundal Height (in cm) भ्रूण की लम्बाई (से.मी.)	Gestational age (wk) if available गर्भ की आयु (सप्ताह में)
7.04	First visit प्रथम दौरा	☐☐/☐☐☐/☐☐	☐☐	☐☐
7.05	This visit (today) आज का दौरा	☐☐/☐☐☐/☐☐	☐☐	☐☐
7.06	Last menstrual period (LMP) *(if recorded)* अंतिम महावारी (एल एम पी) (यदि रिकार्ड में हो)	☐☐/☐☐☐/☐☐		
7.07	Risk Factors Current Pregnancy वर्तमान गर्भावस्था के जोखिम कारक	Anaemia < 7g.dl☐ ?☐ BP > 140/90 ☐ ?☐ Pre-Eclampsia ☐ ?☐ Gestational Diabetes ☐ ?☐ Antepartum Haemorrhage ☐ ?☐ Malpresentation ☐ ?☐		

8. Recent malaria morbidity history (Past week)
तत्कालिक मलेरिया रोग की इतिहास (पिछले सप्ताह)

8.01	Did you have a fever in the past week (if yes, do RDT)	yes ☐ no ☐ ? ☐ हाँ नहीं

	क्या आपको पिछले सप्ताह बुखार था (यदि हाँ तो आर डी टी की किट लगायें)	
8.02	Did you take medicine to treat your fever *(if yes, fill Q 8.03)* क्या आपने बुखार कि दवाई ली थी (यदि हाँ तो प्रश्न संख्या 8.03 भरें)	yes ☐ हाँ no ☐ नहीं ? ☐
8.03	Type of medicine दवाई का प्रकार	malaria medicine ☐ मलेरिया की दवाई ☐..... fever medicine ☐ बुखार की दवाई unknown ☐ मालूम नहीं traditional med. ☐ देशी दवाई
9. Maternal Examination before delivery प्रसव पूर्व माँ का परिक्षण		
9.01	Axillary temperature शारीरिक तापमान	☐☐.☐
9.02	Maternal height (cm) माँ की लम्बाई (से मी)	☐☐☐.☐
9.02b	Mid upper Arm Circumference (MUAC) cm	☐☐☐.☐
9.03	Haemoglobin (Hb) g/dl *If Hb less than 7g/dl refer to DU staff* हीमोग्लोबिन (एच बी) g/dl यदि एच बी < 7 g/dl तो डी यू स्टाफ को सूचित करें	☐☐.☐
9.04	Rapid Diagnostic test (peripheral blood) त्वरित परिक्षण किट (परिधीय रक्त)	Pf pure ☐ शुद्ध पी एफ other malaria sp. ☐ अन्य मलेरिया प्रजाति negative ☐ नकारात्मक
9.05	Maternal finger stick Blood Smear (thick & thin) *(if febrile, or history of fever in past 7 days with negative rapid test read slide in clinic)* माँ की उँगली के रक्त की रक्तपट्टी (थिक / थिन) (यदि बुखार है या पिछले 7 दिन में बुखार का इतिहास रहा है परंतु आर डी टी निगेटिव है तो रक्त पट्टी जाँचे)	positive ☐ धनात्मक negative ☐ नकारात्मक undetermined ☐ अनिश्चित

10 Delivery Details प्रसव विवरण		
10.01	Date of Delivery प्रसव का दिनांक	dd/mm/yy☐☐.☐☐☐.☐☐
10.02	Type of Delivery प्रसव का प्रकार :	spontaneous vaginal☐ स्वत:सामान्य forceps/vacuum☐ चीरा लगाकर C/section☐ आपरेशन के द्वारा
10.03	Number of babies born नवजात शिशुओं कि संख्या:	singleton☐ एक नवजात twins☐ जुड़वा triplets /more☐ तीन या अधिक
10.04	Singleton newborn baby outcome एक नवजात शिशु का स्तर	live birth☐ जीवित जन्मा Born alive died in hospital (7days)☐ जीवित जन्मा पर सातवें दिन अस्पताल में मृत्यु stillbirth☐ मृत जन्मा
10.05	Singleton baby born dead check एक नवजात मृत शिशु का परिक्षड़	Fresh stillbirth☐ ताजा मृत जन्मा macerated☐ मृत एवं सिकुड़ा हुआ unknown☐ अज्ञात

10.06	Singleton baby born dead check (record for cause of death) *e.g: malaria, sepsis, birth asphyxia, foetal distress, etc* एक मृत नवजात शिशु का परिक्षड़ (मृत्यु का कारण जानने हेतु) उदाहरणार्थ: मलेरिया, सेप्सिस, जन्म स्फाइजिया, भ्रुण डिस्ट्रेस आदि	.....
11.Maternal Outcomes मातृ परिणाम		
11.01	Maternal Death : If yes fill Q 11.02 माता कि मृत्यु यदि हाँ तो प्रश्न संख्या 11.02 भरें	yes ☐ हाँ no☐ नहीं
11.02	Specify Cause of Death (check record) Not known state : "Unknown" मृत्यु का कारण स्पष्ट करें (रिकार्ड देखें) यदि पता नहीं है तो अज्ञात लिखें	.....
11.03	Were there any of the following complications during labour *(mark ✓any of following)* क्या प्रसव के समय सामने दी गयी जटिलताओं में से कोई महसुस की (कृप्या कोई एक में ✓लगायें)	No complication ☐ जटिलता नहीं Eclampsia ☐ एकलैम्पसिया Puerperal sepsis ☐ परपियुरल सेप्सिस Uterine rupture ☐ गर्भाशय फटना Pre-eclampsia ☐ पूर्व एकलैम्पसिया Obstructed labor ☐ प्रसव पीड़ा Placental abruption ☐ प्लासेंटल एबरप्शन Post partum Haemorrhage ☐ प्रसवोत्तर रक्तस्राव

12. Examination Live Born Singleton Baby जीवित जन्मा एक बच्चे का परीक्षण		
Date of examination: परीक्षण की तिथि ☐☐.☐☐☐.☐☐		Name of Examiner: परीक्षक का नाम
12.01	Sex of baby बच्चे का लिंग	male पुलिंग ☐ female स्त्रिलिंग ☐
12.02	Baby weight *(grams)* बच्चे का वजन (ग्राम में)	☐☐☐☐
12.04	Ballard Score (total) (Attach form) बैलार्ड संख्या (कुल) (संलग्न रूप)	☐☐
12.05	Gestational age (weeks) (by Ballard Score) गर्भ की आयु (सप्ताह में) (बैलार्ड स्कोर के द्वारा)	☐☐

13. Delivery Unit Blood Samples प्रसव इकाई से रक्त नमुनें				
Date: दिनांक:	Sample नमुना	Code label कोड स्तर		Initial हस्ताक्षर
	Placenta Maternal प्लासेन्टा मैटरनल	M	yes☐ हाँ no ☐ नहीं	
	Placenta Impression प्लासेन्टा इम्प्रेशन	T	yes☐ हाँ no ☐ नहीं	
	Placenta Biopsy प्लासेन्टा बायोप्सी	B	yes☐ हाँ no ☐ नहीं	
	Placenta blood in microtube प्लासेन्टा रक्त (माइक्रोट्युब में)		yes☐ हाँ no ☐ नहीं	
13.01	Peripheral Blood smear taken परिधीय रक्त पट्टी निमार्ण		yes☐ हाँ no ☐ नहीं	
13.02	Antimalarial given मलेरिया रोधी दवाई दी गयी		yes☐ हाँ no ☐ नहीं	
13.04	Haemoglobin result <11g/dl inform DU staff हिमोग्लोबिन का परिणाम यदि <11g/dl प्रसव इकाई को सूचित करें		yes☐ हाँ no ☐ नहीं	
Check all questions are answered : tick mark and initial				☐

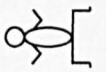
जाँचे कि सारे प्रश्नों की उत्तर मिल गये हैं : ✓ करें तथा हस्ताक्षर करें	

14.Quality Control गुणवत्ता नियत्रंण

Site supervisor to check questionnaire for completeness daily at the end of all interviews
Data entry clerk to initial at the end of every entry
क्षेत्र पर्यवेक्षक को जाँचना है कि क्या प्रश्नावली पूर्ण हो गयी है
डाटा इन्ट्री क्लर्क प्रत्येक इन्ट्री के बाद हस्ताक्षर करें

	Person व्यक्ति	Name or Signature नाम / हस्ताक्षर	Date दिनांक
14.01	Site Supervisor क्षेत्र पर्यवेक्षक		
14.02	Microscopist ensure all blood samples received माइक्रोस्कोपिस्ट को सुनिश्चित करना है कि सारे नमूने मिल गयें हैं		
14.03	Data Entry Clerk 1 डाटा इन्ट्री क्लर्क 1		

Annexe 4: Ballard Examination form

SCORE:	0	1	2	3	4	5	Record Score Here	
Posture								
Wrist	 90°	 60°	 45°	 30°	0°			
Arm Recoil	 180°	140-180	 110-140°	 90-110°	 <90°			
Popliteal Angle	 180°	 160°	 130°	 110°	 90°	 <90°		
Scarf Sign								Total
Heel to Ear								
Skin	Paper thin, transparent, many veins visible	Smooth, still thin but less transparent	Peeling, rash, only a few veins visible	Shallow cracking in some areas, rare veins	Deep cracks, skin rough, no veins seen	Leathery, cracked, wrinkled, old man skin		
Lanugo	None	Abundant	Thinning	bald areas	mostly bald	N/A		
Plantar Creases	No creases (lines)	Faint red marks	Only one crease (line) across front of foot	Creases (lines) over front 2/3 of foot	Creases (lines) over all of foot			
Breast	Nipple very hard to see	Flat areola, no bud	Areola with small dots of dark color, 1-2 mm bud	Raised areola, 3-4 mm bud	Full areola, 5-10 mm bud			
Ear	Very soft, stays folded	Soft, slow recoil when folded	Soft, rapid recoil when folded	Formed, firm, instant recoil when folded	Thick, stiff	N/A		
Genitals (Male)	Scrotum empty, no rugae (lines)	Testes at top of scrotum, rare rugae (lines)	Testes descending few rugae (lines)	Testes down into scrotum good rugae (lines)	Testes hung down low in scrotum, deep rugae (lines)	N/A		Total
Genitals (Female)	Clitoris large, labia minora larger than labia majora	Clitoris prominent enlarging minora	Clitoris visible, labia minora visible under labia majora	Clitoris small, but visible labia minora mostly covered	Clitoris and labia minora completely covered	N/A		
TOTAL SCORE =								

Date of exam		Mother's study ID No	
Date of birth (Baby)		Age in hours (baby)	
Facility Name		Assessed by :	

Table to read gestational age maturity

Total Score	Age (weeks)		Total score	Age (weeks)		Total (score)	Age (weeks)
5	**26**		**20**	**32**		**35**	**38**
6	26		21	32		36	38
7	27		22	33		37	39
8	27		23	33		38	39
9	27		24	33		39	39
10	**28**		**25**	**34**		**40**	**40**
11	28		26	34		41	40
12	29		27	35		42	41
13	29		28	35		43	41
14	29		29	35		44	41
15	**30**		**30**	**36**		**45**	**42**
16	30		31	36		46	42
17	31		32	37		47	43
18	31		33	37		48	43
19	31		34	37		49	43

Annexe 5: Inpatient Surveillance Form

RAPID ASSESSMENT MALARIA IN PREGNANCY

INPATIENT DATA ABSTRACTION FORM

गर्भवस्था में मलेरिया का त्वरित ऑकलन

A. Patient Identification रोगी की पहचान		
A.1	Date: दिनांक	(dd/mm/yy)☐☐/☐☐/☐☐
A.2	Patient Record ID No: *Facility / Unit=W/ Data Record No:* रोगी की रिकार्ड आई डी संख्या सुविधा / इकाई = W/ डाटा रिकार्ड संख्या	☐/☐/☐☐☐☐
A.3	Patient Hospital File No: रोगी का अस्पताल की फाइल संख्या	☐☐☐☐
A.4	Woman's Name महिला का नाम	.....

B. Patient Information रोगी की सूचना			
B.1	Patient's Village / Town Name रोगी का गॉव / कस्बे का नाम	.....	
B.2	Age (years) आयु (वर्ष में)		☐☐
B.3	Age group *(if age unkown in medical record)* आयु समूह (यदि मेडिकल रिकार्ड में आयु अज्ञात हो)	15-19 ☐ 20-24 ☐ 25-29 ☐	30-34 ☐ 35-39 ☐ 40-44 ☐
B.3b	Total number of previous pregnancies		☐☐
B.3c	Date of last delivery		☐☐/☐☐/☐☐
B.4	Last menstrual period (LMP) *If known fill date (dd/mm/yy) or month* *Unknown = ?* पिछली महावारी यदि पता हो तो तिथि लिखें या महीना । अज्ञात = ?	~~~/~~~/~~~~~/..... ?☐	
B.5	Pregnant *(ask patient if not recorded in file)* *if yes fill B.6* गर्भावस्था (यदि रिकार्ड में न हो तो रोगी से पुछें) यदि हॉ तो प्रश्न संख्या B.6 भरे		Y☐ N☐ DK☐
B.6	What is the gestational age *(weeks)* *If unknown tick mark =?* गर्भ की आयु क्या है (सप्ताह में) यदि अज्ञात हो तो ? पर टिक करें		☐☐ ?☐

C. History of Current Illness वर्तमान बीमारी का इतिहास		
C.1	How long has the patient been ill *(days)* कितने लम्बे समय से रोगी बीमार है (दिनों में)	☐☐
C.2	Prior to admission did the patient have: अस्पताल में प्रवेश से पहले क्या रोगी को था	coma Y☐ N☐ DK☐ मुर्छा convulsions Y☐ N☐ DK☐ झटके decreased consciousness चेतना में कमी Y☐ N☐ DK☐
C.3	Did the patient take antimalarial medicine for this illness prior to admission *if yes check record or ask (D.3a)* क्या अस्पताल में प्रवेश से पहले इस बीमारी हेतु मलेरिया रोधी दवाई ली थी यदि हाँ तो रिकार्ड देखें अथवा पूछें *(D.3a)*	Y☐ N☐ DK☐
C.4	Name of medicine दवाई का नाम	Chloroquine Y☐ N☐ DK☐ क्लोरोक्वीन SP/Fansidar Y☐ N☐ DK☐ एस/फैनसीडार Quinine Y☐ N☐ DK☐ कूनैन Primaquine Y☐ N☐ DK☐ प्राइमाक्वीन Artesunate Y☐ N☐ DK☐ आर्टीसुनेट EMAL Y☐ N☐ DK☐

D. Admission Data प्रवेश डाटा			
D.1	Admission diagnosis *(Tick as appropriate)* प्रवेश निदान (समुचित पर टिक करें)	☐ malaria मलेरिया ☐ severe malaria सिवियर मलेरिया ☐ cerebral malaria सेरेबरल मलेरिया ☐ anaemia एनेमिया ☐ others अन्य.....	
D.2	Check records for any of the following *If not recorded mark as NO= n* निम्न के लिए रिकार्ड जाँचे यदि रिकार्ड में नहीं=*n* ना हो तो		
	(multiple) convulsions (मलटीपल) झटके	y☐ हाँ	n☐ नहीं
	↓ conciousness ↓चेतनावस्था	y☐ हाँ	n☐ नहीं
	coma मूर्छा	y☐ हाँ	n☐ नहीं
	respiratory distress श्वसन में दिक्कत	y☐ हाँ	n☐ नहीं
	shock (circulatory collapse) सदमा (सरकुलेटरी कोलेप्स)	y☐ हाँ	n☐ नहीं
	unusual bleeding (petichiae/haemetemesis) असमान्य रक्त प्रवाह (पेटीची/ हेमेटेमेसिस)	y☐ हाँ	n☐ नहीं
	For following check test results before marking as NO = n NO = n मार्क करने से पहले निम्न के लिए परीक्षण का परिणाम जाँचे		
	severe Anaemia गंभीर रक्तालप्ता	y☐ हाँ	n☐ नहीं
	jaundice	y☐	n☐

	पीलिया	हाँ	नहीं
	hypoglycaemia रूधिर में सर्करा की कमी	y☐ हाँ	n☐ नहीं
	pulmonary oedema फेफड़ों में सूजन	y☐ हाँ	n☐ नहीं
	haemoglobinuria हिमोग्लोबीन्युरिया	y☐ हाँ	n☐ नहीं

E. Laboratory Results
प्रयोगशाला परिणाम

Malaria Blood film
मलेरिया कि रक्त पट्टी

E.1	Smear Done स्मीयर बनाये गये	y☐ हाँ	n☐ नहीं
E.2	Results परिणाम	positive धनात्मक ☐ negative नाकारात्मक ☐ undetermined अज्ञात ☐	
E.3	Parasite Count *(if unknown mark n)* परिजीवी की संख्या (यदि अज्ञात हो तो *n* पर टिक करें	.....	n☐
E.4	Malaria Species मलेरिया की प्रजातियाँ		
	P.falciparum only		☐
	P.vivax only		☐
	P.falciparum+ P.vivax		☐
	Others (specify) अन्य (स्पष्ट करें)		☐
	Undetermined अज्ञात		☐

Blood Tests: *(if done write result)* रक्त परिक्षक (यदि किया गया तो परिणाम लिखें)		Value मान	Not done किया नहीं गया
E.5	haemoglobin (lowest) हिमोग्लोबीन (न्युनतम)		n☐ नहीं
E.6	platelet count (lowest value) प्लेटलेट की संख्या (न्युनतम मान)		n☐ नहीं
E.7	blood glucose (lowest value) रक्त सर्करा (न्युनतम मान)		n☐ नहीं
E.8	serum bilirubin (highest value) सीरम बिलीरूबीन (उच्चतम मान)		n☐ नहीं
E.9	creatinine (highest) क्रियेटीनीन (उच्चतम)		n☐ नहीं
Other Tests: अन्य परिक्षण			
Lumbar Puncture Done *(if yes check value)* लम्बर पंचर किया गया (यदि हाँ तो मान जाँचे)		y☐ हाँ	n☐ नहीं
E.10	spinal fluid glucose *(result unknown = n)* स्पाइनल फ्लूड सर्करा (परिणाम अज्ञात = *n*)	.....	n☐ नहीं
E.11	spinal fluid protein *(result unknown =n)* स्पाइनल फ्लूड प्रोटीन (परिणाम अज्ञात = *n*)	.....	n☐ नहीं
Radiology क्ष-रश्मि विज्ञान			
E.12	Chest X-Ray (*if yes check report for)* छाती का एक्स-रे (यदि हाँ तो निम्न के लिए परिणाम देखें)	y☐ हाँ	n☐ नहीं
E.13	pulmonary oedema फेफड़ों में सूजन	y☐ हाँ	n☐ नहीं

F. Hospital Treatment अस्पताल में उपचार			
F.1	Antimalarial medicine given *(if yes check drug)* मलेरिया रोधी दवाई दी गयीं (यदि हाँ तो दवाई जाँचे)	y☐ हाँ	n☐ नहीं
	Drug given: दी गयी दवायें		
	Chloroquine क्लोरोक्वीन	y☐ हाँ	n☐ नहीं
	Quinine IV कुनैन आइ वी	y☐ हाँ	n☐ नहीं
	Quinine tablets कुनैन टैबलेट्स	y☐ हाँ	n☐ नहीं
	SP/Fansidar एसपी/फैन्सीडार	y☐ हाँ	n☐ नहीं
	Artesunate आर्टीसुनेट	y☐ हाँ	n☐ नहीं
	Primaquine प्राइमाक्वीन	y☐ हाँ	n☐ नहीं
	Proguanil प्रोगोनील	y☐ हाँ	n☐ नहीं
F.2	Other treatment given: दिया गया अन्य उपचार		
	Oxygen ऑक्सीजन	y☐ हाँ	n☐ नहीं
	Glucose Infusion (IV bottle) ग्लुकोज इनफ्युजन (आइ वी बोतल)	y☐ हाँ	n☐ नहीं
	Blood transfusion रक्त अदान-प्रदान	y☐ हाँ	n☐ नहीं
	Anticonvulsant medicine झटके रोकने की दवाई	y☐ हाँ	n☐ नहीं
	Diuretic (Lasix) डायुरेटिक (लैसिक्स)	y☐ हाँ	n☐ नहीं
	Vitamin K (injection/tablet) विटामिन के (सुई/ गोली)	y☐ हाँ	n☐ नहीं
	Antipyretics (fever medicine) बुखार की दवाई	y☐ हाँ	n☐ नहीं
	Antibiotics एन्टीबायोटिक्स	y☐ हाँ	n☐ नहीं

G. Admission Outcome (Illness) प्रवेश परिणाम (बीमारी)			
For Pregnant Women only केवल गर्भवती महिलाओं के लिए			
G.1	Did the woman deliver during this admission *If yes* क्या महिला को इस प्रवेश के दौरान प्रसव हुआ यदि हाँ	y☐ हाँ	n☐ नहीं
G.2	Outcome of delivery प्रसव का परिणाम		
	Live birth जीवित जन्म	y☐ हाँ	n☐ नहीं
	Stillbirth मृत जन्म	y☐ हाँ	n☐ नहीं
	Abortion गर्भपात	y☐ हाँ	n☐ नहीं

Disease Outcome (for all women) बीमारी का परिणाम (सभी महिलाओं के लिए)			
G.3	Discharged with full recovery पूर्ण रूप से सही होने पर छुट्टी दी	y☐ हाँ	n☐ नहीं
G.4	Discharged with disease sequelae रोग के लक्षण शेष रहते छुट्टी दी	y☐ हाँ	n☐ नहीं
G.5	Died in hospital अस्पताल में मृत्यु	y☐ हाँ	n☐ नहीं
G.6	Left against medical advice चिकित्सकीय परामर्श के बगैर छुट्टी पर चला गया	y☐ हाँ	n☐ नहीं
Discharge Diagnosis छुट्टी देते समय निदान			
G.7	Malaria मलेरिया	y☐ हाँ	n☐ नहीं
G.8	Severe malaria गंभीर मलेरिया	y☐ हाँ	n☐ नहीं
G.9	Cerebral malaria मस्तिक मलेरिया	y☐ हाँ	n☐ नहीं
G.10	Anaemia रक्ताल्पता	y☐ हाँ	n☐ नहीं
G.11	Other Disease (if known specify) अन्य बीमारी (यदि ज्ञात है तो स्पष्ट करें)	y☐..... हाँ	n☐ नहीं
G.12	Diagnosis unclassified निदान परिभाषित नहीं है	y☐ हाँ	n☐ नहीं
uality Control: गुणवत्ता नियंत्रण			
	Check all information /results filled जाँचे कि सभी सूचनाऐं/ परिणाम भर दिये गये हैं	y☐ हाँ	n☐ नहीं
	Abstractor initial & date साक्षात्कार करने वाले के हस्ताक्षर एवं दिनांक		Date: दिनांक
	Supervisor Initial & date पर्यवेक्षक के हस्ताक्षर एवं दिनांक		Date: दिनांक
	Data Entry Clerk initial & date डाटा इन्ट्री करने वाले के हस्ताक्षर एवं दिनांक		Date: दिनांक

Annexe 6

Pearsons Correlation co-efficient matrix ANC variables

	educ	residenc	SES	Dsite	season	Dhb9	Fever at visit	Fever past wk	Drug use past wk	Antimal past wk	Fever in preg	Drug use in preg	Antimal in preg	Iron-suppl	bedNet use	Net last night
educ	xxx	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Residen	0.25032 <.0.0001	xxx														
SES	0.40024 <.0.0001	0.42431 <.0.0001	xxx													
Dsite	- 0.26166 <.0.0001	- 0.41117 <.0.0001	- 0.29561 <.0.0001	xxx												
Season	0.01736 0.0477	0.01988 0.4083	0.00320 0.8935	0.00916 0.7019	xxx											
Dhb9	0.13041 <.0.0001	0.11140 <.0.0001	0.10103 <.0.0001	- 0.10076 <.0.0001	- 0.10750 <.0.0001	xxx										
Fever at visit	- 0.04051 0.0904	- 0.10372 <.0.0001	- 0.08388 0.0004	0.13484 <.0.0001	- 0.02248 0.3476	-0.06739 0.0048	xxx									
Fever past wk	0.12806 <.0.0001	0.11226 <.0.0001	0.15786 <.0.0001	0.18316 <.0.0001	0.16879 <.0.0001	0.13809 <.0.0001	0.17439 <.0.0001	xxx								
Drug use past wk	- 0.07525 0.0018	- 0.06389 0.0075	- 0.06848 0.0042	0.10651 <.0.0001	0.10061 <.0.0001	- 0.10698 <.0.0001	0.09505 <.0.0001	0.59257 <.0.0001	xxx							
Antimal Past wk	- 0.06645 0.0054	- 0.05011 0.0362	- 0.03202 0.1809	0.06603 0.0055	- 0.03479 0.1460	- 0.01406 0.5570	0.03903 0.0960	0.10039 <.0.0001	0.32660 <.0.0001	xxx						
Fever in preg	0.00788 0.7421	- 0.12285 <.0.0001	- 0.08885 0.0002	0.18585 <.0.0001	0.18223 <.0.0001	- 0.09887 <.0.0001	0.08483 0.0004	0.28081 <.0.0001	0.17247 <.0.0001	0.9898 <.0.0001	xxx					
Drug use in preg	0.04323 0.0708	- 0.05075 0.0339	- 0.00441 0.8537	0.06163 0.0100	0.11361 <.0.0001	- 0.05068 0.0341	- 0.01444 0.5463	0.12695 <.0.0001	0.22760 <.0.0001	0.13415 <.0.0001	0.73562 <.0.0001	xxx				
Antimal in preg	0.03114 0.1931	- 0.02446 0.3068	0.02035 0.3953	0.02925 0.2216	- 0.00597 0.8002	- 0.03088 0.1970	- 0.02881 0.3045	0.08380 0.0005	0.12020 <.0.0001	0.26281 <.0.0001	0.22935 <.0.0001	0.29624 <.0.0001	xxx			
Iron suppl	0.07886 0.0010	0.05771 0.0158	0.16523 <.0.0001	-0.12451 <.0.0001	- 0.17104 <.0.0001	0.04125 0.0847	- 0.05015 0.0980	- 0.08068 0.0007	- 0.04422 0.0845	- 0.03511 0.1423	- 0.04598 0.0548	- 0.00587 0.9810	0.00057 0.9810	xxx		
Bednet use	0.14462 <.0.0001	0.09898 <.0.0001	0.06556 0.0061	- 0.31730 <.0.0001	- 0.10273 <.0.0001	0.08424 0.0001	- 0.00091 0.9697	- 0.08995 0.9697	- 0.03857 0.1266	0.00599 0.8676	- 0.06879 0.0043	- 0.03830 0.1282	0.01358 0.5712	0.08171 0.0006	xxx	
Net last night	0.13576 <.0.0001	0.15715 <.0.0001	0.08431 0.0004	- 0.35659 <.0.0001	- 0.35659 <.0.0001	0.09015 0.0002	- 0.01410 0.5557	- 0.01410 0.5557	- 0.04595 0.0547	0.00340 0.8871	- 0.08703 0.0003	- 0.06333 0.0081	0.00041 0.9862	0.10439 <.0.0001	0.86535 <.0.0001	xxx

Annexe 7: Correlation Coefficient Matrix Deilivery Units Variables

Pearsons Correlation between variables of significance for Placental *falciparum* malaria; N=2620

	Caste category	residence	SES	education	Attend ANC	Possess ANCcard	season	Fever past wk	Drug use past wk	Antimal past wk	Fever in preg	Drug use in preg	Antimal in preg
Caste category	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Residenc	- 0.0043 0.83	xx											
SES	- 0.0889 <0.0001	0.4665 <0.0001	xx										
Educ	- 0.2141 <0.0001	0.1897 <0.0001	0.4394 <0.0001	xx									
Attend ANC	- 0.0380 0.05	0.0739 0.0002	0.1329 <0.0001	0.1156 <0.0001	xx								
Possess ANCcard	0.00004 0.99	0.0154 0.43	0.0272 0.16	0.0843 <0.0001	0.2291 <0.0001	xx							
season	0.0338 0.08	0.0403 0.04	- 0.0119 0.54	0.0387 0.05	0.0013 0.94	0.1870 <0.0001	xx						
Fever past wk	0.0224 0.25	- 0.0562 0.004	- 0.1264 <0.0001	- 0.0560 0.004	- 0.0054 0.78	- 0.0414 0.03	0.0264 0.17	xx					
Drug use past wk	- 0.0480 0.01	- 0.0164 0.39	- 0.0337 0.08	0.0077 0.69	0.0044 0.82	- 0.0226 0.25	0.0482 0.01	0.5534 <0.0001	xx				
Antimal past wk	- 0.0076 0.69	- 0.0025 0.89	0.0045 0.82	- 0.0009 0.96	0.0129 0.50	- 0.0147 0.45	0.0236 0.22	0.1982 <0.0001	0.3582 <0.0001	xx			
Fever in preg	- 0.0249 0.20	- 0.0076 0.69	- 0.0482 0.01	0.0284 0.14	- 0.0033 0.86	0.0642 0.001	0.1057 <0.0001	0.2519 <0.0001	0.1997 <0.0001	0.0757 0.0001	xx		
Drug use in preg	- 0.0439 0.02	0.0264 0.17	0.0188 0.33	0.0785 <0.0001	0.0249 0.20	0.0651 0.001	0.0852 <0.0001	0.2044 <0.0001	0.2356 <0.0001	0.1022 <0.0001	0.7899 <0.0001	xx	
Antimal in preg	- 0.0254 0.19	0.0223 0.25	0.0063 0.74	0.0336 0.08	- 0.0261 0.18	0.0368 0.06	0.0168 0.38	0.1152 <0.0001	0.1666 <0.0001	0.1794 <0.0001	0.35589 <0.0001	0.4410 <0.0001	xx

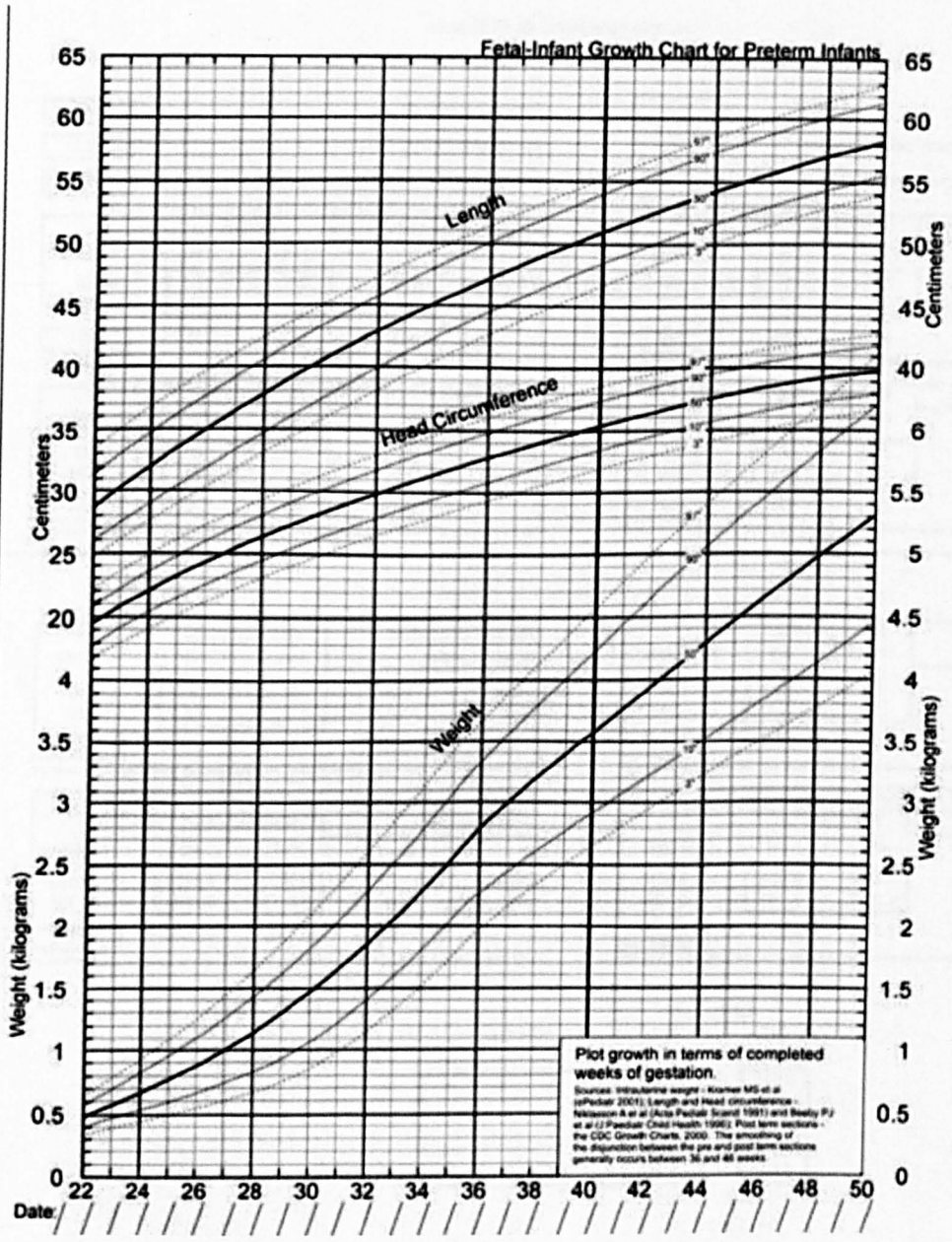
Annexe 8: Truncation Point for LBW

```
TRUNCATION POINT     2500.500
GRUNDY ITERATION NOT CONVERGED BY MAX I
DMU=     .0000000    DSIGMA=     .0000000

TRUNCATION POINT      2500.500
  NO OF ITERATIONS NEEDED=     8
  PREDOMINANT DISTRIBUTION MEAN=   2450.798000
  STANDARD DEVIATION SIGMA=         497.020500
THIS TRUNC POINT TOO HIGH Z=      .100000

TRUNCATION POINT      2250.500
  NO OF ITERATIONS NEEDED=     4
  PREDOMINANT DISTRIBUTION MEAN=   2632.062000
  STANDARD DEVIATION SIGMA=         430.250400
  Z=     -.886836
  ESTIMATED P=     -.043491

TRUNCATION POINT      2000.500
  NO OF ITERATIONS NEEDED=     4
  PREDOMINANT DISTRIBUTION MEAN=   2654.245000
  STANDARD DEVIATION SIGMA=         417.428800
  Z=    -1.566124
  ESTIMATED P=     -.010731


TRUNCATION POINT      1750.500
  NO OF ITERATIONS NEEDED=     4
  PREDOMINANT DISTRIBUTION MEAN=   2672.679000
  STANDARD DEVIATION SIGMA=         401.634600
  Z=    -2.296064
  ESTIMATED P=      .011034

TRUNCATION POINT      1500.500
  NO OF ITERATIONS NEEDED=     4
  PREDOMINANT DISTRIBUTION MEAN=   2665.801000
  STANDARD DEVIATION SIGMA=         409.608700
  Z=    -2.844913
  ESTIMATED P=      .004364
```

TRUNC POINT	ITNS	SUM NI	MEAN	ST DEV	ESTIM P
2500.5000	8	1308	OUTSIDE VALID RANGE		
2250.5000	4	1676	2632.0620	430.2504	-.043491
2000.5000	4	1881	2654.2450	417.4288	-.010731
1750.5000	4	1934	2672.6790	401.6346	.011034
1500.5000	4	1964	2665.8010	409.6087	.004364
1250.5000	2	1971	OUTSIDE VALID RANGE		
1000.5000	2	1977	OUTSIDE VALID RANGE		

```
 TRUNCATION POINTS MAXIMISING MEAN
 MINIMISING STANDARD DEVIATION
     AND MAXIMISING P
```

Annexe 9: Fenton Newborn Growth curve



Annexe 10: Excel sheet for sample calculation for diagnostics

sample calculation for PCR tests

Disease prevalence (%):		5.7	Total sample size:		989
Index test:	*actual sensitivity (%):*	95	*actual specificity (%):*		99.5
Reference test:	*actual sensitivity (%):*	95	*actual specificity (%):*		99.5
		95			

Predicted study results		Actual +ve	Actual -ve	Totals
Index test	+ve	54	5	59
	-ve	2	928	930
	Totals	56	933	989

Predicted study results		Actual +ve	Actual -ve	Totals
Reference test	+ve	54	5	59
	-ve	2	928	930
	Totals	56	933	989

Expected study results		Reference test +ve	Reference test -ve	Totals
Index test	+ve	51	7	58
	-ve	7	923	930
	Totals	58	930	989

Actual study results		Reference test +ve	Reference test -ve	Totals
Index test	+ve	37	6	43
	-ve	3	921	924
	Totals	40	927	989

RESOLVER TEST

Number of resolver tests performed		Reference test +ve	Reference test -ve	Totals
Index test	+ve	47	3	50
	-ve	2	262	264
	Totals	49	265	314

Number of positive resolver tests		Reference test +ve	Reference test -ve	Totals
Index test	+ve	43	2	45
	-ve	1	42	43
	Totals	44	44	88

Resolver +ve (truth = disease present) Probabilities		Reference test +ve	Reference test -ve	Totals
Index test	+ve	0.0342	0.0040	0.0383
	-ve	0.0015	0.1493	0.1508
	Totals	0.0357	0.1533	0.1891

Resolver -ve (truth = disease absent) Probabilities		Reference test +ve	Reference test -ve	Totals
Index test	+ve	0.0032	0.0020	0.0052
	-ve	0.0015	0.7820	0.7835
	Totals	0.0047	0.7840	0.7887

	Estimates: Old	New	95% C.I.
Sensitivity:	0.925	0.202	
Specificity:	0.994	0.993	
+ve predictive value:	0.860	0.880	
-ve predictive value:	0.997	0.839	